The Physical and Psychological Health of X-linked Carriers of Chronic Granulomatous Disease in the United Kingdom

Volume 1 of 1

Alexandra Battersby

N000027753

Institute of Cellular Medicine

Thesis for: Doctor of Philosophy

January 2015

Abstract

Background

Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiency in which there is a defect in one of the subunits of NADPH oxidase resulting in recurrent, severe infection, inflammation and autoimmunity. In the UK, 70% of cases are inherited in an X-linked (XL) manner, with the remainder being autosomal recessive (AR). Patients with CGD have an absent, or significantly reduced, neutrophil oxidative burst (NOB). XL-CGD carriers have a dual population of cells, those that function normally and produce an oxidative burst, and those that do not. XL-CGD carriers have been reported to have higher rates of discoid lupus, but there is little literature about other significant medical problems. Anecdotally, XL-CGD carriers suffer from more significant medical problems akin to that seen in CGD patients.

Methods

XL-CGD carriers were identified from the UK CGD Registry and through consultants caring for patients at the main centres in the UK; Great North Children's Hospital, Newcastle upon Tyne, Great Ormond Street Hospital and the Royal Free Hospital, London. A control group of carriers of Muscular Dystrophy (MD) were recruited from the Great North Children's Hospital.

XL-CGD carriers completed questionnaires about their medical and psychological health. Blood samples were taken for neutrophil oxidative burst, autoantibody panel and cytokine measurement. MD carriers completed psychological health questionnaires.

Questionnaires were compared with population data, where available, and published works about comparable groups. Psychological health questionnaires were compared to the recruited MD carrier control group.

Results

81 XL-CGD carriers were recruited from 62 families, 2 were deceased.

The mean NOB at enrolment was 47% with the majority of XL-CGD carriers falling in the range of 21-60%.

Photosensitivity was reported in 74% of the recruited XL-CGD carriers and 40% reported a DLE-type malar rash. 26% of XL-CGD carriers met 4 or more of the ARA SLE criteria, whilst a further 30% met 3 or more criteria. 23% suffered recurrent or significant infection. 53% suffered from gastrointestinal symptoms and 59% suffered joint symptoms. Other autoimmune phenomena including Raynaud's phenomenon were reported.

66% XL-CGD carriers suffered greater than normal levels of anxiety and 27% suffered depression. The XL-CGD carriers had significantly higher anxiety scores than parents of children with Cystic Fibrosis and had similar anxiety scores to published data about patients with SLE.

50% XL-CGD carriers suffered excessive fatigue. IL-8 levels were significantly higher in XL-CGD carriers compared to healthy controls. IL-8 levels were significantly higher in XL-CGD carriers reporting excessive fatigue than XL-CGD carriers who did not report significant fatigue.

Quality of Life (QoL) Scores were reduced in all domains and significantly worse than UK population data. The XL-CGD carriers had poorer QoL than CGD patients in the social function, vitality and bodily pain domains.

Conclusions

This is the first study to have evaluated the health of XL-CGD carriers, and has demonstrated that XL-CGD carriers experience similar problems to CGD patients, with infection, inflammation and autoimmunity all demonstrated in this study. Excessive fatigue was reported in approximately half of the XL-CGD carriers and was associated with higher levels of IL-8.

The aetiology for the symptoms seen in the XL-CGD carriers in this study is unclear. There was a lack of consistent correlation with degree of residual NOB function, with only recurrent skin abscesses, diarrhoea and abdominal pain being significantly associated with lower values. The raised IL-8 in the fatigued XL-CGD carriers supports the hypothesis of an inflammatory process but further work is required to investigate this. The lack of association with degree of

residual NOB function means identifying XL-CGD carriers at risk of medical symptoms is not possible simply by assessing their NOB function.

Psychological health has also been affected with the high rates of anxiety in the XL-CGD carrier population and significantly reduced QoL scores in comparison to UK population data. This has not been previously demonstrated. The psychological health problems are likely to be multifactorial in aetiology.

This study has clearly demonstrated that XL-CGD carriers must now be considered as potential patients and should be pro-actively assessed and managed. It is not yet clear what the optimal medical management is, and this now needs to be investigated.

Acknowledgements

This study was funded by the Bubble Foundation and promoted by the CGD Society.

I would like to thank all the medical professionals involved in the care of families with CGD for their help in recruitment and particularly the CGD Nurse Specialist. I would also like to thank the participants for taking part in this study.

Particular thanks go to Alex Mann (undergraduate medical student) who undertook the cytokine laboratory work.

Abbreviations for Thesis

AR	Autosomal Recessive
BCG	Bacille Calmette-Guérin
BMD	Becker's Muscular Dystrophy
BMI	Body Mass Index
BP	Bodily Pain (domain in SF36)
CD	Crohn's Disease
CF	Cystic Fibrosis
CGD	Chronic Granulomatous Disease
DHR	Dihydrorhodamine
DLE	Discoid Lupus Erythematosus
DMD	Duchenne Muscular Dystrophy
ER	Emotional Role (domain in SF36)
GH	General Health (domain in SF36)
GI	Gastrointestinal
GNCH	Great North Children's Hospital
GOS(H)	Great Ormond Street (Hospital)
GP	General Practitioner
HADS	Hospital Anxiety and Depression Scale
HRQoL	Health Related Quality of Life
HSCT	Haematopoietic Stem Cell Transplant
HUMARA	Human Androgen Receptor Gene
IBD	Inflammatory Bowel Disease
IL	Interleukin
MD	Muscular Dystrophy

MCS	Mental Component Score (summary score in SF36)
MFSI-SF	Multidimensional Fatigue Symptom Inventory-Short Form
MH	Mental Health (domain in SF36)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate-oxidase
NBT	Nitroblue Tetrazolium
NOB	Neutrophil Oxidative Burst
NPSLE	Neuropsychiatric Systemic Lupus Erythematosus
PCS	Physical Component Score (summary score in SF36)
PF	Physical Function (domain in SF36)
pGALS	Paediatric Gait Arms Legs Spine
PID	Primary Immunodeficiency
PIP	Pediatric Inventory for Parents
PMA	Phorbol-12-Myristate-13 Acetate
PR	Physical Role (domain in SF36)
PRI	Perceptual Reasoning Index
pSS	Primary Sjögrens Syndrome
PSI	Processing Speed Index
QoL	Quality of Life
ROS	Reactive Oxygen Species
SF	Social Functioning (domain in SF36)
SF-36V2	Short Form 36 version 2 (Quality of Life Assessment Tool)
SGRQ	St George's Respiratory Questionnaire
SLE	Systemic Lupus Erythematosus
SOP	Standard Operating Procedure
UC	Ulcerative Colitis
UTI	Urinary Tract Infection

VCI	Verbal Comprehension Index
VT	Vitality (domain in SF36)
WAIS-IV	Wechsler Adult Intelligence Scale version IV
WMI	Working Memory Index

List of Figures

List of Tables

List of Appendices

Chapter 1: Introduction

This chapter will present an overview of chronic granulomatous disease (CGD).

1.1 Definition

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency (PID), in which phagocytes are unable to generate reactive oxygen species (ROS) due to a defect in one of the subunits of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase. This defect results in a failure to kill bacteria and fungi. Patients suffer recurrent, life threatening infection and systemic inflammation.

1. 2 History of CGD

CGD was first described in the 1950s as a syndrome of recurrent suppurative lymphadenitis, abscesses and pulmonary infiltrates in boys, with death almost universal by the age of 7 years. The high early mortality led to the description 'Fatal Granulomatous Disease of Childhood' [1, 2].

The mechanism for this disease and its link to early mortality was unknown until the 1960s, when it was discovered that phagocytes of affected boys had reduced bactericidal activity and a reduced oxidative burst [3]. Further information about the mechanism of this disease was gained, with recognition of the failure of phagocytes to reduce nitro-blue tetrazolium (NBT) during phagocytosis, providing an assessment of phagocyte function. A carrier state was also demonstrated in female relatives with impaired, but not absent NBT reduction [4].

Recognition of CGD as an X-linked (XL) disease, with female carriers demonstrating a dual population of cells due to lyonisation was the earliest understanding of inheritance of the disease in 1967[5]. Lyonisation is the process of random inactivation of one X chromosome in females. Thompson et al [6] as early as 1970 observed that the female relatives of patients seemed to suffer from skin complaints, including recurrent boils. Further studies

demonstrated that XL was not the only mode of inheritance, and an autosomal recessive form was recognised [7].

Further understanding about CGD has been gained from information about clinical features and outcomes that has been collected through disease registries both internationally [8-10] and in the UK [11]. The information gained through these registries has greatly advanced understanding of the condition and contributed to the knowledge of the disease and clinical course. The improved understanding has aided advances, which has improved survival and these advances will be discussed later.

1.3 NADPH Oxidase

NADPH oxidase is a complex enzyme involved in the oxidation of NADPH, found in phagocytes as its predominant role is in killing of microorganisms. In this process, NADPH is oxidised resulting in the production of NADP, a hydrogen ion and a superoxide. This is shown by the equation:

$$NADPH + 2O^2 \rightarrow 2O^{2\cdot-} + NADP + H^+$$

The resultant superoxide is converted into a reactive species such as H_2O_2 or HOCl.

1.3.1 Structure of NADPH Oxidase

NADPH Oxidase comprises 6 different subunits. Knowledge of the components of NADPH oxidase and their role continues to develop.

There are two membrane bound subunits, three cytosolic components and a low molecular weight G protein. The membrane bound components are $gp91^{phox}$ and $p22^{phox}$ and together comprise cytochrome *b558*. The 3 cytosolic components are $p47^{phox}$, $p61^{phox}$ and $p40^{phox}$. The final components are the low molecular weight G proteins, rac1 and rac2, which are required for activation.

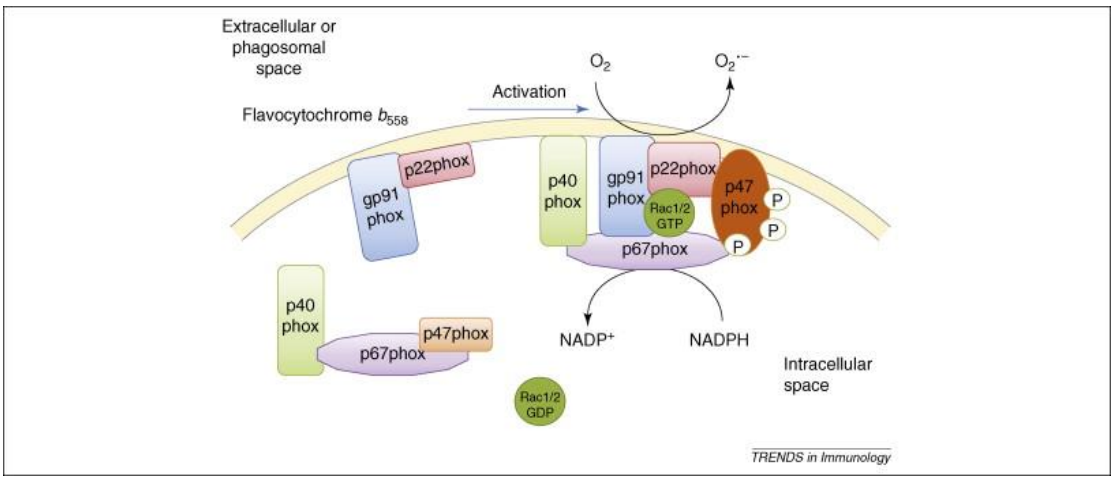
1.3.2 NADPH Oxidase Activation

In order for NADPH-oxidase to be active, the cytosolic and membrane bound components must be brought together. This is a controlled process where the cytosolic components migrate towards the membrane bound cytochrome *b558*

and bind to them. p47^{PHOX} is responsible for carrying the cytosolic proteins to the membrane bound subunits.

Activation of NADPH oxidase may occur by either a receptor-dependant or receptor independent mechanism. Receptor dependant stimuli are short lived, up to 5 minutes, for example by complement fragment C5a or immune complexes, whilst receptor-independent stimuli continue for as long as the stimulus remains, for example phorbol myristate acetate (PMA). The process of NADPH oxidase activation is shown in the diagram in Figure 1-1.

Figure 1-1: NADPH Oxidase Activation [12]



1.3.3 Residual NADPH Oxidase Function

There is considerable heterogenecity of disease severity in CGD, which, in part, may be related to degree of residual NADPH oxidase function. Evidence for this comes from case reports. For example, a recent report describes a boy who at 9.4 years old was diagnosed with XL-CGD due to recurrent pneumonia but no other clinical features of CGD. He was proven to have XL-CGD despite residual NADPH oxidase function [13].

Kuhns et al [14] studied 287 patients with CGD with 154 distinct mutations. Better survival was associated with greater residual NADPH oxidase function and ROS production. Additionally, alongside improved mortality, less severe disease was associated with greater ROS production. The specific mutation was shown to correlate with the degree of ROS production and a reduction in

infectious burden. There was no association found between the degree of ROS production and the presence of colitis.

1.3.4 Mechanism of Hyperinflammation in CGD

The inflammatory complications in CGD are due to an aberrant response to inflammatory stimuli. The exact inflammatory pathology is poorly understood, but there are several hypotheses, which will now be considered.

One of the earliest and simplest potential mechanisms is the failure of clearance of phagocytosed material. CGD phagocytes accumulate microbial material or cellular debris, including apoptosed neutrophils, but due to the lack of functional NADPH oxidase are unable to clear this material, resulting in persistent cell activation and inflammation [15].

Inflammasomes have been indicated in chronic inflammatory disease and Meissner et al [16] evaluated their role in CGD patients. CGD patients had significantly raised IL1β released from monocytes in those who were symptomatic. The levels of IL1β release were particularly significant in those suffering from colitis, but were seen in all symptomatic CGD patients when compared with healthy controls. This study supports the role of capase-1-mediated inflammation as an aetiology of the inflammatory complications of CGD and confirms the findings in the mouse model.

Experimental work on CGD mice and human cells has yielded further information about IL1 dependant mechanisms in the hyperinflammation seen in CGD and have demonstrated a more complex understanding. Autophagy is the process of self-degradation [17] and is important in the removal of intra-cellular pathogens, and has been shown to be defective in CGD patients as it is thought that ROS production is a necessary component [18]. In a recent publication, de Luca et al [19] demonstrated that the defective autophagy, seen in both mice and human CGD patients, was associated with increased release of IL1β. This release of IL1β has been previously studied in mice where autophagy was defective and severe colitis was seen [20]suggesting this has clinical relevance and is important in the inflammatory complications of CGD. Furthermore, de Luca et al demonstrated that this could be used as a therapeutic target. Defective

autophagy was repaired by the use of anikinra. Anikinra is an IL1 receptor antagonist. By blocking IL1, the amount of IL1β secreted was reduced and there was clinical improvement.

A study of tryptophan catabolism in a CGD p47^{phox} knock out mouse model[21] suggested that Indoleamine2,3-dioxygenase (IDO) has an important role in the exaggerated inflammation typical of CGD. The CGD mice were infected with *Aspergillus fumigatus* and the resultant inflammatory lung injury was not only more damaging than the initial infection, but also found to be due to inefficient tryptophan catabolism as a result of blocked IDO function.

1.4 Genetics

There are two modes of inheritance of CGD; autosomal recessive (AR) and X-linked (XL). In the UK, Europe and the United States XL disease is the most common form, accounting for approximately 70% of cases [8, 11]. In areas with higher consanguinity rates, AR disease is more common. For example in Turkey and in India up to 60% of cases are AR[22, 23].

The gene causing XL-CGD is *CYBB*, and is located at chromosome Xp21.1. This gene codes for the gp91^{phox} protein, which is integral to NADPH oxidase function. The gp91phox protein consists of 570 amino acids. Missense mutations in amino acids 1 to 309 have been shown to be associated with higher residual superoxide production when compared with mutations in the later amino acids [14]. Accurate molecular diagnosis of patients has enabled over 681 different mutations to be identified within the gene at this location and shown to be, causing X-linked CGD [24]. Splice-site, insertion, deletion, missense and nonsense mutations are all described. The most frequently occurring mutations are deletions accounting for 35.6% of cases [24].

AR disease is the result of a defect in one of p47^{phox}, p67^{phox}, p22^{phox} or p40^{phox} protein subunits, with defects in p47^{phox} being the most common. Defects of p40^{phox} have very rarely been described in isolation as causing CGD but a recent report confirmed findings from mice [25] that p40^{phox} defects may cause CGD in humans [26]. Table 1-1 shows the different CGD subtypes, their mode of inheritance and relative frequency[27].

Table 1-1: CGD Subtypes and Inheritance [27]

Subunit	Inheritance	Gene	Frequency
gp91^{phox}	XL	CYBB	70%
p47^{phox}	AR	NCF1	25%
p67^{phox}	AR	NCF2	2%
p22^{phox}	AR	CYBA	3%
p40^{phox}	AR	NCF4	Rare

AR-CGD has been described as having a less severe phenotype than XL-CGD. However, this is likely to be an over simplification. The role of residual NADPH oxidase has already been discussed and it is likely that this, along with the specific mutation, accounts for the differing phenotypes rather than simply the mode of inheritance. This furthers the argument for ensuring an accurate genetic diagnosis in all patients.

1.5 Clinical Features of CGD

The clinical features of CGD are recurrent infection, inflammatory complications and a predisposition to autoimmunity [28].

1.5.1 Infection

Patients suffer recurrent, severe bacterial and fungal infection, with particular susceptibility to catalase positive organisms due to their impaired oxidative burst. The most common bacterial organisms affecting CGD patients include *Staphylococcus Aureus* and *Burkholderia Cepacia* [8]. Fungal infections pose a significant problem and represent a leading cause of death [8], with *Aspergillus* species of particular importance [10, 28]. All species of *Aspergillus* are prevalent in CGD, but *Aspergillus Niduluns* shows a predilectation for, and particular virulence in[29], CGD patients even when compared with other PIDs [30].

Children with CGD may present with infection at virtually any site, but the most frequent presentations are pneumonia, abscesses and lymphadenitis [8, 10, 11]. Hepatic abscesses, particularly due to *Staphylococcus Aureus* [10, 31], are a common presentation in CGD and may be multiple and persistent. Less common, but reported, presentations include brain abscesses and osteomyelitis[8, 10].

An increased susceptibility to mycobacterial infection has been seen in both XL and AR CGD patients [32, 33]. Similarly, an increase in localised reactions to BCG (Bacille Calmette-Guérin) vaccination has been demonstrated. In a Chinese study of 17 XL-CGD patients, 7 patients suffered prolonged scarring or abscess formation following the administration of BCG vaccine [23]. This finding was confirmed in the large European study[10], where 8% of the 429 patients were found to have localised skin infection or lymphadenitis following BCG vaccination.

1.5.2 Inflammation

Clinical manifestations of CGD are not limited to recurrent infection. Patients with CGD suffer from abnormal inflammatory responses, which can affect virtually all organ systems and may occur independently of the infective manifestations. Well recognised complications include chorioretinitis [11, 34], colitis and granulomas in multiple sites [8, 10].

Granuloma formation is the hallmark of CGD resulting from an abnormal inflammatory response. Granulomas are often formed when infection has failed to be cleared. They contain inflammatory cells and are usually sterile. Granulomas may be found at any site and can subsequently result in secondary complications such as obstructive symptoms, depending upon their location.

Gastrointestinal manifestations are some of the most frequently seen non-infectious manifestations occurring in up to half of all patients [15, 35]. Colitis is a particularly common feature of CGD [8, 10, 15, 35] and was present in 37% of CGD patients in a comprehensive UK study in 2000 [11]. It has been particularly associated with XL disease and a family history of CGD colitis [11, 36, 37]. Median age of presentation of CGD colitis is 5 years, although it may present throughout life[37].

Symptoms of CGD colitis include diarrhoea, rectal bleeding, nausea, vomiting and abdominal pain[15]. In a study of 46 CGD patients with gastrointestinal involvement, abdominal pain was universal but diarrhoea, constipation, nausea, vomiting and bloody stool were all commonly described [37]. Growth and height attainment are significantly impacted by gastrointestinal involvement [37] and

failure to thrive is frequently present prior to the development of typical gastrointestinal symptoms, associated with anaemia [6, 15].

There is considerable clinical and histopathological overlap between CGD colitis and inflammatory bowel disease (IBD), particularly Crohn's disease, [15, 38, 39] and CGD presentation may mimic IBD. It is not uncommon for a diagnosis of CGD to be delayed if colitis is the presenting feature, as patients may be managed as IBD patients before the diagnosis of CGD is made [39].

The classical findings on gut biopsies of CGD patients exhibiting symptoms of colitis are a high number of eosinophils, decreased neutrophils and lipid-laden macrophages, along with granulomas [15, 35, 37] similar to findings in Crohn's disease. Histopathological findings in CGD colitis include microgranulomas, pigmented macrophages, tissue eosinophilia and acute and chronic inflammation [40]. A study of 7 patients with known CGD and gastrointestinal symptoms found that paucity of neutrophils with a predominantly eosinophilic infiltrate, without an associated rise in peripheral blood eosinophils, were the most specific features of CGD colitis [15]. Alongside this finding was the presence of large pigment-containing macrophages. Granulomas were not present in all patients.

Patient's without colitis symptoms are rarely biopsied, but it is possible that they have subclinical manifestations due to mucosal damage [41], as patients with CGD, irrespective of the presence of symptoms, have been found to have higher levels autoantibodies more typically associated with IBD than a control group [41].

Complications of gastrointestinal involvement may include obstruction at any point along the gastrointestinal tract, but frequently pyloric outlet obstruction. Fistulae and strictures are also common and initial presentation may be with appendicitis, in which granuloma may be demonstrated within the diseased organ when the appendix is removed [8].

Inflammation outside of the gastrointestinal tract is also seen. Within the urinary tract, inflammation is common, with inflammatory cystitis the most common manifestation [42]. Granulomas may be present within the bladder wall, or

throughout the urinary tract, and secondary obstruction may result in hydronephrosis [8, 43].

Pulmonary disease, manifesting as infection or inflammation, was the most common site of disease in the European registry [10]. Chronic respiratory disease was common in the UK registry, with 77% of those undergoing computerised tomographic imaging of the chest having significant abnormalities[11]. Typical features may include diffuse infiltrate, reticulonodular opacities and focal consolidation[28].

Chorioretinitis as a manifestation of CGD has been reported since the 1970s [44]. It affects a significant number of patients but is only very rarely associated with loss of vision [45].

1.5.3 Autoimmunity

Autoimmune phenomena are also described in CGD patients[46]. Juvenile idiopathic arthritis, cutaneous lupus erythematosus (LE) and IgA nephropathy are all reported in the CGD population [46]. Systemic lupus erythematosus (SLE)[47, 48], discoid lupus erythematosus (DLE)[10] and glomuerulonephritis [48] are also described. The pathogenesis of this is not well understood. However, it is likely these are not simply manifestations of disease, but, in fact, represent an increased risk of developing autoimmunity in CGD patients through disordered immune regulation, which may be related to abnormal apoptosis [49] and immune complexes. This will be discussed in greater depth in chapter 2.

1.5.4 Malignancy

There have been case reports of an increased risk of malignancy in CGD patients with Hodgkins lymphoma [50] and glioblastoma [51]reported. Weel et al[52] report 3 CGD patients each suffering from different malignancies (rhabdomyosarcoma, melanoma and retinoblastoma).

These case reports have not been corroborated by the large scale registries[8, 10] and the small number of different malignancies makes it difficult to be certain of a disease-related association.

1.6 Investigations and Diagnosis

Suspicion of a diagnosis of CGD is based upon a clinical history of recurrent infection, particularly with catalase positive organisms or fungi, and inflammation. Clinical suspicion is confirmed by the demonstration of abnormal NADPH oxidase activity or protein expression and subsequent identification of the specific mutation. In families where there have been previous cases of CGD, diagnosis may be made before the onset of symptoms.

Diagnosis of carriers of XL disease may be made by family history. For example, female offspring of affected males will be obligate carriers and carrier status may be suspected in the female relatives of diagnosed patients. Confirmation of carrier status may be made using the same techniques used for diagnosis of patients.

This section will outline the techniques used for diagnosis of patients and carriers of CGD.

1.6.1 NADPH Oxidase Activity

The diagnostic feature of CGD is demonstration of abnormal NADPH oxidase activity and an inability of phagocytes to produce reactive oxygen species (ROS). Neutrophils are routinely used to demonstrate the diagnosis. As NADPH oxidase is inactive in resting phagocytes, the neutrophils must be activated by a stimulus in order to assess NADPH oxidase activity. Phorbol-12-Myristate-13 Acetate (PMA) has become the standard agent but alternatives are available. The neutrophils are stimulated to produce ROS such as hydrogen peroxide [53].

NADPH oxidase activity may be assessed in a number of ways: through oxygen consumption, superoxide generation or hydrogen peroxide production.

Oxygen Consumption

Measurement of oxygen consumption is rarely used in clinical practice as it is time consuming and expensive. However, it does provide a quantitative assessment of oxygen consumption by use of an oxygen electrode[54].

Superoxide Generation

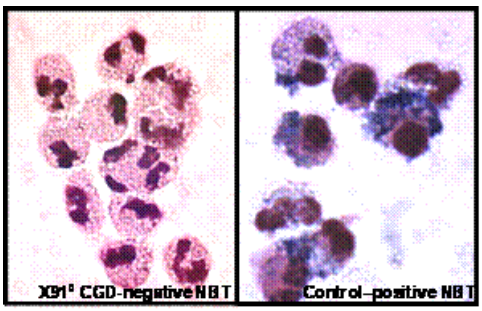
Superoxide generation may be measured by the ability of phagocytes to reduce a known reagent. The most commonly used agent is nitroblue tetrazolium (NBT) in the NBT reduction test.

Nitroblue Tetrazolium (NBT) reduction test

The NBT reduction test is the traditional test used to confirm the diagnosis of CGD. NBT is a yellow dye that is reduced to blue formazan by the superoxide produced as a result of the respiratory burst. Neutrophils are stimulated as described above and NBT added. The reaction takes place intracellularly and the result is read manually. CGD phagocytes, which have phagocytosed NBT, remain yellow as they do not reduce the NBT (as no superoxide is generated) and normal phagocytes show the blue insoluble, precipitate of formazan. XL-CGD carriers demonstrate both colours, as the two populations of cells are present.

Interpretation of results in this test is subjective and detection of XL-CGD carriers can be difficult as the two populations may not be well defined or distinct [55]. However, an experienced technician will be able to quantify the percentage of functioning neutrophils. Figure 1-2 shows a normal and abnormal NBT.

Figure 1-2: Normal and Abnormal NBT Reduction Test Slides[56]



NBT reduction test

Alternatives to NBT include ferricytochrome *c* or lucigenin, but these are rarely used in clinical practice.

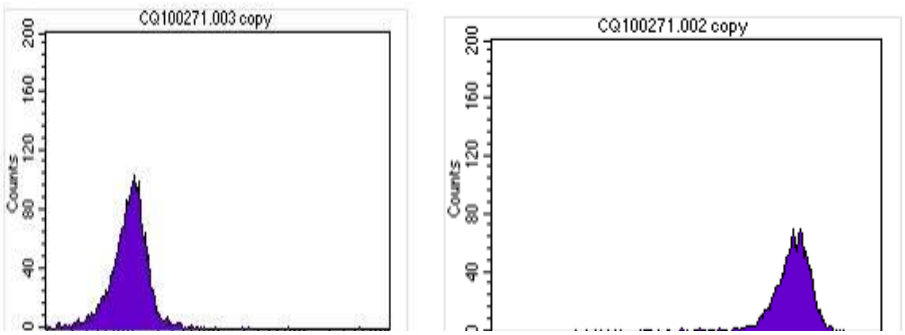
Hydrogen Peroxide Generation

Hydrogen peroxide (H_2O_2) generation may be used to assess the oxidative burst by using flow cytometry and H_2O_2 detecting agents.

Flow Cytometry (DHR)

A flow cytometric method may be used to demonstrate the absence or reduction in the oxidative burst in CGD patients and carriers. Neutrophils are isolated and stimulated in the same manner as for the NBT reduction test. An H_2O_2 detecting agent such as dihydrorhodamine-1,2,3 (DHR) is added and freely enters the cells. DHR is oxidased by the ROI to rhodamine-1,2,3 which emits a fluorescent signal, which is detected by the flow cytometer.

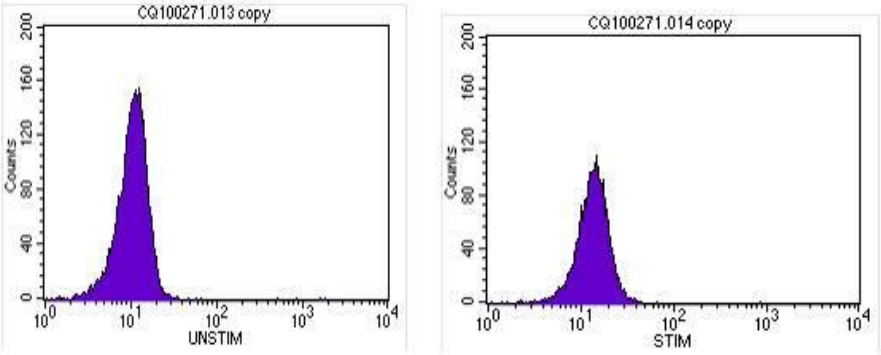
Figure 1-3: Unstimulated and Stimulated DHR in normal control (images courtesy of Dawn Barge, Immunology, Newcastle upon Tyne Foundation Trust)



In healthy controls, all phagocytes produce an oxidative burst, shown as a peak as seen in Figure 1-3.

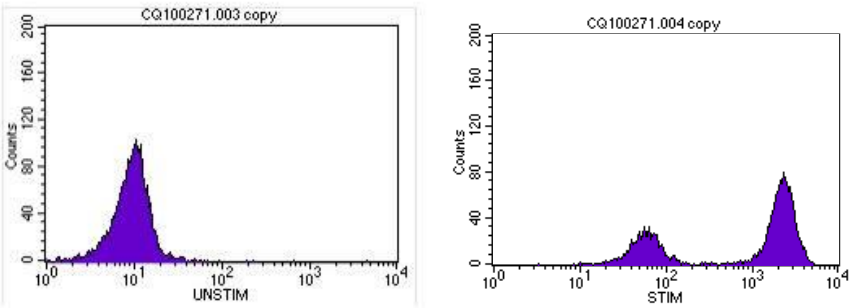
In patients with CGD, the oxidative burst is absent in the majority of X-linked patients or substantially reduced, and no peak is produced upon stimulation as shown in Figure 1-4.

Figure 1-4: Unstimulated and Stimulated DHR in CGD Patient (images courtesy of Dawn Barge, Immunology, Newcastle upon Tyne Foundation Trust)



Carriers of XL-CGD demonstrate two populations of phagocytes, as some phagocytes produce an oxidative burst when stimulated, whilst those in which the mutated X-chromosome is active i.e. $gp91^{phox}$ negative, do not. Most carriers will exhibit between 20-80% normal burst activity. The DHR of an XL-CGD carrier is shown in Figure 1-5.

Figure 1-5: Unstimulated and Stimulated DHR in XL-CGD Carrier (images courtesy of Dawn Barge, Immunology, Newcastle upon Tyne Foundation Trust)



This is a very sensitive and reliable method of assessment and can be performed on a small amount of blood collected with EDTA. It requires less subjective assessment than the NBT reduction test.

The use of DHR and flow cytometry is also dependant on myeloperoxidase oxidase (MPO). MPO deficiency would result in an abnormal result and is more common than CGD but of less clinical significance. G6PD deficiency will also result in an abnormal result by DHR flow cytometry. DHR has also been shown to be inaccurate during acute illness [57].

Alternatives to DHR have been shown to be less reliable. DHR provides the best distinction between normal and abnormal (CGD) phagocytes. When compared

with two other fluorescent probes (2'7'-dichlorofluorescin and 5,6-carboxy-2'7'-dichlorofluorescein diacetate) DHR demonstrated a higher intensity fluorescence and the greatest separation of fluorescent signals between normal and abnormal phagocytes [58]. Another alternative is Amplex Red which, although also sensitive and reliable, does not provide the quantitative value provided by DHR [54].

Practical Considerations

When assessing NADPH oxidase activity, there are several practical considerations to ensure accuracy as erroneous results can occur throughout the process. Samples of blood should be processed within 48 hours, and ideally 24 hours, of venepuncture in order to avoid inaccurate results. Samples should be transported in either an EDTA or heparin medium and kept at room temperature. Samples that are not fresh, are likely to contain degraded neutrophils due to apoptosis. Degraded or apoptotic neutrophils do not produce an oxidative burst and will result in a false reduction in measured NADPH oxidase activity, thereby giving an inaccurate result. This may be particularly important when determining carrier status. A control sample should be taken, transported under the same conditions as the test sample, and processed alongside the test sample.

1.6.2 NADPH Component Expression

Analysis of the individual components of NADPH oxidase may also be used in the diagnosis of CGD. By using FACS analysis of intact neutrophils and 7D5, a monoclonal antibody against $gp91^{phox}$ it is possible to determine the presence or absence of $gp91^{phox}$. This is the standard first investigation of component expression and if normal is followed by immunoblot analysis with antibodies against all NADPH oxidase subunits. One difficulty with this investigation is with the membrane components of NADPH oxidase. As $gp91^{phox}$ and $p22^{phox}$ stabilise each other, if one is absent the other is not detectable. Therefore, if one of the cytosolic components is absent, it is possible to be certain that this is the correct diagnosis. However, if either $gp91^{phox}$ or $p22^{phox}$ are absent, the other will not be detectable and further investigation is required to confirm the diagnosis,

although a family history or the sex of the patient may suggest the more likely diagnosis.

1.6.3 NADPH Component Activity

In rare cases it may be possible to demonstrate the presence of all 5 subunits, but with absent or reduced enzymatic activity. In these cases, it is important to consider variants in which a genetic mutation results in the preservation of protein expression, but the absence of activity.

1.6.4 Genetic Diagnosis

Mutation analysis should be undertaken in all cases of CGD [54], as defining the mutation allows for certainty in diagnosis and more accurate genetic counselling. Precise genetic diagnosis may have implications within the clinical setting. For patients undergoing haematopoietic stem cell transplantation (HSCT) from a family donor, the donor may be accurately screened to ensure they do not carry the defective gene. If gene therapy is to be used it is clearly imperative that there is a genetic diagnosis. In the research setting, the mutation can be used to correlate with clinical severity and specific problems. The precise mutation may also be used to determine association with degree of reduction in NADPH oxidase activity, information that can be transferred into the clinical setting.

Gene Sequencing

PCR amplification and sequencing may be used to analyse *CYBB*, *CYBA*, *NCF2* and *NCF4* genes, encoding gp91^{phox}, p22^{phox}, p67^{phox} and p40^{phox} proteins respectively. The analysis of *NCF1*, encoding p47^{phox}, is more difficult owing to the presence of pseudo-*NCF1* genes either side of the *NCF1* locus.

1.6.5 Carrier Specific Investigations

The identification of carriers of CGD is important for genetic counselling and for identification of further family members at risk. It may also be important for the individual's own medical health.

Whilst the importance of testing for carrier status of AR and XL disease is the same, the techniques required are different. AR carriers should be tested on a

genetic level with knowledge of the specific family mutation. This is required, as in AR disease carriers, NADPH oxidase activity is virtually normal.

XL-CGD carriers differ from AR-CGD carriers as they have variable NADPH oxidase activity owing to lyonisation. Lyonisation is a random process, occurring in early foetal development, which involves the inactivation of one X chromosome in all cells. For female carriers of XL-CGD this means, that in some cells the mutated X chromosome will be active whilst in others the wild type X chromosome will be active. As lyonisation is a random process, there may be an uneven split.

XL-CGD carriers will normally demonstrate a dual population of cells on either NBT or by flow cytometry with DHR, correlating with the degree of residual NADPH oxidase activity. XL-CGD carrier status may also be confirmed by the specific mutation in the *CYBB* gene. However, up to one third of XL-CGD patients have a new germ line mutation [54] and therefore, the absence of an XL-CGD carrier mother does not exclude XL disease in the patient.

X-Inactivation Measurement

It is possible to quantify which X-chromosome is active in carriers by use of the human androgen-receptor gene (HUMARA) assay. This assay measures the degree of methylation at the HUMARA locus [59]. This can be correlated with degree of reduced NAPDH oxidase activity found by either NBT or DHR.

1.7 Management of CGD

Treatment of CGD is based upon prophylaxis against bacterial and fungal infection, aggressive management of established infection and management of the inflammatory complications.

Antibiotic prophylaxis is well established, with the most commonly used agent being co-trimoxazole [11, 60]. Antifungal prophylaxis is part of standard care and itraconazole is increasingly the agent of choice[60]. Management of acute infectious episodes should be aggressive. This involves prompt initiation of antibiotics, thorough investigation for causative organisms, particularly fungi, and use of adjuvant therapies. Examples of adjuvant therapies include

corticosteroids for the treatment of liver abscesses [61] and the use of IFNγ, common place in the United States of America, and granulocyte infusions[42].

Corticosteroids form an important part of the treatment of inflammatory and granulomatous manifestations of disease. Corticosteroids remain first line and mainstay treatment for CGD related colitis [37]. The management of CGD colitis is similar to the treatment of other inflammatory bowel conditions, with agents such as sulfasalazine being used with good results and infliximab becoming increasingly important [15, 37].

The use of infliximab may be considered in those patients with significant CGD colitis despite conventional management. However, whilst there has been success with the use of anti-TNF alpha therapy, serious and even fatal infections are reported [62]. In a case series of 5 CGD patients with inflammatory bowel disease treated with infliximab, all improved significantly, including resolution of fistulae, but all developed significant infection requiring the infliximab to be discontinued [53].

Haematopoietic stem cell transplantation (HSCT) remains the only curative treatment at present. There has been success with unrelated donor stem cell transplantation as well as matched sibling donor transplantation [63] and increasingly HSCT is being viewed as an early treatment option. A recent UK study demonstrated that, not only was HSCT successful in curing CGD patients, but that quality of life in paediatric CGD patients who had undergone HSCT was the same as normal population data [60, 64]. The continued success of HSCT in CGD patients has now led to HSCT being considered the treatment of choice.

Gene therapy remains a salvage therapy at present for CGD, but a promising alternative therapy although currently remains predominantly in the research setting [65-68]. Conservative management remains an option for those who do not undergo HSCT. However, life expectancy for those managed conservatively remains significantly reduced with up to 50% dying before mid-adulthood [11].

1.8 XL-CGD Carriers

Female carriers of XL-CGD possess one copy of the mutated gene and have a mosaic pattern of wild type and mutated X chromosomes, producing a mixture of

gp91^{phox} positive and gp91^{phox} negative phagocytes. This is the result of lyonisation. Lyonisation is the random inactivation of one X chromosome and occurs early in the foetal development of haematopoietic precursor cells. In phagocytes where the X chromosome with the mutated gene is active there is no gp91^{phox} expression whilst, where the wild type X chromosome, is active there is normal gp91^{phox} expression in those phagocytes. As lyonisation is a random process, there is a wide variability of the proportion of phagocytes expressing the mutated gene.

Phagocytes expressing gp91^{phox} have normal NADPH oxidase activity, whilst those which are gp91^{phox} negative, do not. XL-CGD carriers therefore, have overall reduced NADPH oxidase activity. The degree of NADPH oxidase activity depends upon the proportion of phagocytes expressing gp91^{phox} and also the specific mutation [14]. It ranges from near normal to severely depleted. It is possible to quantify the degree of activity and most XL-CGD carriers fall within the range of 20-80% reduction [69, 70].

The reduction in degree of NADPH activity has been considered to have no significant clinical consequences, with XL-CGD carriers considered healthy despite the link with discoid lupus erythematosus (DLE) [71-73]. Currently, most centres in the UK do not routinely offer carriers any clinical review or prophylactic treatment. Logically, however, carriers have a theoretically increased risk of infection and abnormal inflammatory response similar to patients with CGD, if a sufficient percentage of phagocytes are functionally deficient. Early observations found the mothers of boys with the newly described CGD, had rashes resembling lupus [6] and increasingly, manifestations beyond skin disease are being described in XL-CGD carriers, with symptoms similar to classical disease reported. There are numerous case reports of female carriers manifesting symptoms typical of CGD and the associated features [74-77]. The literature on this will be reviewed in the next chapter.

The frequency and percentage of symptomatic XL-CGD carriers is unknown. The overriding hypothesis for symptoms in XL-CGD carriers is that whilst most XL-CGD carriers have fairly equal distributions of the two populations of cells, some appear to have disproportionate levels of inactivation of the unaffected X-

chromosome. This results in a reduction in the degree of NADPH activity to levels more akin to that of patients, making these carriers susceptible to the same spectrum of disease.

The degree of skewing of X-chromosome inactivation and symptoms in XL-CGD carriers is not constant throughout life and the exact relationship to degree of symptoms has not been established. Some XL-CGD carriers appear to have had little difficulty with infection in early childhood, unlike the typical CGD patient [75, 76], whilst others who have significant manifestation at older ages have also suffered recurrent, although less severe, infection in childhood [74]. It is proposed that age-related skewing of lyonisation and a decrease in the percentage of functioning neutrophils may account for the increased susceptibility to opportunistic infection later in life [75], but this has not been corroborated.

The 'breakpoint' at which female carriers become susceptible to infection and symptoms of CGD is uncertain, as symptoms have been described with a range of NOB reduction. It would seem likely that there is point at which functional phagocytes are unable to compensate for the defective population, and it is at this point that symptoms are seen.

Testing for Carrier Status

Testing for carrier status of a genetic disease has ethical and medical implications. The timing of offering and performing genetic testing is an important issue. For CGD, at present, the benefits for an individual in knowing their carrier status relate predominantly to decisions regarding reproduction and having an affected child. As carriers of XL-CGD are currently believed to be healthy there is little to support testing female relatives of index cases, except to confirm inheritance pattern and in screening for potential transplant donors.

It is currently recommended that in children and young people, testing for carrier status of any genetic condition is deferred until an age at which they are able to make an informed decision themselves. Conventionally this is 18 years of age [78]. The benefits of testing versus the benefits of delaying testing are controversial.

Few studies have evaluated the opinions of adolescents themselves, but James et al [79] looked qualitatively at a small cohort of adolescent sisters of patients with CGD and also at their parents. There was discrepancy between the views of the parents and the adolescent girls. The adolescent girls agreed with the recommendation that testing should be deferred. They uniformly felt that they would want to know their carrier status before starting a family, but when offered hypothetical immediate testing none would have accepted this. The parental view differed as the majority felt that testing earlier would be beneficial for the family as a whole.

There is currently not sufficient evidence to support testing for carrier status on medical grounds. Knowledge of carrier status will not alter the treatment received by an individual or their management. However, if it can be demonstrated that XL-CGD carriers are at increased risk of medical problems, there may be an advantage to earlier testing in order that they may receive appropriate investigation and treatment at an earlier stage. My study will provide evidence as to whether there is a benefit to testing for XL-CGD carrier status earlier than currently indicated.

There are psychological aspects to both knowing and not knowing about carrier status. These may be affected by the relationship of the potential carrier to the index case. Those who have a greater understanding of the condition may have a very different viewpoint to a more distant relative who has not seen the full impact of the condition and treatment. This may affect psychological health and indeed quality of life.

1.9 Outline of Thesis

This chapter has outlined information about CGD including genetic inheritance, pathogenesis, diagnosis, clinical manifestations and treatment. Chapter two will review the pertinent literature to the study and provide further background information about XL-CGD carriers. Chapter three will outline the aims and hypothesis underpinning the study and chapter four will describe the methods employed in the study. The results will be presented in chapters five to eight and will be divided into clinical, psychological (including IQ), fatigue and quality of

life findings. Chapter nine will discuss the findings and chapter ten will draw together the final conclusions and make clinical recommendations.

Chapter 2: Literature Review

2.1 Introduction (Manifestations of Disease in XL-CGD Carriers)

There is little published literature about the health of XL-CGD carriers, and it is confined predominantly to case reports, case series or letters. However, within the literature about patients with CGD, references are made to their female relatives and the health of these XL-CGD carriers.

This chapter will examine all the available literature about carriers of XL-CGD. It will also examine the literature surrounding the symptoms described in XL-CGD carriers and about XL carrier status in other diseases.

2.1.1 Anecdotal Experience

Unpublished anecdotal experience in the Great North Children's Hospital (GNCH), Newcastle upon Tyne, highlighted that female relatives of XL-CGD patients were suffering from significant medical problems. Inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE) were seen in two separate families.

2.1.2 Early Descriptions

The earliest observation of symptomatic XL-CGD carriers was in 1969 when Thompson et al reported on 10 cases of CGD in 1969 and observed facial rash, resembling lupus, polyarthritis and Raynaud's phenomenon occurring in 3 of the female relatives [6]. Since 1969 there have been more descriptions of symptomatic XL-CGD carriers and these will be discussed here.

2.2 Infection in XL-CGD Carriers

As previously discussed, CGD patients suffer recurrent infection with particular susceptibility to infection with catalase positive organisms. Despite the reduced number of functioning neutrophils in XL-CGD carriers, recurrent infection has not been reported as a significant problem. There are a handful of cases described where infection has been the main, or an important, feature in an XL-CGD carrier.

Johnston et al[80] described a girl presenting with recurrent infections classical of CGD, including recurrent infective abscesses, pneumonia and multiple isolations of *Staphlyococcus Aureus*, who was found to be a carrier of XL-CGD. Lewis et al [74] described a 16 year girl who presented with persisting pneumonia despite aggressive antibiotic therapy who was found to be an XL-CGD carrier. *Aspergillus fumigatus* was isolated from broncheoalveolar lavage and sputum specimens. She had been previously fit and well, but did have a history of recurrent abscesses suggesting an additional infective burden.

Further to the case described by Lewis et al[74], recurrent skin abscesses requiring antibiotics and, at times, surgical drainage have been described in XL-CGD carriers [70, 76, 77, 81] and have been shown to isolate CGD-associated organisms including *Staphlyococcus Aureus* [82]. Single episodes of infection have also been reported such as the case of persistent systemic salmonella infection in a 34 year old XL-CGD carrier[83]. As a single example of infection, this perhaps does not carry significant weight to the hypothesis of XL-CGD carriers being at risk of similar infective organisms to the patients. However, in the context of the case reports, there is a growing body of evidence that suggests XL-CGD carriers may be at greater risk of the infective manifestations of CGD.

In summary, the literature about infection in XL-CGD carriers is limited predominantly to case reports and the small numbers reported make it difficult to draw conclusions. However, these reports are of interest as they lead us to question if there may be an infection risk associated with XL-CGD carrier status and if there are more cases, as yet unreported, indicating that further investigation is warranted.

2.3 Inflammatory Manifestations of CGD in XL-CGD Carriers

2.3.1 Skin Disease

The most frequently reported problem in XL-CGD carriers is skin disease. An association with discoid lupus erythematosus (DLE) has been well described [69, 72, 84, 85]. The presence of a persistent facial rash resembling DLE in 2 XL-CGD carriers was first described in 1970 [6] and these findings have been

corroborated subsequently, most recently in 2007 when Cale et al [69] found photosensitive skin rashes in 58% of their XL-CGD carrier cohort.

The clinical and histopathological features of rashes seen in the XL-CGD carriers are consistent in the literature, but severity and clinical course are not. Virtually all cases describe the presence of photosensitive eruptions on sun-exposed surfaces with similar, if not identical, histological findings to DLE when biopsies were performed[84]. The most common site described is in the malar distribution and on the hands. Age at onset of skin disease is not constant, with reports of early childhood appearances [81] featuring alongside later presentations [72]. Severity ranges from minor irritation, resolving in childhood, to treatment-resistant disease persisting into late adulthood.

Photosensitivity alone is commonly described in XL-CGD carriers without the presence of classical DLE[84, 86]. Sillevis-Smitt et al described 10 of 16 XL-CGD carriers with relapsing skin eruptions, in whom 7 were provoked by sunlight, but not all had typical features of DLE [72]. Despite the link of DLE with XL-CGD carriers, DLE is unusual in patients with XL-CGD, although has been occasionally reported [87].

Conversely, when XL-CGD carrier status was sought in patients presenting with DLE, no carriers were found [88]. This was, however, a small scale study of 19 patients and the authors acknowledged that it remains important to look for XL-CGD carrier status in women presenting with DLE, particularly in the presence of related symptoms such as recurrent or suppurative infection or a family history of early childhood death. Their findings were corroborated in a further study of 34 patients presenting with DLE, tested for XL-CGD carrier status by performing an NBT test. Again, no XL-CGD carriers were found, but the authors recommended screening for carrier status particularly in the presence of aphthous stomatitis [89].

Alongside photosensitivity and DLE manifestations, aphthous stomatitis is reported with even greater prevalence. Brandrup reported as early as 1981 that 7 out of 9 X-linked carriers suffered recurrent aphthous-like stomatitis[84]. This was supported by a later survey in 1989 when 70% of 16 carriers reported recurrent aphthous stomatitis [72] higher than the 63% reporting recurrent skin

eruptions. In both reports the symptoms have been severe. Case reports also corroborate these findings [70, 75].

Photosensitivity may not always be immediately clinically apparent. Therefore, unless specific enquiry is made, the symptom will be missed. Subsequently, it is likely to be more prevalent than previously described.

2.3.2 Gastrointestinal Disease

Gastrointestinal manifestations have infrequently been described in XL-CGD carriers and there are three reports in the literature. Moltanyer et al [90] described a female carrier who presented at the age of 41 years with proven bronchocentric granulomatosis. Her background history revealed that, at age 13 years, she had been diagnosed with ulcerative colitis and had a history of recurrent infection. On testing, she had 10% functioning neutrophils. This combination of inflammatory bowel disease, recurrent infection, respiratory disease and reduced NADPH oxidase function is remarkable as it occurred in an XL-CGD carrier, but presented in a very similar manner to a CGD patient.

The other gastrointestinal manifestations reported in the XL-CGD carrier population were intermittent diarrhoea in one of 19 XL-CGD carriers [69] and colonic polyposis in a single XL-CGD carrier reported primarily due to skin disease [85].

Due to the overlap of histopathology, it is possible that there could be undiscovered XL-CGD carriers in the IBD population. A recent prospective study screened 120 paediatric patients with a diagnosis of IBD by performing a neutrophil oxidative burst. They failed to find any CGD patients or XL-CGD carriers within the IBD cohort [91]. However, this study is limited as it included children of all ages. The majority of CGD patients are diagnosed by the age of 5 years[11]. The mean age in Jaggi et al's[91] study was 14.8 years, so it would be expected that any CGD patients would have already been diagnosed. The lack of XL-CGD carriers diagnosed may reflect the small scale of the study rather than a lack of association. No study to date has looked at XL-CGD carriers specifically for IBD or gastrointestinal symptoms. Anecdotal reports, including our own

experience at the GNCH, suggest there may be more cases than currently reported in the literature.

Impaired neutrophil function, albeit with normal NBT reduction, has been found in Crohn's patients when compared to healthy controls [92]. No patients or XL-CGD carriers were found, but this finding highlights the close relationship between neutrophil dysfunction and inflammation, particularly within the gastrointestinal tract.

Further to the reported overlap in histopathology and clinical symptoms of IBD and CGD colitis, Casanova and Abel [93] report the concept of CD as a primary immunodeficiency and specifically an immunodeficiency of macrophages thus there is an immunological overlap of CD and CGD. Evidence has accumulated that Crohns disease is the result of impaired clearance of bacteria and impaired inflammatory response resulting in excessive inflammation. Specifically, CD patients are unable to clear *E. Coli* when it is present at high levels and that whilst neutrophils are functionally normal in CD, there is a problem with neutrophil recruitment to the site of infection i.e the gut. The neutrophils, therefore, do not clear the bacteria. The failure of clearance of bacteria within the gut results in granuloma formation and the ongoing accumulation of macrophages and lymphocytes [94], which ultimately results in the prolonged release of cytokines and subsequently local damage and irritation. Despite the finding that the macrophages in CD patients only secrete low levels of proinflammatory cytokines, the accumulation of large numbers of these cells results in ongoing chronic inflammation and damage.

The proposed underlying mechanism in CD outlined above is also attributable to CGD, where there is impaired neutrophil function by the inability to generate superoxide. Thus, the CGD patients are unable to clear bacteria or foreign material from their gut and subsequently may suffer sustained inflammation as a result of accumulation of bacteria.

The similarities in presentation of Moltanyer et al's [90] XL-CGD carrier to boys with CGD, and the incomplete understanding of why some CGD patients suffer from severe colitis whilst others do not, leads us to consider whether there may be clinicopathological overlap between the gastrointestinal disease seen in the

patients and in the XL-CGD carrier case reported, and potentially other, XL-CGD carriers. It is possible that inflammatory bowel disease is more common in XL-CGD carriers than hitherto suspected, but this has yet to be investigated.

Despite the paucity of literature, the published reports raise the question of whether there may be a higher prevalence than expected of XL-CGD carriers in patients with IBD and whether XL-CGD carriers may be at greater risk of inflammatory gastrointestinal disease and manifestations similar to those seen in the CGD patients.

2.3.3 Chorioretinitis

As already discussed, chorioretinitis is a recognised inflammatory complication of CGD. Chorioretinitis in isolation, outside of CGD is rare, although can occur in other conditions such as toxoplasmosis. Although the lesions are usually non-progressive, cases of retinal detachment have been described [95] and it is therefore, an important finding.

In 1999, Goldblatt et al [34] examined the eyes of a cohort of CGD patients and also their XL-CGD carrier relatives and non carrier relatives. Of 38 patients screened, almost 24% had chorioretinal lesions demonstrated. Interestingly, 10% of the XL-CGD carrier cohort had discrete typical lesions, compared to none of the non-carrier, non-patient control group. Lesions were rarely associated with visual disturbances. The lesions seen in both the XL-CGD carriers and the CGD patients were similar to previous descriptions of affected CGD patients; well-circumscribed chorioretinal scars [34]. The use of family members as a control group adds weight to the finding that the lesions seen in the XL-CGD carrier family members were related to their carrier status as it controls for other genetic and environmental factors.

The findings concur with an early case report by Brandrup et al [84] who described an historical case of chorioretinitis in an XL-CGD carriers. There are no other reports of visual symptoms or signs in XL-CGD carriers.

Whilst the findings of chorioretinitis in XL-CGD carriers appear to have been incidental and not to have an adverse or significant effect on the individuals, the finding is nevertheless important. It highlights that there may be similar

pathological processes in the XL-CGD carrier population as seen in the CGD patients.

2.3.4 Other Inflammatory Manifestations

There are no descriptions of other inflammatory manifestations in XL-CGD carriers such as inflammatory cystitis or chronic respiratory disease.

2.4 Autoimmunity in XL-CGD Carriers

Autoimmune manifestations are recognised features of primary immunodeficiencies [96] and CGD is no exception. However, the association with autoimmunity is also described in XL-CGD carriers. Case reports of CGD patients frequently comment that their XL-CGD carrier female relatives suffer from features of autoimmune disease [6, 46, 72].

2.4.1 Symptoms of Autoimmune Disease in XL-CGD Carriers

Reports about autoimmune manifestations in XL-CGD carriers have been published. Polyarthritis, recurrent aphthous ulcers and Raynaud's phenomenon are all reported [6, 72, 73, 84, 90]. The prevalence of these features appears to be more than expected in a healthy population.

Following these reports, Cale et al [69] performed a limited assessment of the physical health of 19 XL-CGD carriers to further investigate autoimmune manifestations. Aphthous stomatitis, photosensitivity and Raynauds phenomenon were demonstrated, with photosensitivity occurring in 58% [69]. 37% of X-linked carriers reported that they suffered from joint pain, with no other cause identified and nearly half of the cohort suffered from excessive fatigue [69]. This was the largest cohort of XL-CGD carriers studied to date, but direct questioning and examination about symptoms of other autoimmune conditions or other manifestations of CGD was not undertaken, although any information volunteered was recorded. It confirmed the smaller scale findings of Sillevis Smitt's study where 70% of the XL-CGD carriers surveyed suffered recurrent apthous ulcers and 63% had recurrent skin eruptions [72].

Autoimmune features were also described in the case reports and series describing XL-CGD carriers with skin disease as the predominant feature. These reported arthralgia [82], polyarthritis [6, 70] and Raynaud's phenomenon [6, 72] highlighting that XL-CGD carriers may be affected by more problems than simple skin disease.

Case reports of CGD patients also frequently comment that female relatives, known to be carriers, suffer autoimmune phenomena [6, 97]. Autoimmune diseases are more prevalent in females, but the association with X-linked carriers of CGD appears to go beyond simply gender predisposition.

2.4.2 SLE and 'Lupus Like' Disease in XL-CGD Carriers

Winklestein et al's [8] registry of CGD patients observed that, within families of boys with X-linked CGD, there was a higher incidence of autoimmune conditions and most notably systemic lupus erythematosus and its variants. The autoimmune phenomena described in the previous section may all be part of SLE, but the descriptions in the literature refer to 'lupus-like' disease due to absence of associated autoantibodies in serum [6].

2.4.3 Autoimmunity and Primary Immunodeficiency

Autoimmune manifestations are a feature of primary immunodeficiencies as a result of immune dysregulation. The interplay between autoimmunity and PID has generated much interest and X-linked PIDs are of particular significance.

Autoimmune features are frequently seen in conditions where there is random X-chromosome inactivation. For example, CGD, X-Linked lymphoproliferative syndrome (XLP) and X-linked Hyperimmunoglobulin M syndrome (XLHM) have clear associations with autoimmunity which are less prevalent where there is non-random X-inactivation for example in X-linked Severe Combined Immunodeficiency (SCID) [98].

CGD patients are particularly at risk of autoimmunity [97] and features of autoimmune disease are recognised within CGD patients.

2.4.4 Autoantibodies

There is little published about routine autoantibody screening being performed in XL-CGD carriers, even in those who are symptomatic.

Early work on the presence of autoantibodies in primary immunodeficiency identified a higher than expected rate of autoantibodies in both patients and female carriers of XL-CGD. Amman et al, in 1979[99], demonstrated that even in the absence of clinical features of autoimmune disease, over 50% of patients had autoantibodies present, of which Rheumatoid factor and anti-poly (Au) were the most commonly found. XL-CGD carriers, who were relatives of the patients, demonstrated similar findings: 50% had positive autoantibodies with rheumatoid factor, ANA and anti-poly (Au) frequently found. The cohort was small, with 11 patients and 8 XL-CGD carriers, but the findings interesting. Within the XL-CGD carrier population, clinical symptoms of autoimmune disease were present and correlated with the positive autoantibodies. Three out of eight XL-CGD carriers clinically had DLE whilst one had SLE.

These early results were supported by a study twenty years later. Martin-Villa et al [98] looked at 5 families each with at least one XL-CGD patient per family. Patients, carriers and non-carrier relatives were all studied for the presence of autoimmune disease and autoantibodies. The findings showed that the XL-CGD carriers and the CGD patients had significantly higher rates of autoantibodies including ANA and anti-smooth muscle autoantibody, present than their non-carrier relatives. Of the eight patients, six had autoantibodies present and 11 of the 13 carriers also had autoantibodies present, whilst only two of the 13 non-carrier relatives demonstrated presence of autoantibodies. Unusually, two CGD patients had evidence of DLE and one XL-CGD carrier also had DLE whilst there were no autoimmune features in the non-carrier family members [98]. The patients with DLE had autoantibodies present.

The early work described has not been replicated in a larger, more recent study. Cale et al's [9] prospective study of XL-CGD carriers investigated the presence of autoimmune features and autoantibodies in 19 XL-CGD carriers. Only five of these XL-CGD carriers had autoantibodies present and all five had a positive ANA, although in three the values were only weakly positive. One XL-CGD carrier

also had a weakly positive DS-DNA antibody present. All of the women with autoantibodies present had clinical features of autoimmunity (four photosensitive rash, one joint pain and recurrent aphthous ulcers without rash). However, overall within this cohort there were features of autoimmunity and lupus like symptoms in over 50% of carriers, but autoantibodies only found in 25%.

An earlier case report supported this finding. Cordona-Guijarro in 2000 reported an individual XL-CGD carrier who manifested features of lupus, but in whom extensive autoantibody testing was negative [100].

On balance, therefore, it would seem the literature does not support autoantibodies as a reliable method for screening for symptoms of autoimmune disease in XL-CGD carriers and the classical patterns seen in autoimmune disease may not be present in XL-CGD carriers. The exact relationship between autoantibodies, autoimmune diseases and XL-CGD carriers has yet to be elucidated.

It is possible that autoantibodies are present in more XL-CGD carriers, but that they are not detected by conventional techniques, and with more sophisticated and advanced methods, autoantibodies would be detected more frequently. If this is the case, there may be a closer correlation with the presence of symptoms than is currently recognised.

Mechanism

There is no consensus over the mechanism for autoantibody production in XL-CGD carriers. A review of the literature reveals several leading hypotheses.

A popular hypothesis is that there is failure to clear debris, which may act as antigenic stimulant, because of abnormal apoptosis of affected phagocytes, particularly macrophages. This results in persistent antigenic stimulation, which leads to autoantibody production. It can be theorised that this may correlate with the degree of reduction in neutrophil function in carriers i.e. that those with greater number of functioning neutrophils are more likely to be able to clear debris and organisms [98].

A further hypothesis is that the defect may lie more specifically with the inability for affected phagocytes to produce superoxide[98].

Finally the interaction of gp91^{phox} with B cells may account for autoantibody development, as there has been demonstrated an interaction between p47^{phox} and this may be transferable[101] .

2.5 Possible Causation

2.5.1 Residual NADPH Function

Historical understanding of CGD severity and survival is that XL-CGD is the most severe manifestation, with AR-CGD presenting later and with a milder clinical course [8, 10]. Increasingly it is realised that this is too simplistic an understanding and does not account for the differences seen.

Kuhns et al [14] reviewed 287 patients from 244 kindreds, of whom 195 patients (169 kindreds) had XL disease. They found that whilst NADPH oxidase function was impaired in all CGD patients, the degree of residual ROI production was variable. From the residual ROI production, four discrete quartiles were identified, without accounting for the clinical phenotype. The authors were able to conclude that residual ROI production was associated with survival. Even small degrees (1% of normal) of residual ROI production improved survival with the best survival seen in patients with the greatest residual production. The specific gene mutation was not as important to survival as the degree of ROI production. Although survival was accounted for by ROI production, there was no association found between residual ROI production and the presence or severity of inflammatory gastrointestinal manifestations.

Further evidence for the clinical implications of residual NADPH oxidase function can be found in case reports. In 2007, a case resembling juvenile sarcoid was reported in a male. He presented with a classical picture of juvenile sarcoid, including a raised ACE, but went on to develop a hepatic abscess. Upon DHR testing, residual NADPH oxidase activity was apparent and [102] genetic testing confirmed the presence of a mutation in the *CYBB* gene.

In 2012 Gutierrez et al[13] reported a 9.4 year old male who presented with recurrent pneumonia. He had no other manifestations of CGD and was well grown. Investigations revealed a dual population of cells upon DHR with only 34% negative for ROI production, thus showing preserved residual NADPH oxidase function. Genetic testing confirmed the presence of a known CGD mutation in the *CYBB* gene. This is an unusual case both in the mild phenotype presented, as he suffered only isolated lung disease, and in the dual population of cells more akin to a female carrier than a typical XL-CGD patient. However, it further supports the evidence about the importance of residual NADPH oxidase function.

2.5.2 Correlation with Neutrophil Oxidative Burst

As not all XL-CGD carriers demonstrate significant problems, interest has been directed towards how to identify those who may be affected. Quantification of the oxidative burst by either DHR or NBT shows significant variation amongst carriers, ranging from 20-80%[69]. Reports from published studies are conflicting. Some studies suggest severity of symptoms and incidence of autoimmune manifestations was associated with lower NBT values [6, 72, 86] whilst patients in Cale et al's study showed symptoms at both ends of the NBT range [69]. It is difficult to draw significant conclusions about the relationship between symptoms and oxidative burst value due to the small number of XL-CGD carriers studied and the discrepancy between timing of oxidative burst measurement and onset of symptoms.

Furthermore, little is known about whether neutrophil oxidative burst is consistent throughout life in carriers, or whether there is variation with age and how this may affect the development of symptoms.

2.6 X-Inactivation and Skewing

Females have two X-chromosomes; one paternally derived and one maternally derived. Only one X-chromosome remains active in a cell with one X-chromosome being 'switched off'. The process by which this occurs is called lyonisation or X-inactivation and occurs in all women early in foetal development. Lyonisation is considered a random process. It is anticipated that

the split between maternally derived and paternally derived active chromosomes would be approximately 50/50, but, as it is a random process, the split may be anywhere between 0 and 100%. Where there is no mutated gene present on the X chromosome, which chromosome is selected to be active is less significant. However, when considering XL conditions such as CGD in female carriers, which X-chromosome (wild type or mutated) becomes inactivated is of particular functional importance for relevant cells in which the gene product is expressed.

Where the distribution of maternally to paternally derived X-chromosome is not equally distributed, it is referred to as skewing. Skewing is considered to be significant when there is a disparity between activation of parentally-derived X chromosomes and where ratios are approaching 80:20[103].

In a study of 1005 females, with no known XL genetic mutation, less than 12% of women had extreme skewing i.e. a ratio of 80:20 or more [104]. Almost all women had near equal distribution. Measuring this ratio at one point in time is useful, but the ratio may not be constant throughout life. It has been hypothesised that the degree of skewing may be related to age and that extreme skewing becomes more likely with increasing age[105]. In the same study, it was shown that there was a greater percentage of extreme skewing in older women compared to newborn cord blood (19.5% > 80:20 in adult women compared to 5.6 % in the newborn group [104]). Extreme lyonisation (>95:5 %) occurred in less than 1% of the newborn population.

It is possible that the ratio varies not only from individual to individual and with age, but also between tissue types. In muscular dystrophy the degree of skewing has not always been equal when muscle and peripheral blood measurements were made simultaneously [106]. Gale et al[107] found differences in X-inactivation between t-lymphocytes and neutrophils in the same patients. In XL-CGD carriers the ratio may differ between different tissue types explaining why there are symptoms in some systems and not others. However, there is no literature about this at present.

2.6.1 Assessment of Skewing

As highlighted from the study results discussed earlier, it is possible to quantify the degree of X-inactivation skewing. In XL-CGD carriers, the degree of skewing may be inferred from the neutrophil oxidative burst result, as those neutrophils producing a respiratory burst express the normal X chromosome. Koker et al's [59]study of one family found that the DHR correlated well with assessment of DNA methylation from leucocytes, although the correlation was less close when buccal swabs were compared.

Simple measures using methylation can look for extreme degrees of lyonisation, but it is also possible to quantify the degree of skewing using the human androgen receptor (HUMARA) assay[108].

2.6.2 Cause of Extreme Skewing

The reason why some women demonstrate such extreme lyonisation is unclear. As discussed earlier, in the 'normal population', up to 14% women have skewed inactivation [109]. Potential causative factors will be discussed here.

Age

As outlined in the study of healthy controls discussed earlier, it has been suggested that there may be an increase in extreme skewing with increasing age. In one family of XL-CGD carriers, three generations of women were assessed[59]. The percentage of functioning neutrophils ranged from 36 to 81% and the highest levels were seen in the youngest generation[59].

A large study of 333 healthy females found that there was an increase in the presence of excessive skewing associated with increasing age[110]. It was found when using a definition of 90:10 or greater, less than 2% of neonates demonstrated extreme skewing compared with 22.7% in the over 60 years age group.

In a similar study, Hatakeyama et al [111] examined 350 females to assess the degree of skewing. They found that whilst there was an increase in the incidence of extreme skewing with increasing age, this was only at a modest level. There was little difference within 20-year age brackets. A smaller study by Racchi et

al[112], failed to find significant correlation between increasing age and degree of skewing.

The flaw with these studies in evaluating the relationship between age and degree of skewing is that none are longitudinal and so the natural history of skewing within an individual cannot be assessed.

Inheritance of Extreme Skewing

It has been hypothesised that X-Inactivation may be controlled by genetic factors[113]. Early work with mice found a region on the X-chromosome was responsible for controlling the inactivation process[114]. This was further supported by work in humans when a different gene (*XIST* gene), located in the same region of the X-chromosome, was found to control the X-inactivation process [113]. Naumova et al [109] add weight to the hypothesis of an inheritability of skewing in their study of 3 generations of 36 families. Their study found that in a family with extreme skewing in the grandmother (90:10 ratio), all 7 of her grandchildren manifested the same degree of extreme skewing. The women were not carriers for any known genetic mutations and statistically the probability of this occurring by chance was insignificant. Further analysis suggested that the most convincing explanation was that the process was controlled by a specific locus on the X chromosome.

Preferential Selection

In some conditions, there may be preferential selection of the non-mutated chromosome as there is a growth or selective advantage.

In a study of X-linked Dyskeratosis Congenita (DC), a rare cause of bone marrow failure, 16 carriers were reviewed. Vulliamy et al[115] found that all 16 XL carriers demonstrated extreme skewing, on peripheral blood, of greater than 90% in favour of expressing the non-mutated chromosome. This supports the hypothesis that, in the case of XL DC, there is a selective disadvantage for the cells in which the mutated X chromosome is active and, therefore, cells in which the wild type X chromosome is active preferentially survive.

Conversely, in a study of a 72 year old female carrier of X-linked sideroblastic anaemia and her family[116], all of the female carriers demonstrated skewed inactivation in favour of the mutated X chromosome. The authors suggested this was the result of congenital skewing. The original patient only presented with symptoms at the age of 64, perhaps supporting a degree of age affected skewing. This, however, was a single family and only tentative conclusions may be drawn. It may also support the case for an inherited component of the X-inactivation process.

This literature suggests that, in certain circumstances, X-inactivation is not a random process. In XL-CGD there appears to be no selective survival advantage for phagocytes containing the mutated or wild-type gene, and it is assumed that the active X chromosome is selected randomly.

2.6.3 Skewing in XL-CGD Carriers

In CGD, cases of extreme skewing in female carriers have been reported. Gono et al[117] described a Japanese female with a de novo mutation of *CYBB* with extreme skewing and a ratio of 93:7, with a corresponding DHR of 9.6% positive cells. She was diagnosed as a result of clinical features similar to CGD with refractory abscesses and recurrent stomatitis. A similar degree of skewing was seen in another female patient who presented with pneumonia caused by *Aspergillus Fumigatus* [74]. She presented at 16 years of age and had only 9.68% functioning neutrophils on DHR, which correlated with assessment from the HUMARA assay where the ratio was 96:4.Two further cases have been reported[82, 118] and both reported significant symptoms associated with the extreme skewing. In the case reported by Anderson-Cohen et al[118], the skewing was so extreme that, upon performing an NBT, only one population of neutrophils was demonstrated. However, further analysis revealed that this young woman was in fact a carrier of a de novo mutation in *CYBB* and had a skewing ratio of 99:1. She had significant symptoms with recurrent infection and abscesses.

In Koker et al's [59] study of three generations of XL-CGD carriers, the oldest carrier demonstrated a 35:65 ratio of skewing, but was not noted to be

symptomatic. All but one of the reported female XL-CGD carriers exhibiting extreme skewing suffered from significant symptoms. However, this may not reflect the XL-CGD carrier population as a whole, as these cases may not be representative of the XL-CGD carrier cohort. There may be asymptomatic women who have a similar degree of skewing, but who have not been investigated. It is uncertain whether there is a correlation between the degree of reduction of NOB and the presence and severity of symptoms and their severity. The association with increasing age is also unclear.

2.7 Carriers of other X-Linked Disease

Female carriers of other XL disorders have some degree of expression of the defective gene. Therefore, they may be at risk of manifesting symptoms. Whilst there is limited literature about XL-CGD female carriers, other XL conditions have been reported and information about XL carrier status may be extrapolated from these.

Muscular Dystrophy (MD) is a well-studied example. MD is a genetic progressive muscle disorder caused by a defect in the dystrophin gene located on the X chromosome. Symptomatic carriers are reported in the literature. Carriers of Duchenne muscular dystrophy (DMD) exhibit manifestations of disease, ranging from subtle weakness elicited only by detailed neurological examination to clinical patterns identical to the disease itself [119]. More significant manifestations, such as sudden death due to cardiac abnormality, have been described in XL carriers of the rarer Emery-Dreifuss Muscular Dystrophy [120]. This occurrence, whilst unusual, is important as it shows carriers may suffer from disease manifestations, including the most significant manifestations.

Female carriers of haemophilia A, in which there is a lack of functioning clotting factor VIII, have been found to have reduced factor VIII levels, when compared to non-carrier controls with considerable variability in the degree of reduction[121]. The reduction in factor levels does not appear to be clinically significant in the majority of cases, however, cases of severe haemophilia in manifesting carriers are described[122]. This further demonstrates that XL carriers may be affected by their carrier status. A similar finding has been

described in an XL carrier of Wiskott-Aldrich where the platelet count was reduced, although to a lesser degree than patients[123] with the disease.

The molecular cause of symptoms in disease-manifesting carriers has been investigated. DMD XL carriers have variable degrees of dystrophin protein expression, but this does not correlate with clinical phenotype[124]. It has been assumed that the reason for manifestations within the DMD XL carrier cohort is skewed X-inactivation and extreme lyonisation, as outlined earlier. Small studies have added support to this argument. Yoshioka et al[125] found that 3 out of their 4 symptomatic carriers had greater than 70% skewing compared to only 6% of the control group. However, as this was a small study, it is not possible to draw definitive conclusions. It has also been suggested that whilst measuring skewing in blood may correlate with other tissues, it may be important to consider measurement on the tissue of interest, in the case of MD, muscle cells [106].

In summary, there is literature to support the hypothesis that female carriers of XL disorders may suffer from similar clinical problems as the affected males, to varying degrees.

2.8 Symptomology similarities between CGD, SLE and IBD

The literature reviewed so far highlights that there may be some overlap between symptoms seen in XL-CGD carriers and other inflammatory conditions. In this section, I will review the pertinent literature about two such conditions; Systemic Lupus Erythematosus (SLE) and Inflammatory Bowel Disease (IBD).

2.8.1 Systemic Lupus Erythematosus

Many of the autoimmune features described in XL-CGD carriers are part of the spectrum of SLE. This section will review some of the literature about symptoms and diagnosis of SLE, in order to be able to evaluate the overlap with the symptoms described in XL-CGD carriers.

SLE is a multi-systemic autoimmune disease.

Risk Factors for Development of SLE

The exact aetiology of SLE is uncertain, but several factors have been shown to be important in the development of this disease. A popular hypothesis is the exposure of susceptible individuals to trigger environmental factors leading to the development of disease [126-130].

Epidemiological studies have shown a female predominance of SLE, with rates up to nine times more common in females [127]. Incidence and prevalence varies with sex and age. In England and Wales in 1982 the prevalence was 12.5 / 100,000 women of all ages and 17.7 / 100,000 in women aged 15 to 64 years [128]. The prevalence in women aged 15 to 64 years was higher in a more recent UK study by Johnson et al where the prevalence was 27.7/100,000 [131].

A significant ethnic variation has also been demonstrated. A higher incidence is seen in the African population and with individuals with African or Asian ancestry[127, 129]. Caucasians also have a predilection to milder disease compared with African patients [129].

Genetics

Genetic susceptibility has been shown with HLA-DQ and DR highly associated [132, 133] and recent insights demonstrate genetic variability may account for the different phenotypes seen in this heterogenous condition [132].

Due to the heterogenecity of the disease, risk factors and associations have frequently been sought.

Environmental factors have been considered in the development of SLE. There have been mixed reports about the effect of cigarette smoking on the development of SLE. Some studies have found that smoking increases the risk of developing SLE and particularly the cutaneous manifestations[134], whilst other studies have found no link between cigarette exposure and SLE [130].

More unusual associations have also been evaluated to a greater and lesser extent. Burry et al's study in the 1960s, attempting to explain the female predominance, found an association between the use of lipstick and SLE [126] but this has not been further supported. A further association was found when

an inverse relationship between alcohol consumption and the development of SLE symptoms was shown[135] in a cross-sectional study. However, it is likely that this represents a reduction of alcohol intake after diagnosis of SLE, rather than alcohol as a genuine protective factor.

Infective triggers have also been hypothesised as a cause of SLE. The most compelling evidence is seen with EBV [133] which supports the genetically susceptible individual suffering a second hit hypothesis.

Symptoms and Diagnosis

Diagnosis of SLE is based upon clinical clusters of symptoms with accompanying biological markers. The American College of Rheumatology published case definition criteria in 1982 [136] which were subsequently updated in 1997. Table 2-1 shows the modified classification criteria. There is a wide spectrum of disease and diagnosis is generally accepted if more than 4 of the criteria are met.

Table 2-1: Modified 1997 ARA Criteria for SLE

	Criteria	Additional Description
1	Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2	Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging
3	Photosensitivity	Skin rash as a result of unusual reaction to sunlight (history or observation)
4	Oral ulceration	Oral or nasopharyngeal ulceration (usually painless)
5	Arthritis	Non-erosive arthritis involving two or more peripheral joints, characterised by tenderness, swelling or effusion
6	Serositis: Pleuritis Pericarditis	 Convincing history of pleuritic pain or rub heard by physician or evidence of pleural effusion Pericarditis documented by ECG or rub or evidence of pericardial effusion
7	Renal Disorder: Persistent proteinuria Cellular casts	 Proteinuria >0.5g/day or greater than +++ if quantification not performed Red cell, haemoglobin, granular, tubular or mixed casts
8	Neurological disorder	See Table 2-3
9	Haematological disorder	Haemolytic Anaemia with reticulosis Leucopenia <4000/mm^3 total on 2 or more occasions Lymphopenia <1500/mm^3 total on 2 or more occasions Thrombocytopenia <100,000/mm^3 in the absence of offending drugs
10	Immunological disorder	Presence of Anti-DNA antibody, Anti-Sm antibody or abnormal serum level of IgG or IgM anticardiolipin antibodies A positive test for lupus anticoagulant A false positive serological test syphilis
11	Antinuclear Antibody (ANA)	Abnormal ANA titre by immunofluorescence or an equivalent assay at any time point in the absence o f drugs known to be associated with 'drug induced lupus'

SLE is a heterogeneous disease and there is debate on going as to whether it remains a group of diseases or a single disease with a broad spectrum of manifestations[137]. Table 2-2 shows the prevalence of different symptoms in SLE patients in a large European cohort (1000 patients).

Table 2-2: Prevalence of SLE Symptoms in European Cohort

	Euro-Lupus Cohort [129] Number (%)
Malar Rash	311 (31.1)
Discoid Lesion	78 (7.8)
Photosensitivity	229 (22.9)
Oral Ulcers	125 (12.5)
Raynaud's Phenomenon	163 (16.3)
Arthritis	481 (48.1)
Serositis	160 (16.0)
Nephropathy	279 (27.9)
Neurological Involvement	194 (19.4)
Death	68 (6.8)

Neuropsychiatric symptoms are also observed in SLE (NPSLE) and they are diverse and numerous. A summary of the features observed was published in 1999 [138] and their findings are reproduced in Table 2-3.

Table 2-3: Neuropsychiatric Features of SLE

Central Nervous System	Peripheral Nervous System
• **Aseptic Meningitis** • **Cerebrovascular disease** • **Demyelinating syndrome** • **Headache (including migraine and benign intracranial hypertension)** • **Movement Disorder (chorea)** • **Myelopathy** • **Seizure Disorder** • **Acute confusional state** • **Anxiety Disorder** • **Cognitive Dysfunction** • **Mood Disorder** • **Psychosis**	• **Acute inflammatory demyelinating polyradiculopathy (Guillain-Barré Syndrome)** • **Autonomic disorder** • **Mononeuropathy** • **Myasthenia Gravis** • **Neuropathy, cranial** • **Plexopathy** • **Polyneuropathy**

Included in the manifestations of NPSLE are mood disorder and anxiety disorder which are neither specific to SLE, nor are they limited to SLE, highlighting the broad range of symptoms observed and difficulties differentiating between NPSLE and other psychological disease processes. The proportion of patients with SLE with NPSLE symptoms ranges from 20 to 50%[139].

Features outside of the core diagnostic criteria are also commonly seen in SLE. Fatigue has been reported as a predominant symptom in SLE. Up to 80% of SLE patients report fatigue as a significant complaint [127, 140, 141]. Fatigue is also reported as significant in patients in whom their lupus was otherwise considered quiescent [140], although there has been correlation demonstrated between degree of fatigue and level of disease activity.

Gastrointestinal symptoms are also reported in SLE patients [142]. However, these symptoms are usually attributed to an infective cause or the result of an adverse drug reaction. A study of 105 female patients with SLE in 2013 demonstrated that up to 50% of these women met the Rome Criteria for a diagnosis of IBS [143], which was associated with reduced HRQoL. Gastrointestinal symptoms do not form part of the diagnostic criteria for SLE, but appear to be prevalent in the SLE population and impact upon quality of life[144].

There is considerable overlap with SLE and anti-phospholipid syndrome (APS), a syndrome in which individuals suffer thrombosis (arterial and venous) and may also suffer spontaneous abortion[145]. It is classically associated with the presence antiphospholipid antibodies. Recurrent miscarriage is seen in patients with APS, both with and without associated SLE [146].

The presence of APS, alongside SLE, results in a significantly increased risk of morbidity and premature death[127]. In SLE there is also an apparent increase in cardiovascular risk, which is separate to classical risk factors for heart disease[127, 129] and accounts for some of the early mortality seen in SLE patients.

Natural History of SLE

The clinical course of SLE is one of relapsing and remitting nature with acute episodes of flare. Outcome is extremely variable with permanent remission to early death reported. Survival of patients with SLE is improving and a recent review highlighted that the survival rate has increased significantly over the past 40 years, with a five year survival rate of 93% compared to the 50% seen in the 1950s[129].

Autoantibodies in SLE

Autoantibodies form part of the diagnostic criteria of SLE (Table 2-1). They may be present before the onset of symptoms[129], but may also be positive in the healthy population [147, 148] highlighting their lack of specificity. The positive predictive value (PPV) of ANA at a titre of 1:640 was 6% in one retrospective American study of 232 patients referred for a positive ANA result [149].

Autoantibodies are not only important in the detection and assessment of SLE, they may also play a direct role in the pathogenesis of the disease. The pathogenesis of SLE has not been completely elucidated, but there is increasing evidence supporting the hypothesis that organ damage is mediated by immune complexes [150]. Lupus nephritis is one of the clearest examples of this with IgG-complex deposition leading to early disease [150]

The role of autoantibodies in the development of NPSLE has been demonstrated in murine models. When anti-ribosomal antibodies were injected into mice, the mice developed depressive symptoms [151]. When anti-dsDNA antibodies were injected directly into the mouse brain, these mice developed problems with cognition and associated emotional disturbances [152]. Both these studies support the pathogenic role of autoantibodies in the development of SLE symptoms.

A Japanese study of 597 health hospital workers found up to 20% of healthy volunteers were reported to be Antinuclear Antibody (ANA) positive [153]. However, the difficulty when assessing healthy individuals is that the follow-up time is often insufficient. As it is known that ANA may be positive years before the onset of clinical symptoms, the follow-up on healthy controls recruited to studies would need to be many years (and ideally lifelong) in order to be certain they did not develop symptoms and this is seldom the case. Despite this difficulty, there still appears to be compelling evidence that there may be a high level of autoantibody positivity in individuals without apparent symptoms. Dellavance et al [154] in 2005 evaluated 10,000 samples which were positive for anti-DFS70 ANA (dense, fine speckled antigen) and found that whilst they were common in those with autoimmune disease, particularly thyroiditis, they were in fact more common in those without any features of autoimmune disease. There

was a four-year follow-up of healthy individuals in this study. A more recent study by Mariz et al [155] confirmed this finding when they found that the dense fine speckled pattern was seen in 33% of ANA positive healthy individuals compared to none of the ANA positive individuals with autoimmune diseases.

Measurement of Autoantibodies

There are a number of techniques that may be used to detect the presence of autoantibodies. Immunofluorescence (IF), ELISA, and immunoblot are three of the most commonly used. The ARA still recommends the use of IF as the screening tool for ANA positivity [156].

The degree of positivity is usually quoted as a titre. The level at which a titre is decided as positive is laboratory-dependant.

An American study in 2013 [149] evaluated 227 patients who had been referred to a rheumatology service with a positive ANA result. The positive predictive value (PPV) of a positive ANA for a diagnosis of a defined ANA associate disease when the tire was 1:40 was less than 10%. When the titre accepted as positive was increased to 1:640 the PPV rose to 26.9%. No patients with a positive ANA at less than 1:640 had an ANA associated disease at the time of the study, suggesting that the clinical significance of a positive ANA at lower titres is at best uncertain, and at worst unhelpful.

2.8.2 Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a term encompassing Crohn's disease (CD) and ulcerative colitis (UC). CD has an incidence in the UK population of approximately 4 per 100,000, whilst UC is more common with an incidence of 10 per 100,000 [157]. Onset of IBD is usually in late adolescence or early adulthood (around 15 to 30 years). Presenting symptoms are similar to CGD colitis with abdominal pain, diarrhoea and rectal bleeding all prominent.

Extra-gastrointestinal manifestations are also described. Arthritis, particularly ankylosing spondylitis and large joint involvement [158] has been seen and fatigue is described in both active disease and in patients in remission [159].

Ocular manifestations such as uveitis[158] are also part of the disease, demonstrating IBD may affect multiple systems.

Histological findings in CD and UC differ. In CD there is transmural inflammation with granuloma. This may affect any part of the intestine and the inflammation may not be continuous with classical skip lesions leaving some areas spared. UC may affect any part of the colon, but typically favours the rectum and the inflammation is continuous but limited to the mucosa. The presence of granuloma in CD has led to difficulties in distinguishing CD and CGD-related colitis. As already considered histopathologically and clinically IBD and CGD-colitis may appear very similar.

Genetics

The exact aetiology of IBD has not been completely elucidated but the overriding hypothesis is that there is abnormal immune response to normal gut commensals in those with a genetic predisposition, which results in chronic inflammation [160].

IBD appears to have a polygenetic basis and genetic susceptibility has been demonstrated in family studies, which have led to the discovery of predisposing gene loci. Recent advances in genetic techniques have identified new loci associated with IBD. Genes associated with UC and CD have been identified in the interleukin-23-Th17 pathway (such as STAT3, IL23R and IL12B), the innate immune responses (such as NOD2) and also in other regions (such as MHC) [161]. Further loci continue to be identified. Further to this, genes have been identified which put the affected individual at greater risk for having a complicated disease course including the development of fistulae and stenosis. Any of the NOD2 variant alleles and also JAK2 have been shown to confer this risk [162].

Neutrophils and IBD

An association of CD and leucocytes was described as early as the 1970s [163].

Impaired neutrophil function, albeit with normal NBT reduction, has been found in Crohn's patients when compared to healthy controls [92]. No patients or XL-

CGD carriers were found, but this finding highlights the close relationship between neutrophil dysfunction and inflammation, particularly within the gastrointestinal tract.

Neutrophil dysfunction has also been associated with IBD in glycogen storage disorders [164, 165] further highlighting the, as yet, incomplete understanding of the interplay between neutrophils and gut disease.

NADPH Oxidase and IBD

The NADPH Oxidase complex's role in CGD has already been described. Given the similarities between CGD colitis and IBD, the role of NADPH oxidase has been evaluated in CD patients. An early study of 43 patients found there was diminished H_2O_2 production, although preserved O_2^- production, in untreated CD patients compared with a control group [166]. There was a significant negative correlation between H_2O_2 production and assessment of disease activity and H_2O_2 was normalised in treated CD patients. This highlights the potential similar pathogenesis in IBD and CGD colitis, as failure to clear foreign matter results in granuloma production.

A more recent study by Muise et al [167] further evaluated the link between the NADPH oxidase complex and IBD. The aim of this study was to establish if the NADPH oxidase components are important in the development of IBD. The authors were able to draw several important conclusions. Firstly, they identified novel mutations in *NCF2,* which encodes $p67^{phox}$, which were associated with very early onset IBD but without evidence of immunodeficiency. *NCF2* is known to cause autosomal recessive CGD. Secondly, they found mutations in *NCF4*, which encodes $p40^{phox}$ and is a very rare cause of AR-CGD, were strongly associated with ileal CD, but again without evidence of immunodeficiency. Finally, they identified a novel mutation in *RAC2*, associated with CD. *RAC2* plays an important role in NADPH oxidase activation.

In summary, associations between defects in the NADPH oxidase complex and the development of IBD have been proven, thus highlighting the overlapping nature of IBD and CGD, and perhaps offer a potential reason why XL-CGD carriers may be at similar risk of bowel disease.

Microbiota

Whilst an infectious cause of gastrointestinal involvement in CGD has not been proven[37], disturbance of the gastrointestinal normal flora has been hypothesised to be involved in the pathogenesis of IBD. Subsequently, evaluation of the microbiota has been considered to be a potentially non-invasive insight into affected individuals. The normal flora of the GI tract in paediatric patients with IBD was examined in a large cohort [168]. Significant differences were found in the microbiota of the gastrointestinal tract in patients with IBD when compared to age-matched controls. The value of this is in both diagnostic utility and potentially aiding understanding of the pathogenesis of gastrointestinal disease. This has not been evaluated in CGD patients, but the clinical and pathological overlap suggests that there may be similar findings.

2.9 Fatigue

Fatigue is a commonly used term in both medical and non-medical settings. It is an instantly recognisable feeling, yet defining fatigue in a clinical or research setting proves difficult. This is due to the complex range of mental and physical symptoms it includes.

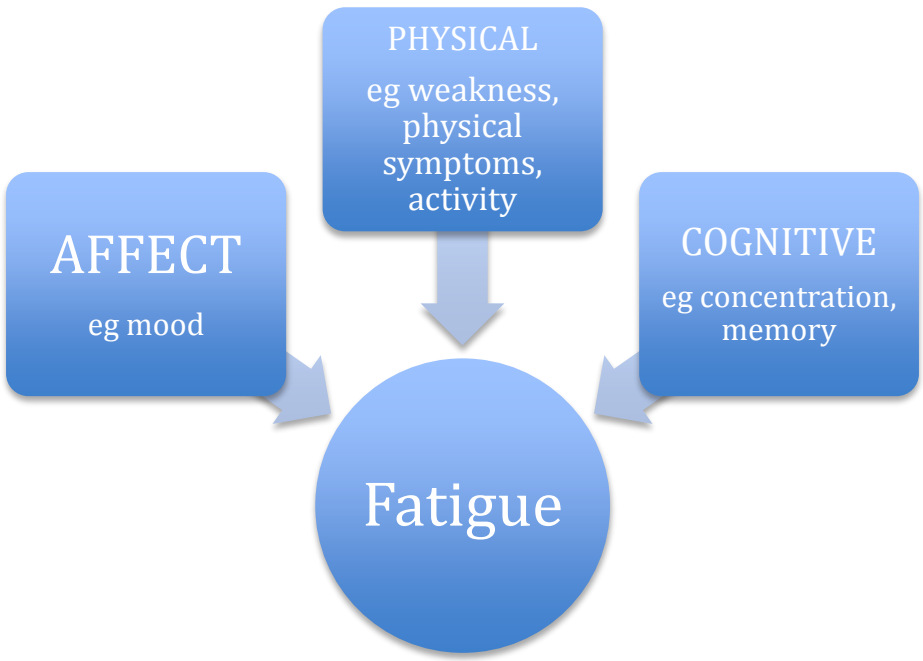
Fatigue encompasses a broad range of symptoms. It may be used to describe a sensation of mental or physical tiredness, a lack of motivation to participate in activities or lack of energy to complete activities of daily living or simply the feeling of being 'washed out'. It may also be used to describe abnormalities of sleep including insomnia, failing to be refreshed from sleep or disturbed sleep. The need to define fatigue is important in both clinical and research settings.

Van Langenberg defined fatigue as 'a persistent, overwhelming sense of tiredness, weakness or exhaustion resulting in a decreased capacity for physical and/or mental work'[169]. However fatigue is formally defined, the importance is to distinguish abnormal fatigue from everyday, transient tiredness that affects all individuals. The features differentiating abnormal fatigue are the overpowering nature causing it to impact upon daily activities, including, but not exclusively, ability to work or perform simple tasks. The other crucial difference is the persistent nature.

Although fatigue may be difficult to define and considered by some as an indistinct entity, its importance to patients should not be underestimated as it represents a significant problem to some individuals. Fatigue is often described by patients as debilitating, distressing, and even overwhelming. In the 1980s, a study in IBD patients showed they considered fatigue to be as significant a symptom as abdominal pain and diarrhoea [170]. Studies in other autoimmune disorders such as Rheumatoid Arthritis (RA) [171] and ANCA-associated vasculitis [172] have corroborated these findings. Not only has it been identified as a significant symptom, fatigue has been shown to impact upon health related quality of life (HR-QoL)[171, 173] reinforcing its significance to the patient.

There is controversy over whether fatigue should be viewed as a unique entity or whether is solely a response to an underlying symptom such as pain or psychological co-morbidity. Fatigue is perhaps best conceptualised as a multidimensional model with physical, cognitive and affective components all contributing to the sensation experienced. Individual components may be affected differently in different individuals. This model of fatigue is progressively being more accepted. Figure 2-1 illustrates how the three components interlink.

Figure 2-1: Conceptualisation of the Multidimensional Model of Fatigue



A small qualitative study by Glaus et al [174] demonstrated that fatigue was described differently by patients than by healthy individuals [174], further

highlighting that fatigue is more complex than simply present or absent; there is a qualitative aspect to the sensation.

Physical factors contributing to the development of fatigue may include pain, anaemia and symptoms of a specific disease process. Pain can be used to illustrate the multidimensional model. Pain may contribute directly to the sensation of fatigue but also through disturbance in sleep, anxiety or low mood associated with not being able to function at the desired level. If pain is the only symptom, then removal of pain through effective analgesic agents or treatment of the cause should resolve the fatigue. Where pain improves, but fatigue persists, this evidences the presence of fatigue as an independent symptom, which may be affected by other factors, but exists independent of them. The same is true of other physical symptoms including anaemia where the associated feeling of lethargy may improve with treatment or may persist despite biochemical makers showing resolution of anaemia and confirming the presence of fatigue. In a study of IBD patients, fatigue was present in 40% of patients who were defined as being in clinical remission [175] strengthening the argument that fatigue may exist in the absence of physical symptoms.

Cognitive consequences of fatigue include inability to concentrate and poor working memory. In addition, psychological factors may contribute to fatigue. Biological symptoms of severe depression include disturbed sleep. Therefore, reports of fatigue may be directly due to depression or mental health complaints. Equally low mood and a lack of motivation may be part of fatigue in the absence of overt depressive symptoms and they may not correlate. Symptoms may be interlinked, but the origin of the symptoms remains important in order to identify and ultimately treat the cause. Fatigue may lead to depressive symptoms rather than all fatigue being the result of psychological co-morbidity.

2.9.1 Assessment of Fatigue

As already discussed, definitions of fatigue often lack specificity and the desired scientific certainty. Subsequently assessment of fatigue provides a significant challenge. Ideally, assessment of fatigue would include both subjective and objective components. Formal assessment must attempt to overcome the

imprecision of definition. Self-assessment of fatigue as with all symptom assessment is likely to be affected by recall bias, whilst physician assessment is likely to be an underassessment.

The simplest assessment method is by asking the patient directly about their degree of fatigue. The disadvantage of this is the lack of reproducible quantification. Hence, to reliably and consistently assess and quantify fatigue, standardised questionnaires have been derived.

Initial attempts to assess fatigue were unidimensional with scales such as the Fatigue Severity Scale (FSS) being introduced to assess fatigue in conditions including SLE [176], chronic hepatitis C [177] and immune-related polyneuropathies[178]. The appreciation of the multidimensional nature of fatigue as shown in Figure 2-1 has led to the realisation that when assessing fatigue it is necessary to incorporate all dimensions; physical, cognitive and affective. Fatigue has also formed part of questionnaires about quality of life including in the SF-36.

Multidimensional assessment scales were developed to reflect this change in understanding and to address the qualitative as well as quantitative aspects of fatigue. These scales began to dominate in the late 1990s and early part of this century [179] and validation of them in both generic terms and in disease specific situations is on-going.

Disease specific tools have also been developed. The disadvantage with this is that they are not generalizable and subsequent comparisons with other patient groups are not possible. They also focus on disease specific aspects, which may not be the sole contributing factor to any fatigue. Therefore, they may be an inaccurate overall representation of the fatigue suffered.

The large number of questionnaires to assess fatigue implies a lack of consensus over how to define fatigue and subsequently how to assess it. This uncertainty was highlighted in a systematic review in 2007. Hjollund et al found there were 252 different patient self report scales for fatigue published between 1975 and 2004 [179]. Of these 252 different scales, 150 had only been used once, making judgements about reproducibility and reliability virtually impossible.

Correlation of fatigue assessments with assessment of other symptoms including psychological features may help to establish the predominant feature. If an alternative diagnosis such as depression is present, this must be considered when interpreting any assessment as outcome and treatments will be different.

2.9.2 Fatigue and the General Population

Evaluation of fatigue as a symptom in the general population reveals that it is a relatively commonly reported problem. In one study in the UK, 10.6% of women reported excessive fatigue [180] lasting over a month. A community based UK study of 15,283 responders (31,651 contacted) revealed even higher rates of fatigue with 18% reporting excessive fatigue lasting longer than 6 months [181]. Despite the large size of that study, there was only a 50% questionnaire return rate making it difficult to interpret the findings. However, this confirmed other reports[180] that women were more like to report suffering from fatigue than men. A greater number of women suffered from psychological disorders, but the authors were not able to establish the exact nature of this relationship and suspect that the two overlap rather than have a strict causative link.

It has also been shown that age plays an important role in fatigue levels with a linear relationship being reported[182]; as age increases so do fatigue levels.

2.9.3 Fatigue in Chronic Illness

As demonstrated by the large of number of fatigue assessment tools, interest in fatigue in the context of chronic illness has dramatically increased over the past twenty years. Whilst there is very little literature about XL-CGD carriers and fatigue, there is a large body of literature about chronic illness and fatigue. This is perhaps in part due to the weight the symptom is given by patient groups. Fatigue is often reported to be of greater significance than other symptoms including pain and has been shown to have a negative effect on quality of life [171, 172]. Some of this literature will be reviewed in this section.

Fatigue is repeatedly described as a significant and prominent feature of autoimmune disease and chronic inflammatory conditions, particularly in rheumatoid arthritis (RA) [171], sarcoidosis [182], IBD[170, 173, 183, 184], multiple sclerosis [185] and primary biliary cirrhosis [186].

Fatigue is reported in approximately half of IBD patients[183] of whom many suffer from chronic fatigue [173, 175, 184] and at greater rates than the general population [173].

The presence of fatigue in chronic conditions is important for several reasons. Firstly, fatigue is defined by patients as a significant problem and often, surprisingly, is the most severe problem they suffer. In one study of 116 patients with PBC, 85% reported suffering from fatigue and in half of these patients it was reported to be the worst or one of the worst symptoms they suffered [186] clearly highlighting the impact of the symptom. This is a similar rate to that reported in sarcoid where rates exceeding 70% are reported[182].

Secondly, fatigue has been found to adversely affect health related QoL (HRQoL). A reduction in all domains of HRQoL was shown in IBD patients suffering from chronic fatigue [173, 184]. This was also found in a study of PBC patients where fatigue impacted upon QoL[186]. Fatigue has also been shown to be an independent factor in HRQoL of IBD patients [175] and in sarcoid patients[187].

Finally, it is currently not well treated and represents a domain to which treatments should be targeted.

2.9.4 Factors Affecting Fatigue

There appears to be a lack of consensus about the most important factors affecting fatigue in chronic illness and it is likely that the aetiology of fatigue is multifactorial. It would seem logical to suspect that disease activity may impact upon fatigue levels.

Disease Activity

Disease activity may be important in the presence of fatigue, but it maybe difficult to distinguish definite clinical activity with subclinical disease in many situations.

A large study of 425 IBD patients found that fatigue levels were significantly higher in patients with active disease compared with those in clinical remission [183]. This finding has been corroborated with two smaller studies in 2011 showing higher rates of chronic fatigue in patients with active disease [173, 184].

Surrogate markers of disease activity may also be used in an attempt to correlate with fatigue levels. For example, Michielsen et al[187] found that sarcoid patients using corticosteroids had higher levels of fatigue than those who did not perhaps reflecting those with more active disease.

Whilst disease activity has been shown to correlate with fatigue levels, there remains uncertainty about the nature of the link. In IBD patients, fatigue was still present at high levels in patients deemed to be in remission [175] with 40% reporting high fatigue. This was a large study of 707 patients, suggesting other factors may play an important role or that there may be a cumulative or delayed effect. The lack of correlation with disease activity was also supported by a study of 116 patients with primary biliary cirrhosis (PBC) where there was no association with disease severity and fatigue levels [186]. However, this study differs from studies of other diseases, as it was predominantly women (89%), which may have impacted upon the fatigue levels reported.

Reversible causes such as anaemia may be assumed to impact on fatigue levels, but this was not the case in Bager et al's [183] study of IBD patients where there was no correlation with overall fatigue and anaemia except in a very small subset of patients. Conversely Romberg-Camps found that anaemia did impact on both fatigue and HRQoL[175].

Psychological Co-Morbidity

Many studies have not measured or looked for depression when examining fatigue, which makes it difficult to examine the link between them. Huet et al[186] were surprised to discover that when they screened 116 patients with PBC, 85% of whom reported fatigue, 50% met the criteria for a diagnosis of depression. They were unable to evaluate the direction of the association.

Two studies in rheumatoid arthritis found that there was significant correlation between fatigue and depressive symptoms [188, 189]. However, again it is difficult to be certain of the direction of the association.

Psychological co-morbidity does not appear to account for all fatigue described in chronic illness. Basu et al[172] studied patients with ANCA-associated vasculitis and found that whilst there was greater fatigue in those suffering with

mood disorder, this did not account for all those suffering with fatigue thus suggesting an alternative mechanism.

Demographic Factors

Age has been shown to be an important determinant in fatigue levels, irrespective of disease, with younger patients (defined as less than 60 years) suffering greater fatigue. This was demonstrated in IBD [183].

Age also appears to be an important factor in the reporting of fatigue in sarcoid patients. A Dutch study [182] demonstrated that the greatest differences between the general population and patient cohort were seen in young patients (less than 60 years), who reported the highest levels of fatigue. Age has not always been found to be a significant finding particularly in smaller studies [184].

Being female has been associated with greater fatigue both in patient groups including IBD [175, 183], sarcoidosis [182, 187] and in the general population[180, 181]. However, gender has not always been found to be a significant contributor when assessing fatigue[184].

Employment status has also been shown to impact upon fatigue levels, with those who are unemployed reporting higher rates of fatigue that those in any type of employment[182]. This relationship may be bidirectional.

In summary, fatigue appears to be a relatively common problem both in the general population and more so in chronic disease. The literature about fatigue in chronic illness highlights that it is a highly prevalent and important symptom. Fatigue also appears to adversely impact upon self reported HRQoL in individuals with chronic illness. There is some evidence to suggest fatigue may be associated with disease activity, although this is not always the case and assessment of disease activity may sometimes be difficult. Fatigue appears to be more prevalent in younger patients and in women.

2.9.5 Inflammation and Fatigue

As evidence accumulates to support the presence of fatigue as an independent symptom, interest is increasing in causation. The presence of fatigue in chronic

inflammatory conditions, as discussed in the previous sections, suggests an intrinsic inflammatory mechanism. Evidence from clinical observation supports this.

In chronic inflammatory conditions where fatigue has been studied, it has been found that there are higher levels of fatigue when disease is active as opposed to clinical remission, for example in IBD[173] and that disease severity correlates with fatigue levels [175]. This may suggest that greater levels of inflammation lead to greater fatigue.

However, fatigue has also been found in 40% of [183] patients with IBD when they are in clinical remission. Van Langenberg puts forward a popular hypothesis in his review of fatigue in IBD that this may be due to on-going subclinical inflammation[169] as clinical remission in IBD does not always correlate exactly with microscopic resolution. The patient may report no gastrointestinal symptoms, but mucosal healing of the gut may not be complete despite apparent resolution of symptoms. This may explain the persistence of fatigue even when other symptoms have improved as there may be on going inflammation.

Further clinical evidence supporting an inflammatory process causing fatigue can be found from the use of anti-TNF agents in patients with rheumatoid arthritis and Crohn's disease. When patients with rheumatoid arthritis were treated with the anti-TNF agent adalimumab, fatigue improved[190]. It is possible that this improvement is the result of the anti-inflammatory effect of adalimumab.

Pro-inflammatory cytokines have been studied with regards to causation of fatigue and appear to support an inflammatory mechanism in the development of fatigue. Cancer patients have reported fatigue as a significant symptom[174] and research into this cohort of patients has been undertaken. Raaf et al [191] found that patients with advanced cancer had significantly higher fatigue scores in both physical and mental domains. Physical fatigue was associated with significantly higher levels of the pro-inflammatory cytokines IL-6, IL-1ra and neoplerin. However, this was not replicated with mental fatigue, where only IL-1a was significantly associated with greater fatigue, perhaps suggesting slightly differing mechanisms.

IL-6 has been implicated in sleep regulation and fatigue as well as chronic or low grade inflammation and also plays a crucial role in the interaction between the immune system and central nervous system[192]. Further to this blockade of IL-6 by tocilizumab has been shown to reduce fatigue in patients with rheumatoid arthritis[192, 193], although improvement in other symptoms including pain, may account for some of the improvement seen in fatigue.

Conditions outside of rheumatoid arthritis have also found a correlation between IL-6 and fatigue levels. In Primary Sjogrens Syndrome, where patients report fatigue as an important symptom[194], levels of IL-6 have been found to be raised in tears, saliva and blood of patients[195], although these have not always correlated directly with fatigue scores [196].

Bower[197] reviewed their own and other data about fatigue in cancer patients, and specifically those undergoing radiotherapy. They found that cancer patients who suffered from fatigue had higher serum levels of the pro-inflammatory cytokines IL-1β and IL-6 during radiotherapy treatment and that this was correlated with the degree of fatigue reported by patients. Further work by Bower et al[198] did not corroborate this finding, as there was no association between IL-1, IL-6 and fatigue in post radiation cancer patients. However, they did find an associated rise in CRP and IL-1 receptor antagonist suggesting that there was a role for systemic inflammation.

IL-8 is a pro-inflammatory cytokine. One of the major functions of IL-8 is as a chemoattractant with a particularly strong propensity for neutrophils[199]. IL-8 has been implicated in fatigue. Sorenson et al [200] studied patients with chronic fatigue syndrome (CFS) and compared them to healthy controls and fatigued controls who did not meet the criteria for CFS. IL-8 was significantly raised in both fatigued groups compared to the healthy controls. In the CFS group, IL-8 levels were higher than those in the fatigued, non-CFS group, correlating with higher levels of reported fatigue.

Whilst the findings may not be consistent, there is a consistent message that there is an inflammatory aetiology to the fatigue suffered by patients with chronic disease and cancer. Pro-inflammatory cytokines appear to play an important role.

2.9.6 Fatigue and XL-CGD Carriers

Cale et al [69] commented in their study of 19 XL-CGD carriers that excessive fatigue was reported, but there was no quantification of this or further information available.

In summary, female carriers of XL-CGD may be at risk of fatigue for many of the reasons discussed in this section. Potentially, XL-CGD carriers are at risk of fatigue for a number of reasons: a potential inflammatory process, manifestation of SLE or associated with being a carer for children with a chronic disease in some cases. There is little research into the presence or causation of fatigue in XL-CGD carriers and this requires further investigation.

2.10 Psychological Health

There are many factors that may impact upon the psychological health of XL-CGD carriers, but there is no published literature specifically about this group. Factors that may impact upon the psychological health of XL-CGD carriers include being a caregiver for a child with chronic illness, genetic guilt, the presence of anxiety and depressive symptoms and potential ill health themselves. Whilst there is no literature specifically about XL-CGD carriers in this area, there is literature from other conditions, which may be extrapolated to be relevant to XL-CGD carriers. This literature will be reviewed in this section.

2.10.1 Caring for a Child with a Chronic Condition

There is no literature about the impact of having a child with CGD on parents. However, there is a growing body of literature about the psychological effects of care giving. Since many XL-CGD carriers are confirmed following the diagnosis of CGD in their relative and often a son, many known XL-CGD carriers are caring for at least one child with a chronic illness. Subsequently, one contributing factor to the psychological health and quality of life (QoL) of XL-CGD carriers may be the impact of care giving.

Children with a PID have higher rates of psychosocial difficulties including emotional and behavioural problems [64, 201]. What is less clear is the impact their diagnosis has on their parents and specifically mothers.

It can be anticipated that caring for a child with a chronic illness increases levels of stress. It is less clear what the impact on other markers of psychological health including the presence of anxiety and depression may be. Barlow et al[202] demonstrated that having a child with a chronic condition impacted upon the psychological health of the family and this has been shown to impact upon the health outcomes of the child[203].

Studies examining the impact on parents of a child with PID have focussed on those in which the patients have undergone HSCT. McdDowell et al [204] performed a qualitative study examining parental stress and perceptions at 1 year post-HSCT. The themes highlighted showed that caring for a child with a life threatening illness unsurprisingly increased perceived stress. These feelings of isolation and uncertainty seemed to persist after transplant. However, the parents did not appear to have clinical levels of psychological distress including mood. This was a small study of only four couples. There may have been an element of selection bias with those recruited being the ones who were motivated to respond to the invitation, as they felt they had suffered distress. It was not designed to provide generalisable answers, but simply to draw out themes. No formal assessment of levels of anxiety and depression were made and degree of distress was not quantified, but it does suggest that caring for a child with a life limiting condition, particularly during HSCT, may impact upon psychological health.

A Dutch study [205] evaluated parental stress following HSCT for malignant disease and primary immunodeficiency. They found that, whilst mothers were more prone to general stresses even five years after their child's HSCT, they did not report higher stress scores when compared with reference groups. The parents were described as resilient and mothers of children with non-malignant disease suffered less stress than the malignant disease group. This perhaps conflicts with the McDowell study [204] where the higher levels of stress had persisted and it may suggest factors outside of HSCT may play an important role.

Manne et al [206]showed that maternal age may also contribute to the degree of distress, with younger mothers suffering from higher rates of distress compared with older mothers.

These studies have focussed on HSCT, which is an acute event requiring intense medical treatment and prolonged hospital stay. Not all children with CGD will have undergone HSCT and it is important to evaluate the stresses of caregiving in everyday life, rather than solely during the acute period of HSCT. As previously stated, there is little about parental stress in PID outside of HSCT. To further evaluate the impact of caring for a child with a chronic illness, information from conditions outside of PID will now be examined.

Caregivers in muscular dystrophy (MD) have also been studied. A recent study from the US looked at 1238 women caring for someone with MD (either Duchenne MD or Becker MD)[207]. The authors found that, despite high levels of resilience in nearly 90% of the women, the rates of significant psychological distress were considerably higher in the caregivers than would be found in the general population. However, they also identified protective factors, i.e. factors associated with lower stress levels, including good family support and a higher income, which perhaps suggests that, whilst psychological distress is common, it is not inevitable in carers of children with chronic conditions. This was a large study, but the authors acknowledged that ethnic minorities may have been under represented due to the method of recruitment and that subsequently psychological distress may be under reported. However, the principles remain true and to extrapolate data for the UK, where there are far fewer Hispanic residents, is still possible.

Genetic guilt may account for some of the psychological distress seen in the carers of genetic disorders. If this were the main cause, one would expect to see lower levels of distress in conditions where there is not such definitive inheritance. This does not appear to be true. A study published in 2013 of paediatric IBD patients and their parents found that there were high levels of caregiver stress [208]. The highest levels were seen where the disease was most active. Disease severity was also important in the MD study where, once children became non-ambulant, distress seemed to increase [207]. This again highlights the many factors that may contribute to distress in caregivers. It is not only severity of disease that influences the degree of distress for caregivers. Pain has been shown to be one of the most difficult aspects of care for caregivers when

Sickle Cell Disease (SCD) was studied[209]. A small scale study of SCD and HIV caregivers corroborated the findings in other disease with high rates of depressed mood in the caregivers of the SCD and HIV patients. The rates were higher than in parents of healthy children who recruited as controls. Interestingly, this study identified that the amount of time perceived to be spent caring also impacted on psychological distress, but that time spent caring did not equate to severity of disease. Nereo et al [210] also highlighted that stress levels are not constant and, in their study of MD caregivers, were highest at time of diagnosis. This may suggest that caregivers adjust to a diagnosis and that the stresses may decrease as time goes on making time since diagnosis an important factor.

Caregiving is not restricted to looking after children. Studies have also evaluated caregivers caring for adult patients. One such study evaluated 100 caregivers of patients with brain tumours. All patients and caregivers were over 18 years of age. This study demonstrated that the carers had reduced quality of life and particularly in the mental health domain. They also suffered from greater levels of anxiety and depression than would be expected for the general population[211]. The authors suggested that one possible cause for the higher anxiety levels relates to fears about supporting the family alone and performing unfamiliar domestic tasks. This reflects that the majority (66%) of participants were a spouse or partner. This may therefore not be reflective for mothers and it is therefore, difficult to extrapolate too much from adult based studies.

Assessment and quantification of the stresses of having a child with a chronic illness will be discussed in the next chapter.

In summary, overall the literature about caregiving suggests that caring for someone with a chronic illness increases general stress levels. However, psychological health and well being is not solely dependent on this, with other factors playing an important role. Psychological distress has important implications for the carer, the patient and the family as a whole.

2.10.2 Anxiety and Depression

Anxiety and depression are two distinct but overlapping entities. They include a range of biological and psychological symptoms, including low mood, sleep and appetite disturbance and difficulties in functioning in daily activities.

The aetiology is uncertain but there are known to be contributing and protective factors. Female gender, a family history and significant loss at a young age are all known to predispose individuals to psychological ill health[212]. Support networks including family may be protective.

Chronic illness is associated with higher rates of anxiety, depression and psychological distress. For example, in SLE mood disturbance forms part of the diagnostic criteria [139] and in conditions such as RA and IBD there are high rates of depression [213, 214]. CGD patients specifically have been demonstrated to have more emotional and behavioural problems than the general UK population [64].

The exact pathophysiological origin of depressive symptoms and diseases remains uncertain. Why some individuals appear to be more vulnerable suggests that there are factors out with external forces that are of importance.

A biological explanation has long been looked for. Increasingly a role for an immune modulated or inflammatory cause has been hypothesised.

Evidence from animal models supports the possibility for an inflammatory cause. When inflammatory agents are injected in to mice, sickness behaviour is induced which resembles depression with the mice demonstrating the core biological symptoms including anhedonia, poor appetite and sleep disturbance [151, 215]. Van Gool et al[216] reported that when human subjects were given IFNα, 45% reported depressive symptoms.

Further support for an inflammatory or immune modulated cause of depression is found by the observation that an increase in production of pro-inflammatory cytokines is associated with depression, in particular levels of IL1β, IFNγ and TNFα[217].

The presence of depression in association with chronic diseases has been argued to support many differing hypotheses for the cause of depression. The link in Crohn's disease (CD) is well established with increase in disease activity and increased anxiety and depressive symptoms occurring simultaneously. Further to this, not only is depression associated with reduced remission rates in CD [218] but is there is a high rate of psychopathology in patients with CD prior to diagnosis.

However, it may be that the symptoms in CD are the cause of depressive symptoms as they are inherently distressing. The association of depression and increased symptoms does not exclusively suggest an inflammatory cause of the psychological component. A small study by Guloksuz et al [217] attempted to examine the potential link between immune activation and depressive symptoms in CD patients. Their cohort of 15 patients were all treated with anti-TNFα therapy. There was an increase in immune activation, as defined by raised positive acute phase proteins and decreased negative acute phase proteins, in patients with a history of past or present depressive episodes even after correcting for level of disease activity. This suggests an association between inflammatory response and depressive symptoms albeit on a small scale.

When anti-TNFα agents, such as infliximab, have been used to treat inflammatory conditions including RA and CD, improvements in depressive symptoms as well as medical symptoms were seen[190] and these studies are ongoing to evaluate anti-TNF agents in treatment resistant depression.

Kishida et al [219] studied gp91^{phox} knock out mice as a surrogate for XL-CGD patients and did not find them to suffer from anxiety or depressive symptoms. There have been no published studies about human patients with CGD and the presence of anxiety and depression.

2.10.3 X-linked Carriers and Psychological Health

Whilst there is no literature about the psychological health of XL-CGD carriers, it can be hypothesised that XL-CGD carriers may be at higher risk of symptoms of anxiety and depression due to a number of factors.

SLE

Mood disturbance is one of the features of neuropsychiatric SLE (Table 2-3). As XL-CGD carriers have been reported to suffer other features of SLE, as discussed earlier, they may be at risk of the neuropsychiatric manifestations as well.

A mouse study discovered that depressive symptoms might precede the onset of other SLE symptoms[215], which perhaps supports the hypothesis that depression is a fundamental part of SLE as opposed to a reactive process resulting from chronic illness. In the same study, the lupus mice did not exhibit anxiety, but depressive symptoms correlated with the presence and level of autoantibodies, specifically ANA titres and anti-ribsomal P antibodies. Depression was also more common in the female lupus mice than their male counterparts. The depressive symptoms presented early in the disease process and were at times the primary manifestation of disease. Despite the correlation with autoantibodies, they were not diagnostic of NPSLE, but MRI was also not informative in the diagnosis of NPSLE. These results were supported by a further study by the same group.

Chronic Illness

XL-CGD carriers may also be at risk of psychological ill health due to their own medical problems. On going, potentially untreated medical problems may contribute to the development of depression and anxiety but this has yet to be demonstrated.

Genetic Guilt

The inheritance of a medical condition results in complex feelings within a family. For the carrier there may be feelings of guilt associated with the passing on of a gene whilst being unaffected themselves by the faulty gene. There may also be subconscious or conscious blame attributed by other family members and the affected individual. Mode of inheritance may contribute to these feelings. CGD is in a unique position, as it may be inherited in either an autosomal recessive manner or in an X-linked manner. This allows for feelings about inheritance to be studied.

James et al[220] examined the different aspects of genetic guilt in their 2006 study of CGD families. AR and XL families were included in the study allowing for comparisons to be made. The feelings of guilt and self blame were more strongly associated with XL disease than AR. Mothers of boys with XL disease blamed themselves for their son's condition more than AR mothers. The fathers of boys with XL disease also blamed the mothers more than in AR disease. This feeling of guilt may affect psychological health.

2.11 Quality of Life

Quality of life (QoL) represents an overlap of medical and psychological health. Quality of life is difficult to define and attempts at definition tend to be nebulous or ill fitting. However, it is a concept with which the majority of the population of the developed world are inherently familiar.

Aristotle made an early reference to the concept of quality of life, 'Both the multitude and persons of refinement….conceive 'the good life' or 'doing well' to be the same thing as 'being happy'. But what constitutes happiness is a matter of dispute…some say one thing and some say another, indeed very often the same man says different things at different times: when he feels sick he thinks health is happiness, when he is poor, wealth." [221]

Aristotle identifies the difficulties in assessing quality of life. Firstly the lack of universality of definition and secondly the speed at which an assessment of QoL may change.

2.11.1 Measurement of QoL

There are several methods available to formally assess QoL in a clinical or research setting.

In its simplest form, assessment of QoL may involve asking the patient how they would rate their QoL. However, different interpretations of a simple question mean that information gained from this method is limited and lacking in reproducibility and validity. Comparisons between different groups are difficult and even comparisons of the same patient at different time points may not be valid. As such, tools have been developed to overcome these difficulties.

Tools assessing QoL may be generic, disease specific or aspect specific.

Generic tools for assessing QoL have been designed to be used by all irrespective of the medical condition or circumstances and may also be used for healthy individuals. The advantage of this is that it allows for direct comparisons to be made between groups irrespective of disease using the same tool.

An example of a generic tool is the Medical Outcomes Study 36-Item Short Form (SF-36). The SF-36 was developed in 1993 by Ware et al [222] and was designed to evaluate general health status. The SF-36 has become one of the most widely used QoL tools in clinical research [221]. As with many generic tools, the SF-36 assesses multiple health concepts. The 36 items are categorised to provide scores for each of these domains in addition to providing a score for summary measures. This allows for comparisons to be made, not only in overall QoL, but also within different domains that have been predefined.

Another example of a generic tool is the EuroQoL, which provides an overall QoL assessment, but given its brevity requires an additional tool such as a disease specific tool for more in-depth assessment [223].

A disadvantage of a generic tool is if the question about quality of life relates directly to disease specific symptoms or manifestations this is not addressed. As such, disease specific tools have been developed.

Disease specific tools focus on the relevant factors for the disease and predominantly include symptoms. For example, a QoL tool for a gastrointestinal disease would pay particular attention to diarrhoea and urgency. The advantage of disease specific tools is that they may detect subtle or small changes with respect to the specific factors deemed to be important. The disadvantage is that while generic tools make few preconceptions about what may impact upon QoL, disease specific tools have pre-defined the symptoms or factors that are likely to impact upon QoL.

There may be aspects of QoL which are of particular interest and they may require more detailed examination than a generic tool allows. For example, fatigue, pain and anxiety and depression may be relevant across diseases. These aspects of QoL are often of interest, independent of the disease process and

therefore require individual assessment. Examples of these surveys include the Hospital Anxiety and Depression Score (HADS)[224] and the MFSI [179].

2.11.2 Importance of QoL Assessment

In some situations, a crude assessment of alive or dead is sufficient to determine if a treatment or intervention is successful. However, increasingly more subtle changes are being required to determine the benefits of an intervention especially as survival improves. QoL is increasingly a primary endpoint in its own right. By assessing QoL, it enables researchers or clinicians to detect positive and negative aspects of treatments.

Patient assessment of QoL does not always agree with physician interpretation. This makes formal assessment even more imperative. Additionally, by assessing QoL, particularly when using a generic model, important factors, which have not been previously considered by the clinical team may become apparent. Once uncovered, these factors may then be addressed.

On a population level, with an ageing population, chronic disease is likely to become increasingly significant. In many situations, chronic disease may not be completely curable, and therefore, advances must be measured in a manner other than survival. QoL is a more comprehensive and accurate assessment than absence of symptoms.

QoL may also provide information about social and emotional functioning rather than only physical symptoms. This may be beneficial when assessing the holistic aspects for a patient.

QoL assessment provides important information in both the research and clinical setting. In this study of XL-CGD carriers, mortality is unlikely to be a useful endpoint, whereas QoL may uncover more subtle difficulties relating to physical and mental health.

2.11.3 The Literature: What has been seen in other conditions?

Evaluation of the QoL of patients with CGD showed that patients with CGD have poorer QoL than the general population[60]. The study also demonstrated that children undergoing curative treatment for CGD, namely HSCT, had near normal

QoL after treatment. There has been no study to evaluate the quality of life of XL-CGD carriers, but if XL-CGD carriers manifest symptoms similar to that described in CGD, it is plausible that they may have reduced QoL and that the reductions may be in similar domains. QoL has been assessed in other PIDs with similar findings. Soresina et al [225] evaluated children and adolescents with X-liked Agammaglobulinaemia (XLA) and compared them to an age matched control group. They found that overall global health related QoL was lower in the XLA group than the healthy controls, despite there being no difference in the perceptions of physical health. This highlights the discrepancy between physical health and overall QoL and indicates the many factors that may impact on QoL rating.

There is a growing body of literature regarding QoL in parents and families of children with chronic illnesses. As with the psychological stresses of caregiving, it can be anticipated that there may be a reduction in QoL in parents and families where there is a child with a chronic illness. Cystic Fibrosis (CF) has been well studied and whilst there are differences with CGD, there are significant similarities in disease severity and medical involvement for conclusions to be transferrable. Driscoll et al [226] demonstrated high levels of anxiety and depression in female and male caregivers of children with CF. These high levels of anxiety and depressive symptoms correlated with reduced QoL. Interestingly, reduced QoL has not been demonstrated in the siblings of children with CF, who report well-preserved QoL [227]. Reduced QoL was also seen in a study of 266 caregivers of children with special needs [228], thus corroborating the findings in CF parents.

As outlined, the evidence suggests that caring for a child with chronic illness reduces QoL. However, the hypothesis of this study into XL-CGD carriers is that there are unmet medical needs for the carriers themselves. Therefore, it is important to consider the impact of a chronic condition, such as SLE, on the QoL of the individual.

A recent study examining QoL and presence of anxiety and depressive symptoms in SLE patients demonstrated that QoL was reduced and there were higher rates of anxiety and depression, but only in patients that reported high levels of pain

[229]. In SLE patients that were classed as 'low pain', the rates did not significantly differ from the healthy controls. This implies that it is not simply the presence of a chronic condition which effects QoL, but that there are more subtle factors involved.

2.12 Cognitive Function

Potential difficulties with learning in CGD patients were noted by Pao et al [230] in 2004. They performed a retrospective review of 26 CGD patients referred to their unit. This was prompted by the observation that many CGD patients had IQ scores of less than 70. The mean IQ was 89 (lower than the UK average) and 23% had an IQ of less than 70 meeting the criteria for learning impairment. The number found to have significantly lower than average IQ was more than the quoted 1-3% of the general population. However, the study suffered from significant selection bias. In particular, all included patients had been referred due to concerns about behaviour or education, which may account for the high rates of deficits. Alternatively, it may be that the impairment is more discrete in the remainder of the population, or that there are problems with specific areas of cognition such as memory and learning.

Cognitive function is the result of the interplay between multiple factors and chronic illness may predispose patients to a number of psychosocial difficulties including cognitive dysfunction. However, a reduction in IQ has not been demonstrated in all chronic illness. For example when children with Cystic Fibrosis (CF) were studied, there was no difference in IQ [231]. Therefore, Pao et al's findings may suggest that the CGD disease process itself may be involved in cognitive dysfunction.

A more recent study by Cole et al[232] recruited all known CGD patients from the UK and Ireland to a study including an IQ assessment. This found that there was no significant difference between CGD patients and UK norms. The study did not have the recruitment bias demonstrated in Pao's original study, but the assessment was not as comprehensive. However, the assessment tool used was a validated tool for IQ assessment and would have detected gross differences such as those found by Pao et al.

Causation of Reduced IQ

Pao et al [230] hypothesise that the reduction in IQ and cognitive function may be due to recurrent infection or due to the lack of functional NADPH oxidase and the resultant loss of superoxide within neuronal cells.

An important part of mammalian learning is thought to involve long-term potentiation (LTP) in the hippocampus[233]. Superoxide is thought to be an important signalling molecule in this process [234] and that the removal of superoxide adversely affects this process[235]. Superoxide has also been demonstrated to be important in associative memory [234]. NADPH oxidase is potentially an important contributor to the production of this superoxide[219].

The clinical observation of reduction in IQ in CGD patients has led to exploration about possible causation and CGD mice provide a useful model.

Kishida et al's [219] study of CGD (gp91^{phox} and p47^{phox} knock out) mice drew several important conclusions. The first was that NADPH oxidase is an important factor in hippocampal long-term potentiation (LTP). This appears logical as superoxide is known to be required for hippocampal synaptic plasticity and specifically for LTP and hippocampal dependant memory [236]. The second is that whilst the hippocampus in CGD mice has a grossly normal structure, the function is impaired as CGD mice have mild memory impairment in hippocampal learning. CGD mice were also found to have a mild deficit in spatial learning and interestingly, in gp91^{phox} knockout mice but not p47^{phox} knockout mice, there were significant impairments in both motor co-ordination and motor memory [219].

Therefore, there is a theoretical reason why CGD patients, and potentially XL-CGD carriers, could have problems with learning despite the findings by Cole et al[232] that CGD patients did not suffer reduction in IQ.

2.13 Conclusions and Summary

The anecdotal observations and what has been described in the literature formed the basis for this study and the need to fully evaluate the health of XL-CGD carriers.

Chapter 3: Objectives of the Study

3.1 Hypotheses

The main hypotheses for the study were:

1. XL-CGD carriers suffer from medical problems similar to those suffered by CGD patients
2. XL-CGD carriers suffer from problems similar to SLE
3. XL-CGD carriers have an average of 50% normally functioning neutrophils as defined by neutrophil oxidative burst
4. There is a correlation between the reduction of neutrophil oxidative burst and any medical problems suffered in XL-CGD carriers
5. Psychological health in XL-CGD carriers is worse than the UK population and mothers are more significantly affected than other XL-CGD carrier relatives

3.2 Objectives

The objectives of the study were:

1. To identify and define the type and prevalence of medical problems in XL-CGD carriers in the UK
2. To define the average and range of neutrophil oxidative burst value seen in XL-CGD carriers in the UK
3. To establish if medical problems in XL-CGD carriers are associated with the degree of reduction in neutrophil oxidative burst
4. To evaluate the psychological health of XL-CGD carriers in the UK
5. To compare the psychological health of XL-CGD carriers in the UK with a control group of XL-carriers of MD
6. To evaluate the quality of life in XL-CGD carriers and to compare this with population data
7. To evaluate IQ in XL-CGD carriers

Chapter 4: Methods

4.1 Recruitment

The inclusion criteria for the study were:

- Resident in the United Kingdom
- Female carrier of XL-CGD
- Aware of own carrier status

4.1.1 Identification of Participants and Recruitment

XL-CGD families were identified from the UK CGD Registry and by consultants caring for families with CGD at the main treatment centres for CGD in the UK, the Great North Children's Hospital (GNCH), Newcastle upon Tyne and Great Ormond Street Hospital (GOS) and the Royal Free Hospital, London. Other sites included in the study were Birmingham Heartlands Hospital and Manchester Children's Hospital as families were identified at these sites and subsequently recruited.

XL-CGD carriers were also identified and recruited through the CGD Society. The CGD Society is a charity set up to support families and patients with CGD. The project was advertised via the charity website and participants responded directly to this. The annual 'Family Day' and 'Ladies' Carrier Day', hosted by the CGD Society, included presentations about the research project and XL-CGD carriers were recruited from here.

All eligible families were contacted either in person at a clinic appointment or by post, with the exception of where the index case was deceased, when the clinical team made a decision as whether it was appropriate to contact the family or not. There were two families where the index case had died shortly before the study commenced and it was decided that it was insensitive to contact these families for research purposes. One index case died during the course of the study. His mother had already enrolled in the study, but was not followed up for missing data. Additional XL-CGD carriers, within a family, were identified at the initial interview with the recruited participants.

Families not attending clinic appointments were contacted by post to participate in the study.

If an XL-CGD carrier was deceased, they were counted in the total number of carriers. Where the next of kin was available, consent was obtained to include the deceased carrier in the project and to access any medical records. Information about these XL-CGD carriers was limited. The researcher recorded information volunteered by the next of kin about the deceased XL-CGD carrier.

There will be XL-CGD carriers in the general population who are not known about, as they have not had children or where the diagnosis in family members has not yet been made. These constitute the 'unknown unknown' and it is not possible to include these carriers in the study or to quantify them.

4.1.2 Confirmation of Carrier Status

Individuals were considered to be a carrier if they had an abnormal neutrophil oxidative burst demonstrating a dual population of neutrophils by either NBT or DHR and a family history of XL-CGD.

Deceased carriers were included if they had been tested prior to death, or if they were an obligate carrier i.e. the daughter of an affected father.

The exact genetic mutation was not tested in the XL-CGD carriers due to the expensive nature of the test. It was assumed that the mutation in the XL-CGD carriers was the same as that found in the index case. It is highly unlikely that a different CGD causing mutation would occur in the same family. After discussion with the genetics department at the Royal Victoria Infirmary, Newcastle upon Tyne, it was felt that this was an acceptable assumption.

As different mutations are associated with different amounts of residual NADPH oxidase function [14], knowledge of the mutation was important in order to correlate medical symptoms with this. Information regarding the specific mutation in the index cases was sought from the original registry data[11] and recorded where the information was available.

4.1.3 Recruitment of Controls

A control group was recruited in order to compare the psychological aspects of the study and to ascertain whether any psychological problems identified were related to being a carrier of XL-CGD specifically or if they were associated with being the relative of a child with a chronic, XL inherited condition. Additionally, small-scale studies [220] suggest there are greater feelings of guilt and blame felt by mothers who are XL carriers, rather than mothers who are AR carriers, which may be important when considering psychological effects. It was also important that the index cases had a significant disease, which was life limiting and required frequent trips to hospital. The control group carriers needed to be accessible to the researcher in order that they may be approached for inclusion.

Muscular Dystrophy (MD) met the required characteristics for the control group. Muscular Dystrophy was chosen as the control group for several reasons. It shares some similarities with CGD; it is X-linked inheritance, it has a severe course, the children have frequent trips to hospital and is life limiting. BMD and DMD classically have different severity patterns. Including XL carriers of both BMD and DMD meant a range of disease severity in the index cases was represented, which is similar to the broad spectrum of disease severity seen in CGD patients. MD is more common than CGD with an incidence of 1 in 3,500 newborn males for DMD and 1 in 17,000 for BMD [237], meaning that recruitment of a control group of similar number to the study population was potentially possible.

The Great North Children's Hospital, Newcastle upon Tyne, is a leading centre in the UK for the diagnosis and management of MD, which is therefore comparable with CGD and means that there is a similar geographical spread for the control group as the XL-CGD carrier group. For both conditions, the Great North Children's Hospital receive referrals from the whole of the UK meaning that any potential regional differences should be similar for both XL-CGD and MD carrier groups.

Carriers of XL-MD were recruited from the MD clinic at the Great North Children's Hospital, Newcastle upon Tyne to form a control group. Carrier mothers, grandmothers and sisters presenting to this clinic with an MD index

case were approached for inclusion into the study. XL carriers of either Duchenne (DMD) or Becker's Muscular Dystrophy (BMD) were eligible for inclusion in the control group. All eligible carriers were approached, at a clinic appointment, for inclusion in the study.

4.1.4 Exclusions

Girls under the age of 16 years who did not know their carrier status were not included in the study. The testing of children for genetic disease is controversial. At present, the guidance remains that where there is no health benefit for the individual, children under the age of 16 years should not be tested for carrier status[78]. A study of the adolescent sisters of boys with CGD found that they concurred with this recommendation and favoured testing for carrier status later in life [79].

Carriers who were aware of their carrier status were eligible and included irrespective of their age.

In families where the index case was deceased, the families were contacted if the consultant caring for the family deemed it appropriate or if there was continuing contact with the family.

There were no other exclusions.

4.1.5 Consent

Written consent was obtained from all participants prior to enrolment in the study. Participants under the age of 16 years were enrolled after consent from their parent or guardian and assent from themselves. Next of kin consent was taken where the carrier was deceased. Consent and assent forms are included in the appendices.

4.2 Assessment of Health

For each enrolled participant a family pedigree was constructed detailing cases, carriers and where carrier status was unknown. Information was recorded about family history of disease and the general health of first-degree relatives. Known medical conditions, regular medications and hospitals attended were recorded.

Smoking status (never, current or ex-smoker) and weekly exercise were recorded.

4.3 Medical Health

4.3.1 Respiratory Symptoms

Participants were asked to complete the St George's Respiratory Questionnaire (SGRQ) to assess respiratory status. This is a questionnaire designed and validated for use in both fixed and reversible airways disease and has been used as a research tool for respiratory conditions [238-241]. It assesses the presence and impact of symptoms. Participants answer a series of questions in the questionnaire relating to cough, shortness of breath and effect on their activities of daily living. Answers were inputted into the licensed scoring software. Once the data had been entered, a numerical score was generated for symptoms, impact and activity. An overall score was also generated. The higher the score for each domain, the more affected an individual is. UK population data were available for comparison and published norms available [242].

4.3.2 Gastrointestinal Symptoms

Participants were initially asked an open question about their medical health and if they suffered from any medical problems or conditions. They were then specifically asked if they suffered from any gastrointestinal symptoms such as recurrent abdominal pain, diarrhoea or rectal bleeding.

If the participant had undergone any gastrointestinal investigations the type and result were recorded. This information was corroborated with medical records either in primary or secondary care where possible.

Children with CGD demonstrate poor growth, which maybe the presenting feature of their disease[8, 11] and may relate to gastrointestinal manifestations of disease such as colitis, or on going diarrhoea of another aetiology. Measurement of growth in children is used as a surrogate marker for health and height and weight may be plotted on per centile charts. In adults, BMI forms part of the standard assessment of nutritional status. BMI was categorised into the 6

categories published in the NICE guidelines [243]. The categories are shown in Table 4-1.

Table 4-1: BMI Category [243]

Category	BMI Range (kg/m^2)
Underweight	< 18.5
Healthy	18.5 – 24.9
Overweight	25-29.9
Obesity 1	30 – 34.9
Obesity 2	35 – 39.9
Obesity 3	> 40

All patients were asked to complete the Inflammatory Bowel Disease Disability Index. This is an assessment tool which evaluates quality of life in relation to bowel symptoms and provides an assessment of severity of symptoms [244]. A numerical score is generated from the answers given. A higher score indicates a greater impact of gastrointestinal symptoms on quality of life. Details about bowel habit were also recorded from this. Gastrointestinal symptoms are frequently not volunteered by patients in consultations. This questionnaire looked for 'hidden symptoms' that have potentially not been previously disclosed by individuals and assessed the severity of symptoms already known about.

4.3.3 Autoimmune Features

To evaluate the presence of symptoms related to SLE, such as photosensitive rashes, a questionnaire based on the American Rheumatology Association (ARA) clinical criteria for diagnosis of SLE [136] was used. The criteria were phrased as questions, which were asked by the researcher if the participant was recruited in person, or completed by the participant themselves if recruited by post.

Information about other autoimmune features, which were volunteered, was recorded and participants were specifically asked about features of Raynaud's phenomenon.

4.3.4 Fatigue

Initial findings indicated that XL-CGD carriers were suffering from what they perceived to be excessive fatigue. Formal assessment of fatigue was subsequently undertaken using the Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) and the vitality component of the SF-36 QoL questionnaire. If participants volunteered (unprompted) that they felt they suffered from excessive fatigue during the medical history taking, this was also recorded.

The MFSI-SF questionnaire comprises 30 statements. Participants rate each statement from 0 (not at all) to 4 (extremely). The questions are ordered so that positive and negative statements are intermingled. The 30 questions comprise five domains; general, physical, mental, emotional and vigour. Each domain has 6 questions attributed to it.

Adding the scores from the general, physical, emotional and mental domains, and subtracting the score for vigour calculated a total score. Each individual domain may receive a maximum score of 24, making the highest possible score 96.

The MFSI-SF was derived from the Multidimensional Fatigue Symptom Inventory (MFSI). The MFSI was designed to assess five domains of fatigue; global, somatic, cognitive, affective and behavioural. The MFSI has been found to have good reliability in test and retest and internal consistency [245]. It is not disease specific and can therefore be used in individuals with a disease and in healthy individuals. Therefore, it is appropriate for use both in the XL-CGD carrier population and in the control group. The design is such that questions relating to a specific domain of fatigue are not consecutive so as to ensure that each question is considered on its merits in order to attempt to gain maximal accuracy. It does not rely on medical symptoms allowing an assessment of fatigue where the cause of fatigue is uncertain.

One disadvantage of the MFSI is its relative length, with 83 questions, which may adversely affect how well it is completed, particularly in individuals suffering from cognitive fatigue. In order to rectify this, an abbreviated version was created, the Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-

SF). This 30-point questionnaire covers the same domains as the longer version, but takes approximately 5 minutes to complete, providing an advantage over longer, potentially more daunting questionnaires.

The MFSI-SF does not assume the presence of fatigue and has been validated for use in a study of 304 cancer patients when compared with both another fatigue scale and specific fatigue components of the widely validated SF-36 Quality of life questionnaire [246]. Further validations have been made [247].

The choice of the MFSI-SF to assess fatigue was based upon several factors. A multi-dimensional tool was preferable as the cause of any fatigue in the XL-CGD carrier group is uncertain. It is useful to have a detailed and broad assessment of the different aspects of fatigue for to conclusions to be drawn about which specific aspects are a concern. The MFSI-SF satisfies this criterion by assessing 5 domains. As this study required participants to complete a number of questionnaires it was important to keep the questionnaires brief where possible so that they were completed as accurately as possible, hence why the MFSI-SF was chosen over the full version.

Where questionnaires were not returned participants were contacted in writing to in order to complete data collection.

4.4 Medical Records

Consent was obtained to access medical records. Upon enrolment into the study, participants were asked at which hospitals they had been seen as a patient. It was not possible to obtain medical records from all hospitals, but consultants caring for participants were contacted and clinic letters requested.

Data were abstracted from the hospital records by a single researcher using a standardised proforma. Detailed information was obtained about hospital admissions and referrals. Information was recorded about reason for admission, treatments including surgery and outcomes. The data abstraction proforma used was based upon the proforma used for collecting data about the patients enrolled in the original registry[11].

4.4.1 Primary Care

The General Practitioner (GP) of each participant was contacted by post. They were informed of enrolment of their patient into the study. They were also asked to provide a patient record summary and return it by post or fax. GP records were used to confirm information recorded from patients and confirm regular medications.

Consent was also obtained from each participant to inform their GP if significant medical problems arose during the course of the study requiring referral or treatment.

4.5 Psychological Health

Assessment of psychological health was undertaken, in the same manner, in both the XL-CGD carriers and the control group of XL-MD carriers. The study design for the psychological component was discussed with the psychologists attached to the paediatric immunology team at the GNCH, Newcastle upon Tyne and GOS hospital, London.

In order to assess psychological health, participants completed questionnaires about self-esteem, quality of life, presence of anxiety and depression symptoms. Participants who were mothers also completed the Pediatric Inventory for Parents (PIP).

IQ was assessed in the XL-CGD carriers using the Wechsler Adult Intelligent Scale (WAIS).

4.5.1 Symptoms of Anxiety and Depression

Assessment of symptoms of anxiety and depression was made using the Hospital Anxiety and Depression Scale (HADS). The HADS is a questionnaire comprising 14 questions; 7 looking for anxiety symptoms and 7 for depression. The individual answered each question using a four-point scale (0-3). There is a maximum score of 21 for anxiety and 21 for depression with higher scores showing more severe symptoms. The HADS is easy to use and can be completed quickly. The original design was for use in individuals with a chronic health condition, therefore in order for the scale to remain generic and universally

applicable, physical symptoms were excluded from the design. It may be used as a screening tool, or as an assessment of severity.

The score generated after completion of the questionnaire, for both anxiety and depression may be categorised and compared to population data [248]. Cut-off values were available from population data and results were evaluated categorically and as a raw score. Categories are shown in Table 4-2 and are the same for anxiety and depression.

Table 4-2: HADS Categories [248]

Category	Score
Normal	0-7
Mild	8-10
Moderate	11-14
Severe	15-21

The HADS alternates between positively and negatively phrased questions. The strength of this is that it ensures as far as is possible, that an individual considers each question on its own merits, rather than simply choosing the same option for each question. By asking about specific symptoms and their frequency rather than using a one dimensional 'are you depressed' question, respondents were more likely to be honest and a more accurate representation of the presence of anxiety and depression should be achieved. Furthermore, many people may not consider themselves depressed or anxious even with symptoms. The HADS provides an objective assessment of the subjective responses. The use of multiple questions means an average response can be obtained.

The HADS was developed in 1983 as a screening tool for anxiety and depression. It was designed to be a cost effective tool for use in patients without known psychiatric disorders. The questions were formulated to exclude anxiety and depressive symptoms related to somatic disorders such as fatigue or headache. It does not attempt to explore the cause of the symptoms but merely screens for their presence. As the tool is a subjective assessment, it may be affected by how the individual perceives they should feel and relies on honesty upon completion.

In 1996 Herrmann [249] reviewed 200 papers studying 35,000 individuals using HADS. His conclusions were that the scale is acceptable to patients. There is good internal consistency and validity and reliability are sound.

Bjelland et al [250]updated this review in 2002. They included 71 studies in their review that met the required criteria. They confirmed Herrman's[249] findings that the HADS was a reliable and screening tool for anxiety and depression. They were able to conclude that the HADS was valid not only in medical patients but also when used in the general population.

Population data for the UK were available for comparison [248] along with published cut off values. Data from parents of children with CF were also used for comparison [226, 251]. Data was also compared to published work about patients with SLE [140, 229].

The acceptability, simplicity and brevity of the HADS along with the validity and reliability were the reasons for its choice in this study to assess the presence of anxiety and depression in XL-CGD carriers. Whilst it does not provide a cause for any symptoms identified, it provides a quantification of the distress. This was then correlated with the other assessments of psychological health to explore which factors impact the most on psychological health.

A copy of the HADs questionnaire can be found in the appendices.

4.5.2 Self-Esteem

An association between anxiety and low self-esteem has been described[252]. Self-esteem is an important part of psychological health. Self-esteem was assessed using the Rosenberg Self-Esteem Scale. This is a 10-point questionnaire and respondent rates their level of agreement with each statement from strongly agrees to strongly disagrees using a 4-point scale. The questions relate to how the respondent feels about their self worth. The normal range has been defined as a score of 15-25 [252, 253]. It has been validated in both adults [252, 253] and school aged children and adolescents [254].

Preserved self-esteem is an important part of psychological health assessment and may be affected by feelings of depression or guilt amongst other things.

A copy of the Rosenberg Self-Esteem Scale can be found in the appendices.

4.5.3 Pediatric Inventory for Parents

The majority of the carriers recruited to the study were mothers of children with CGD. Being a parent of a child with a chronic illness, who requires frequent trips to hospital or medical intervention, may contribute to psychological health. Assessment of the stresses of being a carer may be difficult to isolate from other factors that may impact on psychological health. In order to account for this, recruited mothers were asked to complete the Pediatric Inventory for Parents (PIP).

The PIP is a 42-point questionnaire designed to assess the impact of having a child with a chronic illness. It assesses both the severity and frequency of problems associated with being the parent of a child with a chronic condition. Each statement asks the parent completing the form to rate 'how much of a problem' and 'how often it is a problem' on a 5 point scale where 1 is 'no problem at all' and 5 is 'a significant problem'. The questionnaire includes questions about difficulties specific to medical care such as giving medications and is therefore, more specific than a generic parenting stress survey. The 42 statements cover four domains; medical care, communication with the health care team and the child, role functioning and emotional functions. These domains reflect the multidimensional nature of parental stress and that stresses may be additive. A numerical score is calculated for frequency and severity. These are added together to give an overall total. The maximum possible score is 420 and frequency and severity are equally weighted. The higher the score obtained, the more severe the stress experienced.

The PIP was originally designed for use in parents of oncology cases [255], but has been used in other chronic illnesses [205]. The PIP assesses the degree of stress associated with having a child with a chronic illness and can then be correlated with results from the HAD and other questionnaires assessing psychological distress. Data were compared with published work from parents of children with an oncological diagnosis [255].

A copy of the PIP can be seen in the appendices.

4.6 Quality of Life

Quality of life was assessed using the Short Form 36 version 2 (SF-36V2). If the index case had undergone HSCT, the SF-36 was delayed until a year had passed since the transplant. As outlined in chapter 2, HSCT is an intense period for family and patient alike and the stresses associated may be evident for a prolonged period after the transplant is completed [204]. It was, therefore, felt to be appropriate to delay the assessment of QoL until a period of time had passed in order to get a truer reflection of QoL in XL-CGD carriers. As this study was limited to 3 years, there was also a practical component to the decision. Phipps et al [256] suggest that the anxiety and distress associated with HSCT for parents is transient and lasts for up to six months. Therefore, on balance it was felt delaying QoL assessment for 1 year would be appropriate.

The SF-36V2 is a standardised quality of life assessment tool, which comprises 36 questions. Component scores are generated for 8 separate domains: physical functioning (PF), physical role (PR), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), emotional role (RE) and mental health (MH). A score is also generated for mental component score (MCS) and physical component score (PCS). Each domain receives a numerical score with a minimum of 0 and a maximum of 100. The higher the score the better the function. It was developed in 1993 to assess quality of life in a manner not specific to age, disease or treatment group [222].

The SF36 was chosen, as it is a generic tool, which may be applied to healthy individuals and across all age groups. It provides a subjective assessment of general health and focuses on physical, social and emotional functioning. The SF36 is the most commonly used QoL tool in clinical research. Additionally, the SF-36 may be self-administered, allowing it to remain valid even when posted out to participants.

Once the questionnaire was completed the responses were inputted into the licensed scoring software. Each domain results in a total score and there are normal values which are age and sex matched available within the software to provide comparison for the values. However, one disadvantage is that the

software uses US norms as the comparison data. As all the participants in this study are from the UK, this may not be the most appropriate comparison. However, there are published data on UK norms for an additional comparison to be made[257]. Data are also available from published works about different groups of patients, including carers of patients with brain tumours[211] and SLE patients [229], to which comparisons were made. Data about adult CGD patients[258] were also compared to the XL-CGD carrier data.

A copy of the SF36v2 can be found in the appendices.

4.7 IQ Assessment

As outlined in chapter 2, there is limited research to suggest that boys with CGD may have reduced IQ compared to the population[230] and the factors behind this are uncertain, although it has been hypothesised that it is related to their disease state. Therefore, it can be hypothesised that a similar reduction may be seen in XL-CGD carriers and therefore, IQ assessment was undertaken in the XL-CGD carriers.

IQ was assessed using a standardised assessment tool, the WAIS-IV. It involves 10 subsections which when scored provide an assessment of overall IQ and 4 different domains. The different domains are Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI) and Processing Speed Index (PSI). Together they provide an overall assessment of intellectual functioning. The test is validated for individuals aged between 16 years and 90 years 11 months.

IQ was assessed by an individual researcher who performed all but one of the IQ assessments. The other IQ assessment was performed by a clinical psychologist in the presence of the researcher. The researcher was trained in the use of the WAIS by an educational psychologist.

4.8 Blood Investigations

4.8.1 Neutrophil Oxidative Burst

Blood was taken at enrolment into the study from each carrier and a neutrophil oxidative burst performed. Neutrophil oxidative burst was performed at the recruiting centre due to the time-dependant nature of the investigation. DHR was used at all sites.

All sites followed their standard operating procedure. The standard operating procedure (SOP) for the GNCH, Newcastle upon Tyne is shown in the appendices.

Results are presented as per cent normal functioning neutrophils.

4.8.2 Autoantibodies

All serum samples for autoantibodies were sent to the Immunology Laboratory at the Royal Victoria Infirmary, Newcastle upon Tyne. A routine panel of autoantibodies was sent from each XL-CGD carrier at time of enrolment. Previous results were obtained from medical records if they had been tested.

The panel included:

- ANA pattern
- Anti Gastric parietal cell antibody
- Anti nuclear antibody
- Anti mitochondrial antibody
- Anti smooth muscle antibody

All samples were tested in the same laboratory and the local values were used to determine if they were normal or abnormal. The SOP is shown in the appendices.

A sample of serum has been stored in a freezer at -80 degrees. This sample was stored for use in future work on XL-CGD carriers.

4.8.3 Cytokines

Cytokine analysis was undertaken by a third year medical student and I received the raw data to analyse.

Serum from 52 of the recruited XL-CGD carriers was analysed alongside serum from 15 healthy controls and 20 Sjogren patients, 10 of who reported high fatigue and 10 who reported low fatigue. Using a cytometric bead array immunoassay, levels of IL1α, IL5, IL8, IL10, IL17, IFNα and IFNγ were assessed by a Biosciences LSRFortessa™ cell analyser. Data were compared for significant differences using STATA.

4.9 Ethical Approval

A favourable ethical opinion was obtained for the study from Newcastle and North Tyneside REC 1. All amendments to the design of the study were prospectively approved by the committee. Local research and development approval was obtained from all hospitals involved.

4.10 Statistical Analysis

All data were analysed using STATA. All data underwent testing for normality of distribution using skewness and kurtosis assessment and the null hypothesis that the data are normally distributed was rejected if the combined p-value was <0.05.

Where data were normally distributed, mean values and standard deviations are presented. Where data were not normally distributed, median and interquartile ranges (IQR) are presented. Where data were normally distributed, parametric tests were used. Where data were skewed or not normally distributed, non-parametric tests were used.

The QoL questionnaires were inputted into the licensed software, which generated total scores for each domain. Each domain could score a maximum of 100. Scores when the compared by the software with population data to produce norm based scores (NBS).

Spearman's correlation was used to assess factors associated with anxiety and depression scores. Data are presented with rho correlation coefficient and p-values.

Where published data were available for comparison, means were compared using a one-sample t-test. For non-parametric data, the Mann-Whitney test was used. The per cent or proportions affected were compared by a one-sample test of proportion. P-values are presented, a value of ≤ 0.05 was considered significant.

In order to assess the impact of the age of a child with chronic illness, the index cases were categorised into age ranges to fit with developmental stage. The age categories were then used in comparisons for analysis of the psychological assessments. A one-way ANOVA test was used to assess differences in PIP scores across the 5 different age categories. A one-way ANOVA test was used to make comparisons across more than one group.

Chapter 5: Clinical Results

This chapter will present the results of the tests for normality of the data, information about recruitment and characteristics of the participants and the clinical manifestation of disease results.

Data were tested for normality by skewness and kurtosis. A combined p value was produced and the hypothesis that data were normally distributed rejected if $p<0.05$. These results are shown in Table 5-1 and Table 5-2.

Table 5-1: Skewness, kurtosis and test for normality of data

	Skewness	**Kurtosis**	**p-value**
Participant Age	0.45	4.38	0.03
Index Case Age	2.27	6.98	<0.0001
HSCT Age	1.01	2.93	0.07
Neutrophil Oxidative Burst Values			
Enrolment NOB	0.011	2.13	0.17
Historical NOB	0.35	2.28	0.53
St George's Respiratory Questionnaire			
Symptom Score	1.14	3.17	0.0063
Activity Score	1.38	4.13	0.0008
Impact Score	1.65	4.45	0.0001
Total Score	1.41	3.97	0.0008
Gastrointestinal Assessments			
IBD Disability Index	0.39	2.89	0.40
BMI	0.53	2.87	0.16

The data for participant age, index case age and SGRQ were not normally distributed. NOB values, IBD disability index and BMI were normally distributed

Table 5-2: Skewness, kurtosis and test for normality for psychological assessments

	Skewness	Kurtosis	p-value
Psychology Assessments			
HAD-A	0.39	2.88	0.39
HAD-D	0.64	2.69	0.11
Rosenberg	0.005	1.73	0.0003
PIP-Total	-0.13	3.5	0.49
PIP-F	-0.98	3.76	0.35
PIP-T	-0.003	2.60	0.98
Fatigue			
MF Total	0.27	2.03	0.043
MF Gen	0.19	1.69	0.0001
MF Physical	0.78	2.48	0.044
MF Emotional	0.47	2.14	0.055
MF Mental	0.76	3.38	0.044
MF Vigor	0.45	3.29	0.22
Quality of Life			
PF	-1.56	4.23	0.0002
RP	-0.84	2.30	0.021
BP	-0.43	2.09	0.040
GH	- 0.10	1.78	0.001
VT	0.17	2.10	0.084
SF	-0.60	2.29	0.067
RE	-0.68	2.38	0.057
MH	-0.35	2.89	0.46
PCS	-0.74	2.46	0.05
MCS	-0.69	3.64	0.04

HAD-A, HAD-D, PIP-total and PIP-F were normally distributed whilst Rosenberg and PIP-S were not normally distributed. MF Vigor was normally distributed but

all other fatigue domains were not normally distributed. In the QoL assessment, the PF, RP, BP and GH domains were not normally distributed but all other domains were normally distributed.

Table 5-3 shows that all of the cytokine data were skewed and not normally distributed.

Table 5-3: Skewness, kurtosis and test for normality for cytokine data

	Skewness	**Kurtosis**	**p-Value**
IL1α	4.99	26.49	<0.0001
IL5	4.14	19.79	<0.0001
IL8	6.30	43.25	<0.0001
IL10	5.09	28.89	<0.0001
IL17	5.02	26.93	<0.0001
IFNα	4.91	27.22	<0.0001
IFNγ	1.71	9.21	<0.0001

5.1 Recruitment of XL-CGD Carriers

There were 94 XL-CGD families identified. 20 were excluded; 4 were not approached due to deceased index case, 11 were non-UK residents and 5 known to have a new mutation in the index case. All families were approached either in person at a clinic appointment or by post. One family declined to participate and 11 families did not respond. There were two participants who were originally included, but were excluded after they were found to have a normal neutrophil oxidative burst and are not counted in the numbers or presented in this thesis.

There were 81 XL-carriers recruited from 62 kindreds, who participated in the study. Of these, 19 were lost to follow up or did not return questionnaires despite reminders, 1 withdrew (due to death of index case). Complete data were available for 61 XL-carriers. Two XL-CGD carriers were deceased and therefore, only limited information was available.

A summary of the recruitment of XL-CGD carriers is shown in Figure 5-1 and Figure 5-2.

Figure 5-1: Identification and Recruitment of XL-CGD Carriers

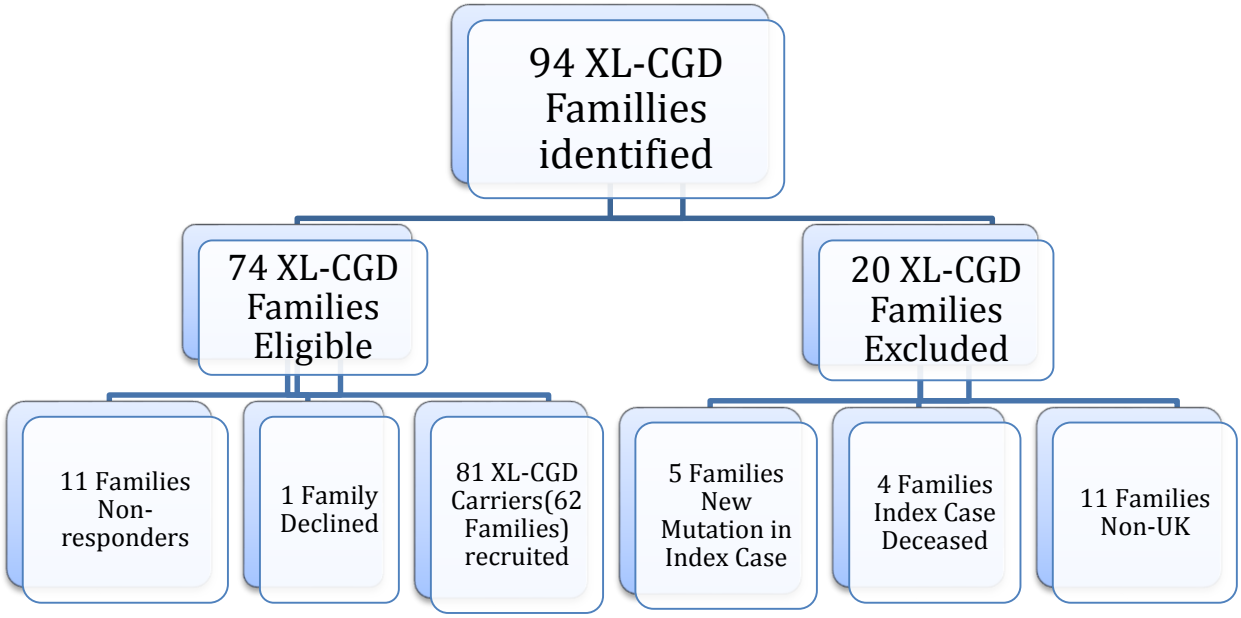
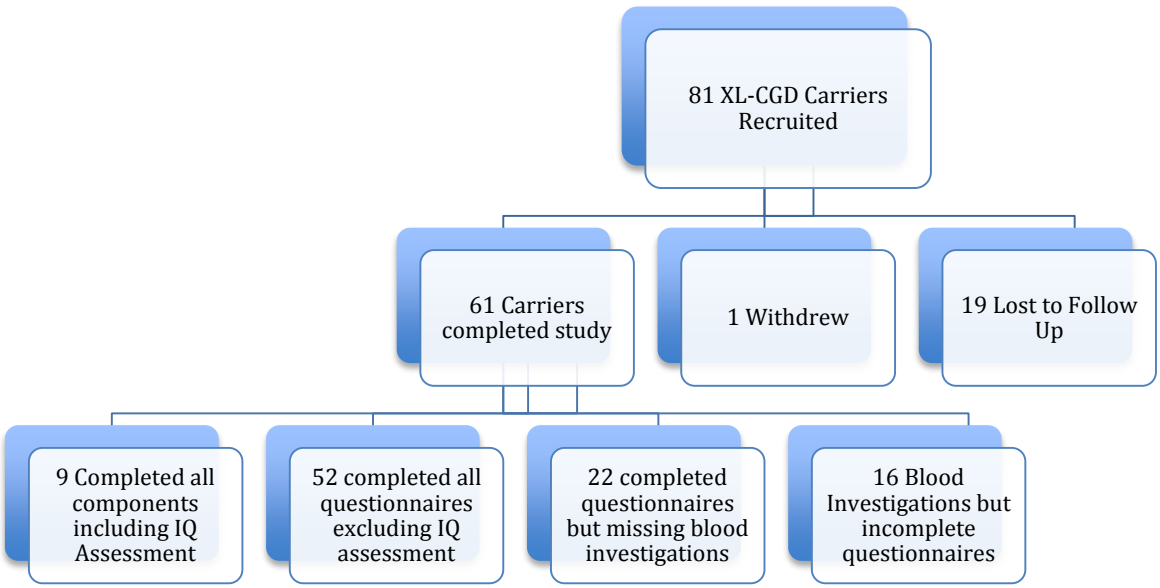


Figure 5-2: Recruited Participant Flow Chart



Demographics

The baseline demographics for enrolled participants are shown in Table 5-4. There were 81 XL-CGD carriers from 62 families, with a median age of 41 years. Ethnicity of XL-CGD carriers was recorded at enrolment. 96.2% (76) of the XL-CGD carriers were white British.

Table 5-4: Baseline Characteristics of recruited XL-CGD Carriers

	XL-CGD Carriers
Number of Carriers	81
Number of Kindreds	62
Median Age (Range)	41 (3-77)
Number Alive (%)	79 (97.5%)
Number Deceased (%)	2 (2.5%)
Median Age Index Case (Range)	12 (1-43)
Number of Children per index case (Mean)	1.7
Number of Affected Children (Mean)	0.84

The age of the index case was recorded at the point of enrolment of the participant into the study. Index case age was categorised based upon developmental stage. This breakdown is shown in Table 5-5, with the majority of index cases being aged between 7 and 18 years, at the time of enrolment.

Table 5-5: Age Classification of Index Case

Age of Index Case	XL-CGD Carrier Group Participants Number (%)
Infant (<2 years)	7 (8.9)
Young Child (2-6 years)	8 (10.1)
Middle Child (7-12 years)	20 (25.3)
Adolescent (13-18 years)	169(24.0)
Adult (>18 years)	17 (21.5)
Deceased	8 (10.1)
New Mutation	1 (1.2)

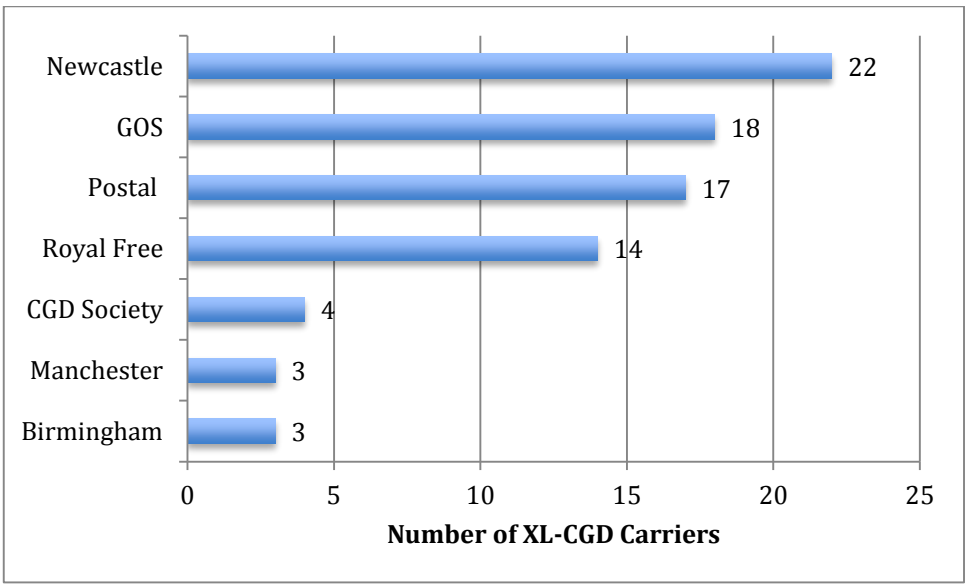
Recruitment Location

XL-CGD carriers were recruited in person from 5 centres; Newcastle upon Tyne, The Royal Free and GOS Hospitals London, Birmingham and Manchester and also

through the postal approach. The breakdown of sites is shown in Figure 5-3, with the majority being recruited from the three main centres; Newcastle upon Tyne, the Royal Free and Great Ormond Street.

In total, 81 GPs were written to for medical information about the recruited XL-CGD carriers. 55 replied with information. 22 did not reply and in 3 cases there was no GP recorded and so they could not be contacted. In 1 case the GP was incorrectly recorded.

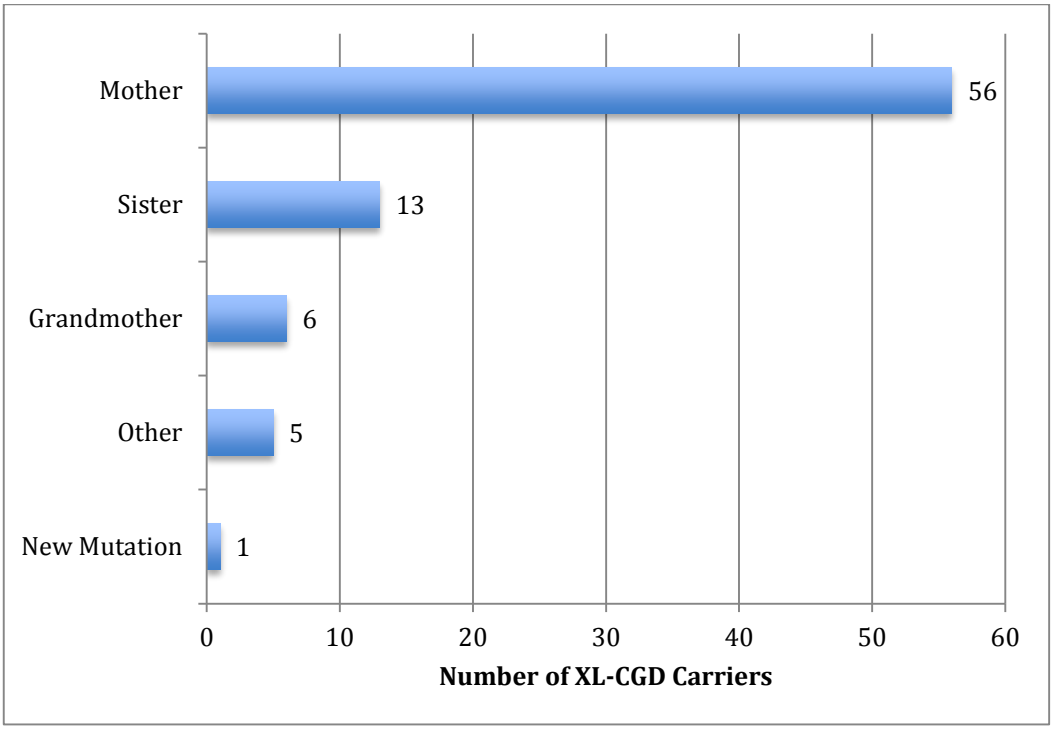
Figure 5-3: Recruitment Site of XL-CGD Carriers



Relationship to Index Case

Once an index case was identified, all known carriers within the family were invited to participate in the study. The relationship of the recruited XL-CGD carrier to the index case is shown in Figure 5-4. The majority of XL-CGD carriers recruited were mothers of the index case.

Figure 5-4: Relationship to Index Case of Recruited XL-CGD Carriers



Mutations

The exact mutation was known for 20 of the XL-CGD carriers. Due to the limited number the mutation was known in, no further analysis was undertaken about correlation of symptoms in XL-CGD carriers with the specific mutation.

Transplant

In 40 of the XL-CGD carriers, the index case had undergone HSCT. There was one index case that had undergone gene therapy and there was 1 XL-CGD carrier in which there was no index case as the XL-CGD carrier was the first identified in the kindred. The remainder were managed conservatively. The demographics of the HSCT and non-HSCT groups are shown in Table 5-6.

Table 5-6: Baseline Characteristics of HSCT vs. No HSCT in index Case Cohorts

	Index Case Underwent HSCT	**No HSCT**
Number of Carriers	40	40
Mean Age Carrier (years)	39.0	45.5
Mean Age Index Case (years)	11.0	16.5

Controls

The control group of carriers of XL Muscular Dystrophy (MD) were all recruited from the GNCH, Newcastle upon Tyne.

There were 58 families with XL MD (Duchenne or Becker) were identified at GNCH. Of these 22 were a new mutation in the index case (and so no carriers in the family) and 4 were non-UK and therefore, not eligible for recruitment to the control group. Additionally, 3 of the index cases were adopted or not living with biological parents and therefore, contacting carriers was not appropriate. 19 MD carriers were approached for recruitment. There were 7 MD carriers who agreed to participate and returned questionnaires, 4 MD carriers declined to participate. There were 8 MD carriers who agreed to participate but did not return completed questionnaires.

All eligible carriers were approached in person. The demographics and a comparison with the XL-CGD carrier cohort are shown in Table 5-7.

Table 5-7: Baseline Characteristic Comparison of XL-CGD Carriers vs. MD Carrier Control Group

	XL-CGD Carrier Group	Control Group
Number	81	7
Mean Age (Range)	42.5 (3-77)	39.6 (36-48)
Number Alive (%)	97.5	100
Number Deceased (%)	2.5	0
Median Age Index Case (Range)	12 (1-43)	9.3 (2-13)
Mean Number of Children per participant	1.7	1.7
Mean Number of Affected Children per participant	0.84	1.3
Ethnicity (% WB)	96.2	100
% Index Cases Ambulant	100	50

The relationship to the index case was recorded for the MD control group in the same manner as the XL-CGD carriers. This is shown in Table 5-8. All of the recruited controls were mothers of the MD patients.

Table 5-8: Relationship to Index Case in XL-CGD Carrier and MD Carrier Control Groups

	XL-CGD Carrier Cohort	Control (MD) Group
Mother	56	7
Grandmother	6	0
Sister	13	0
Other	5	0

As with the XL-CGD carrier cohort, the age of the index case was recorded and categorised for the control participants and this is shown in Table 5-9.

Table 5-9: Classification of Index Case Age in XL-CGD carrier and MD carrier control group

Age of Index Case	XL-CGD Carrier Group	MD-Carrier Control Group
Infant (<2 years)	7 (8.9)	0
Young Child (2-6 years)	8 (10.1)	1
Middle Child (7-12 years)	20 (25.3)	4
Adolescent (13-18 years)	169(24.0)	2
Adult (>18 years)	17 (21.5)	0
Deceased	8 (10.1)	0
No Index Case	1 (1.2)	0

5.2 Clinical Results

This section will present the clinical findings in XL-CGD carriers.

5.2.1 Information about Deceased Carriers

Two of the XL-CGD carriers were deceased at the time of enrolment into the study as consent for inclusion was gained from their next of kin. The clinical information obtained about these XL-CGD carriers is shown in Table 5-10.

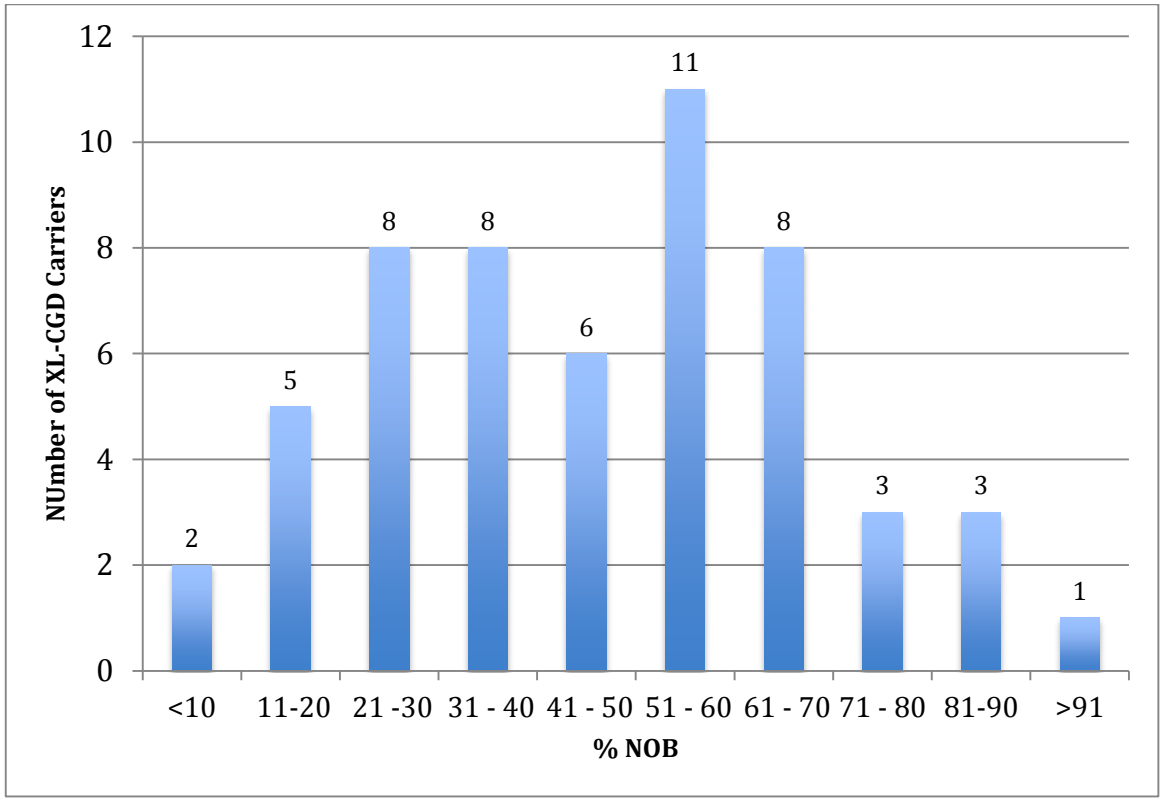
Table 5-10: Clinical Information about deceased XL-CGD Carriers

Carrier ID	Information Available
24	Died in late 30s Recorded cause of death 'lupus'
28	Died age 30 years Recorded cause of death: Ischaemic Heart Disease at post mortem. Suffered from diarrhoea and abdominal pain. 'Mild Lupus' with joint pain and photosensitivity. Asthma. Recurrent skin abscesses particularly in groin.

5.2.2 Neutrophil Oxidative Burst

Neutrophil Oxidative Burst results were available for 54 of the XL-CGD carriers. Results are given as percentage of normal function. The mean value was 47.01 % with a standard deviation of 21.67. The absolute range was 7 – 94%. The majority of XL-CGD carriers fell in the range of 21 – 60% functioning, and this distribution is shown in Figure 5-5.

Figure 5-5: Distribution of NOB in XL-CGD Carriers



Historical NOB results were available for 26 of the recruited XL-CGD carriers. Historical and enrolment NOB means were compared using a paired t-test and the results are shown in Table 5-11. Historical NOB values were significantly higher than those performed at enrolment.

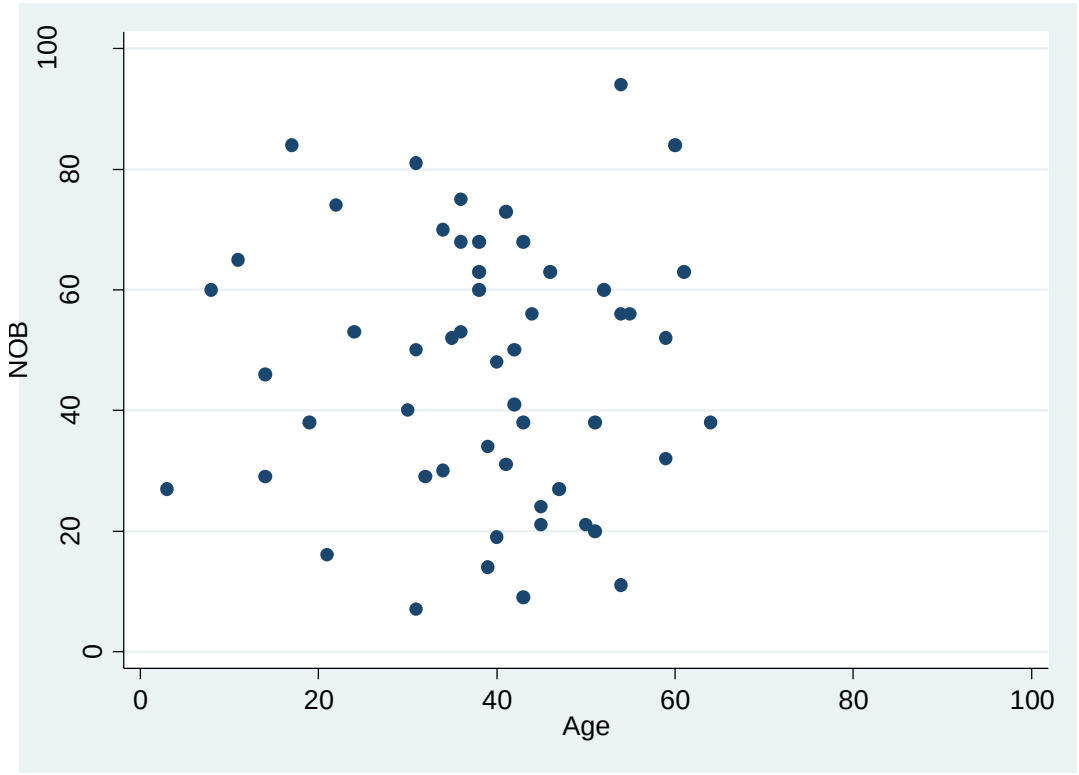
Table 5-11: Historical and Enrolment NOB in recruited XL-CGD Carriers

	Enrolment NOB	**Historical NOB**	**Comparison**
Number of Carriers	54	26	P = 0.022
Mean	47.0	51.7	
SD	21.7	18.6	
Absolute Range	7-94	24-92	

Age against NOB

The age of recruited participants was compared to the percent of normal NOB and a scatter graph of these variables is shown in Figure 5-6. There was no significant correlation ($p = 0.73$) (spearman's correlation rho = -0.048).

Figure 5-6: Age (years) against Per Cent Normal NOB in Recruited XL-CGD Carriers



5.3 Infection

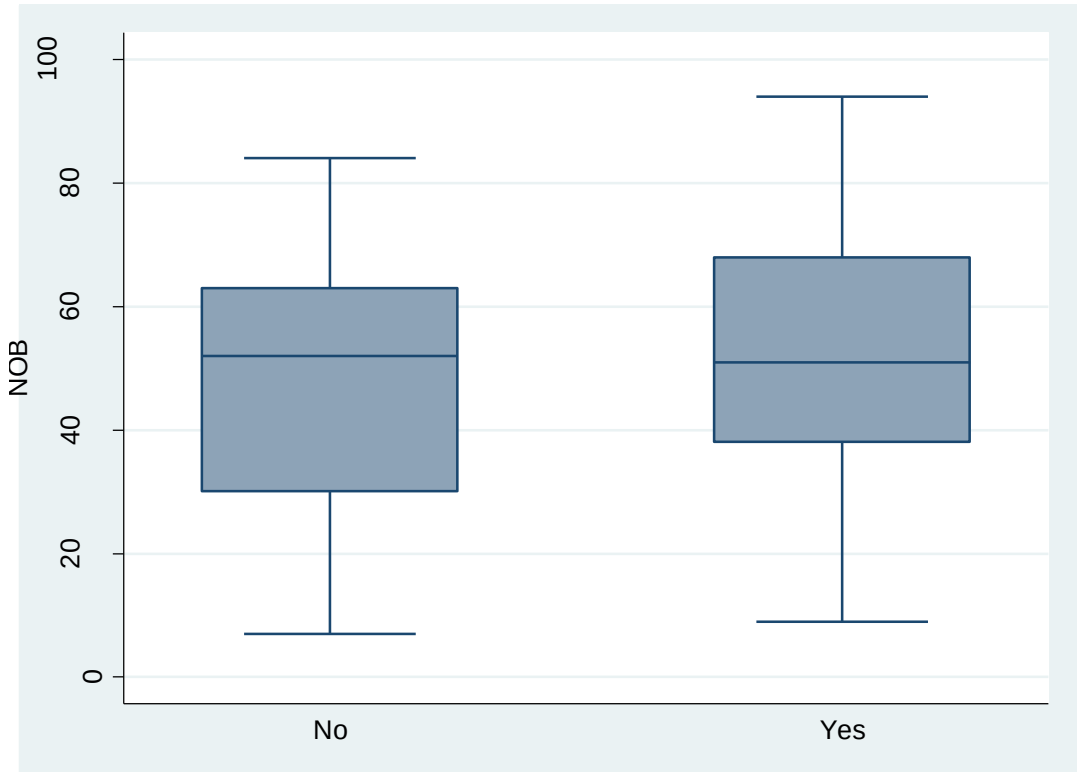
Infection was considered significant if it was potentially life threatening e.g. pneumonia or meningitis or if it were recurrent. The infectious complications in XL-CGD carriers are shown in Table 5-12.

Table 5-12: Significant Infections in XL-CGD Carriers

	Number of XL-CGD Carriers (%)
Any Significant Infection	19 (23.5)
Fungal	1 (1.2)
Pneumonia	4 (4.9)
Meningitis	2 (2.5)
Recurrent Abscesses	14 (17.3)
Sinus	2 (2.5)
Lymphadenitis	4 (4.9)
Recurrent UTI	6 (7.4)

The neutrophil oxidative burst values in those affected and unaffected by significant infection were compared (Figure 5-7). There was no significant difference when the groups were compared by a two sample t-test (p=0.6).

Figure 5-7: NOB Values in those affected and unaffected by recurrent or significant infection



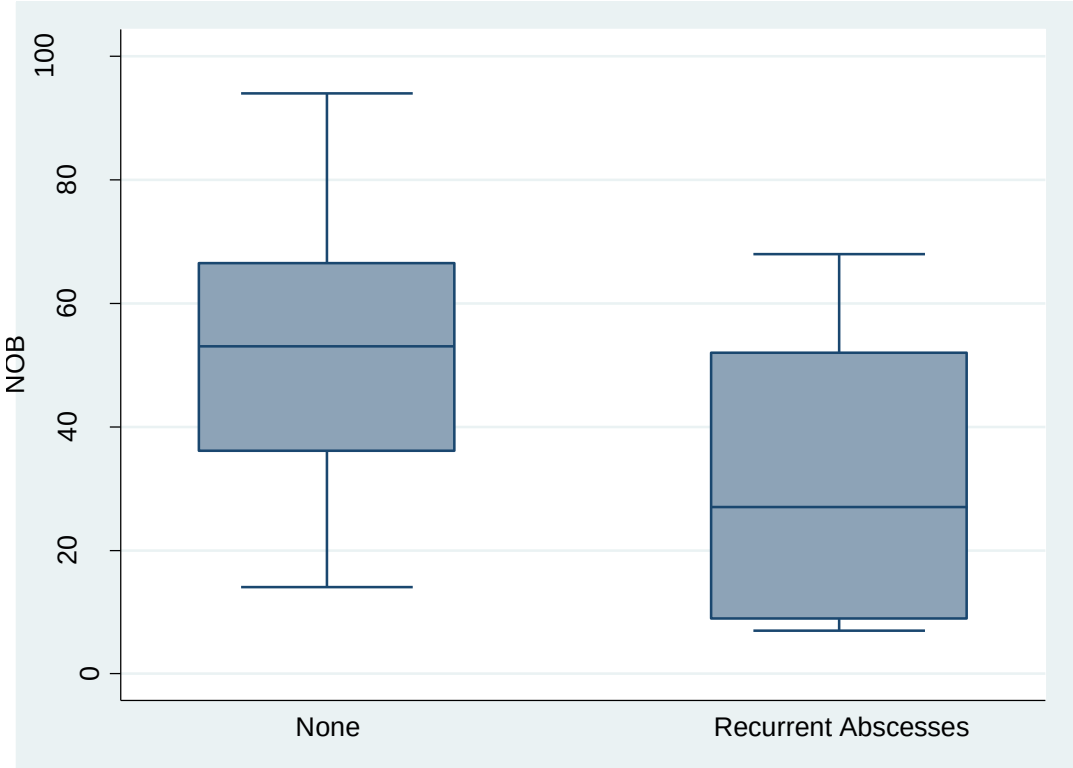
XL-CGD carriers were specifically asked about mycobacterial infection. Information was also volunteered by 3 XL-CGD carriers about reaction to BCG and this information is shown in Table 5-13.

Table 5-13: Mycobacterial Complications in XL-CGD Carriers

	Number Of XL-CGD Carriers Affected (%)
Definite TB Infection	0 (0)
Possible TB Infection	1 (1)
Discharging BCG Site	3 (4)

Recurrent abscesses were considered separately and the neutrophil oxidative burst values were compared in those affected and unaffected by a two-sample t-test and a significant difference was found (p=0.0088). The NOB was significantly lower in those affected by recurrent abscesses. These results are shown in Figure 5-8.

Figure 5-8: Neutrophil Oxidative Burst Results in XL-CGD carriers with and without recurrent abscesses



5.4 Inflammatory Manifestations

5.4.1 Gastrointestinal Symptoms

Growth

Height and weight measurements were available for 69 of the recruited XL-CGD Carriers. These measurements were used to calculate BMI. Classification of BMI was taken from the NICE assessment of BMI [259] and the results are shown in Table 5-14. A comparison of the proportion of XL-CGD categories in each category with the proportion of UK females in each category is shown in Table 5-14. There were significantly more XL-CGD carriers classified as underweight and significantly fewer in the overweight category. In all other categories there was no significant difference between the XL-CGD carriers and UK female population data.

Table 5-14: BMI of XL-CGD Carriers compared with the UK Population

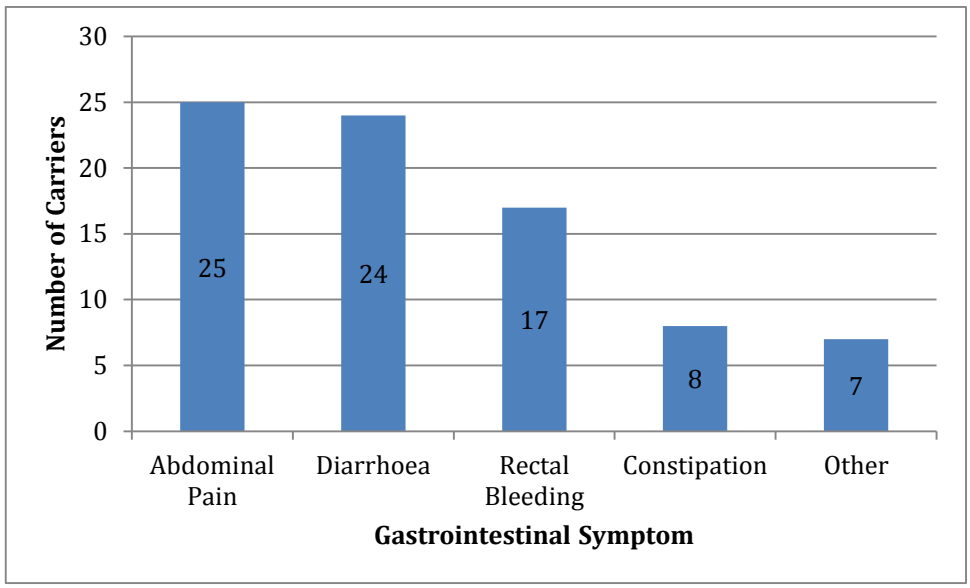
	BMI Range (kg/m^2)	**Number of Carriers (%)**	**Female Population Data (2008-2010) (%)[259]**	**p-value**
Underweight	<18.5	5 (7.3)	1.2	0.0002
Healthy	18.5-24.9	29 (42.0)	36.8	0.18
Overweight	25-29.9	16 (23.2)	34.4	0.025
Obesity 1	30-34.9	10 (14.5)	24.0	0.5
Obesity 2	35-39.9	7 (10.1)		
Obesity 3	>40	2 (2.9)	3.5	0.39
Missing	n/a	13 (16.3)	n/a	

Gastrointestinal Symptoms

All recruited XL-CGD carriers were asked about the presence of gastrointestinal (GI) symptoms. Gastrointestinal symptoms were present in 40 (53%) of the XL-CGD carriers.

The type of gastrointestinal symptom suffered and the number of XL-CGD carriers affected is shown in Figure 5-9 with abdominal pain and diarrhoea the most frequently occurring.

Figure 5-9: Gastrointestinal Symptom Type and Frequency in XL-CGD Carriers



A comparison with the published data about GI symptom frequency in CGD patients is shown in Table 5-15 along with a one-sample test of proportion. Rectal bleeding and constipation were significantly more common in the XL-CGD carriers whilst abdominal pain was significantly less common. Diarrhoea occurred in similar proportions.

Table 5-15: A Comparison of frequency of gastrointestinal symptoms in XL-CGD carriers compared to published data about CGD patients

	XL-CGD Carriers	CGD Patients [37]	p-value
Number in Study	81	140	
Abdominal Pain	34%	46 %	0.018
Diarrhoea	33%	33 %	0.51
Rectal Bleeding	23%	6 %	<0.0001
Constipation	11%	4 %	0.001

Gastrointestinal Investigations

GI investigations undertaken in XL-CGD carriers were recorded. The distribution of the type of investigation is shown in Table 5-16 and the findings are shown in Table 5-17. The most common investigations were endoscopy and colonoscopy.

Table 5-16: Gastrointestinal Investigations in XL-CGD Carriers

	Number of XL-CGD Carriers
Any Investigation	15
Referred for Investigation	2
OGD	4
Colonoscopy	4
CT	1
USS	3
Other	2

Table 5-17: Results of Gastrointestinal Investigations and Results in XL-CGD carriers

Carrier	Investigation	Investigation Finding
3	Colonoscopy	Colitis consistent with CGD colitis
	Endoscopy	Hiatus hernia
4	Colonoscopy	Minor, non-specific inflammation (reported as not significant)
11	Labelled white cell Small Bowel Follow Through Endoscopy USS	Normal Normal Hiatus hernia and gastritis Normal
13	Gastroscopy	Normal duodenal biopsy. Telangiectasia. Normal GI tract
20	USS	Normal
21	Colonoscopy	Crohn's disease
34	Body scan	Diverticular Disease
36	Colonoscopy	No significant abnormality
39	Colonoscopy	Crohn's disease Patchy inflammatory abscesses with cryptitis and crypt abscesses. No convincing granulomas. Consistent with mild/moderate chronic inflammation
	Sigmoidoscopy	Mild non-specific inflammation Indeterminate colitis
	Colonoscopy and endoscopy	Patchy inflammatory changes. Acute inflammation with cryptitis and crypt abscesses. Mild chronic inflammation in left colon. No convincing granulomas. The appearances are of patchy mild and moderate active chronic inflammation, in keeping with clinical history of Crohn's disease.
42	Sigmoidoscopy	Normal
53	Endoscopy	Oesophagitis (Early Barrets)

Episodes of gastrointestinal surgery were recorded where the information was available and the findings are shown in Table 5-18.

Table 5-18: Surgical Interventions in XL-CGD carriers

Surgery Type	Number XL-CGD Carriers (%)
Colectomy	1 (1.2)
Appendicectomy	4 (5)

The IBD Disability Index was completed by 61 XL-CGD carriers. The higher the score on the IBD Disability scale, the greater the effect the symptoms have upon quality of life. The mean score was 28.7 (+/-10.94) in the XL-CGD carrier cohort.

Scores in the symptomatic group were higher than those not suffering from any GI symptoms ($p<0.01$), which is expected.

Gastrointestinal Diagnosis

Not all XL-CGD carriers who suffered from GI symptoms had a formal diagnosis. In those where a diagnosis was available it was documented. These findings are shown in Table 5-19. The majority of XL-CGD carriers did not have a gastrointestinal diagnosis. Of those that did, irritable bowel syndrome was the most common diagnosis, affecting nearly 10% of the XL-CGD carrier cohort.

Table 5-19: Gastrointestinal Diagnoses in XL-CGD carriers

Diagnosis	**Number (%)**
Inflammatory Bowel Disease	2 (2.5)
CGD Colitis	1 (1.2)
Irritable Bowel Syndrome	8 (9.8)
Other	2 (2.5)
None	66 (81)
Referred for Investigation	2 (2.5)

Correlation with Neutrophil Oxidative Burst

The neutrophil oxidative burst value in the affected and unaffected groups were compared using a paired t-test. The results are shown in Table 5-20. The NOB was significantly lower in those suffering from diarrhoea and also in those suffering from abdominal pain. This was not found in those suffering from rectal bleeding. The significance was not found when GI symptoms were considered overall, although it was approaching statistical significance.

Table 5-20: Neutrophil Oxidative Burst Value in Symptomatic and Asymptomatic XL-CGD carriers

Symptom	Average NOB in Affected Group	Average NOB in Unaffected Group	Paired T-test P-value
Any GI Symptom	43.6	52.3	0.08
Abdominal Pain	39.8	51.8	0.05
Diarrhoea	36.5	53.5	0.009
Rectal Bleeding	43.5	49.6	0.16

The proportion of XL-CGD carriers with any positive autoantibody tested by immunofluorescence was also compared between the affected and unaffected groups and these results are shown in Table 5-21. The proportion of individuals with positive autoantibodies was not significantly different between the affected and unaffected groups.

Table 5-21: Autoantibodies and GI Symptoms in XL-CGD Carriers

	% Any Autoantibody Positive	PR Test of Proportion
GI Symptoms	22	p = 0.76
No GI Symptoms	27	

Factors contributing to the development of gastrointestinal symptoms were considered and the correlation is shown in Table 5-22. Age, the presence of an index case with colitis, anxiety and depression scores, fatigue and joint symptoms were all significantly correlated with gastrointestinal symptoms.

Table 5-22: Correlation of Gastrointestinal Symptoms with Potential Contributing Factors

	Correlation Coefficient	p – value
Age	0.37	0.0010
Index Colitis	0.46	0.032
Smoking	0.18	0.12
Anxiety (HAD-A)	0.31	0.015
Depression (HAD-D)	0.28	0.028
Fatigue (MFTotal)	0.33	0.0098
NOB	-0.17	0.23
Autoantibodies (IF)	0.10	0.48
Joint Pains	0.99	<0.0001
SLE Criteria	-0.03	0.75
Total PIP	0.16	0.34
IBD Disability Score	0.53	<0.0001
Ulcers	0.18	0.12

Regression: GI Symptoms

A logistic regression analysis was undertaken in relation to the presence of any gastrointestinal symptoms and the results from this are shown in Table 5-23. The presence of colitis in the index case, a higher anxiety and depression score and a higher number of SLE criteria met were all significantly associated with the presence of gastrointestinal symptoms.

Table 5-23: Regression Analysis of Gastrointestinal Symptoms in XL-CGD carriers

	Odds Ratio	95% CI	P-Value
Age	1.01	0.985, 1.037	0.41
Index Colitis	8.67	1.049, 71.56	0.045
Smoking	1.69	0.8607, 3.305	0.13
Anxiety (HAD-A)	1.19	1.026, 1.370	0.021
Depression (HAD-D)	1.18	1.012, 1.372	0.034
Fatigue (MFTotal)	1.03	1.006, 1.057	0.014
NOB	0.98	0.95, 1.01	0.23
Autoantibodies (IF)	1.60	0.442, 5.787	0.47
Joint Pains	1.84	0.708, 4.79	0.21
SLE Criteria	1.37	1.00, 1.88	0.05
Total PIP	1.01	0.994, 1.016	0.33

5.4.2 Respiratory Symptoms

Specific respiratory diagnoses or complaints in the XL-CGD carriers and their frequency are shown in Table 5-24. The most common diagnosis was asthma.

Table 5-24: Respiratory Diagnoses and Symptoms in XL-CGD Carriers

Diagnosis/Symptom	Number of Carriers (% Carriers)
Asthma	10
Bronchitis	1
Pleural Inflammation	1
Cough	3
Other	1

There were 59 XL-CGD carriers who completed the St George's Respiratory Questionnaire (SGRQ). The data were not normally distributed. The median scores and ranges for each domain are shown in Table 5-25 along with published normal values. It can be seen that the scores are similar for all domains.

Table 5-25: Comparison of Results of SGRQ in XL-CGD carriers with published data

	XL-CGD Carriers Median (IQR)	Published Norms Median (IQR)
Symptom Score	12.7 (0-39.8)	12 (9-15)
Activity Score	12.17 (0-35.2)	9 (7-12)
Impact Score	1.63 (0-13.6)	2 (1-3)
Total Score	7.32 (1.6-21.8)	6 (5-7)

5.4.3 Skin Disease

Photosensitivity was seen in 74% of the XL-CGD carriers. Other dermatological conditions were seen in 56 of the XL-CGD carriers and the frequencies of these diagnoses are shown in Table 5-26. The most commonly seen was eczema followed by acne of adult onset. A history of poor wound healing was volunteered in the medical history by 2 XL-CGD carriers.

Table 5-26: Skin Disease in XL-CGD carriers

	Number of Carriers (%)
Photosensitivity	57 (74%)
DLE/malar rash	30 (40%)
Eczema	11 (14%)
Psoriasis	3 (4%)
Adult acne	8 (10%)
Erythema multiforme	2 (3%)
Dermatitis	5 (7%)
Allergic/hives	5 (7%)
Rosacea	5 (7%)
Malignant	1 (1%)
Other	12 (16%)
Poor wound healing	2 (3%)

An association was sought between NOB value and the presence of the most prevalent skin disease. These results are shown in Table 5-27. It can be seen that

there were no significant differences between NOB value in the affected and unaffected groups.

Table 5-27: NOB Values in affected and unaffected groups of XL-CGD carriers with skin disease

	Mean NOB in affected group	Mean NOB in unaffected group	p-value
Photosensitivity	46.4	48.5	0.61
DLE/Malar Rash	46.2	47.5	0.58
Eczema	57.5	46.6	0.12

5.5 Autoimmune Features

SLE Symptoms

The frequency of the different SLE symptoms in XL-CGD carriers is shown in Table 5-28 alongside published data from a European cohort of SLE patients [129]. There were significantly higher rates of mouth ulcers, photosensitivity and Raynaud's phenomenon in the XL-CGD carrier cohort. The death rate was also significantly higher in the XL-CGD carrier cohort.

Table 5-28: SLE Symptoms in XL-CGD carrier cohort compared with published data of European SLE cohort

	Euro-Lupus Cohort [129]	XL-CGD Carrier Cohort	PR Test p-value
Geographical Area	**Europe**	**United Kingdom**	
Number of Patients (%)	1000	66	
Malar Rash	311 (31.1)	30 (39)	0.0681
Photosensitivity	229 (22.9)	57 (74)	<0.01
Oral Ulcers	125 (12.5)	57 (76)	<0.01
Raynaud's Phenomenon	163 (16.3)	27 (35)	<0.01
Arthritis	481 (48.1)	48 (62)	0.01
Serositis	160 (16.0)	3 (3.8)	0.003
Nephropathy	279 (27.9)	3 (3.8)	<0.001
Neurological Involvement	194 (19.4)	3 (3.8)	<0.005
Death	68 (6.8)	2 (3)	<0.0001

A diagnosis of SLE is made using the ARA criteria as previously described. 4 of the 11 criteria are required to make a diagnosis. Over half of the XL-CGD carriers met at least 3 of the ARA SLE criteria. The number of criteria met is shown in Table 5-29.

Table 5-29: Number of ARA SLE Criteria met in XL-CGD carriers

Number of SLE Criterion Met	Number of Carriers (%)
None	10 (12.3%)
1	9 (11.1%)
2	17 (20.9%)
3	24 (29.6%)
4 +	21 (25.9%)

Neuropsychiatric SLE symptoms were evaluated in addition to the other SLE criteria. The frequencies of these manifestations are shown in Table 5-30. The most commonly occurring were headache and anxiety.

Table 5-30: Neuropsychiatric SLE features in XL-CGD carriers

Symptom	Number of Carriers Affected (%)
Aseptic Meningitis	0
Cerebrovascular disease	2
Demyelinating syndrome	0
Headache (including migraine and benign intracranial hypertension)	15
Movement Disorder (chorea)	0
Myelopathy	0
Seizure Disorder	0
Acute confusional state	0
Anxiety Disorder (HADS>10)	26
Cognitive Dysfunction	0
Mood Disorder	5
Psychosis	0
Peripheral Nervous System	0

Joint Symptoms

Participants were asked about the presence of joint problems. Participants described episodic involvement with pain, swelling and associated fatigue lasting

up to 3 days at a time. There were 48 (59%) XL-CGD carriers who suffered from recurrent joint symptoms with 36 reporting more than one joint being affected. Participants were asked which joints were affected and these results are summarised in Table 5-31. Where multiple joints were affected in an individual participant they are all included. There were 36 XL-CGD carrier who reported symptoms in more than one joint.

Table 5-31: Affected Joints in XL-CGD carriers

	Affected (% of all XL-CGD carriers)
Any Joint	48 (59)
Multiple Joints	36 (44)
Fingers	13 (16)
Small Joints of Hand	14 (17)
Wrist	8 (10)
Shoulder	11 (14)
Neck	3 (4)
Jaw	1 (1.2)
Back	12 (15)
Hips	15 (19)
Knees	26 (32)
Ankles	6 (7)
Feet	4 (5)
Toes	2 (2)

The neutrophil oxidative burst values and autoantibody results were compared between the affected and unaffected groups. These results are shown in Table 5-32. There was no significant difference found between the groups. Those affected by joint symptoms were significantly older than those without joint symptoms. There was no difference in smoking status between the affected and unaffected groups.

Table 5-32: Neutrophil Oxidative Burst Values in XL-CGD carriers affected and unaffected with joint symptoms

	Joint Symptoms Present	Joint Symptoms Absent	p-value
Mean NOB (% normal)	47.26	49.94	0.468
Autoantibody Positive (%)	10	3	0.625
Median Age (years)	43.3	36.9	0.0345
Smoking (smokers/total)	7/44	2/28	0.535

Autoimmune features outside of SLE found in the XL-CGD carriers are shown in Table 5-33 with Raynaud's phenomenon being the most commonly described.

Table 5-33: Autoimmune Features in XL-CGD carriers

	Number XL-CGD Carriers (%)
Raynaud's Phenomenon	27 (35.5)
Alopecia	6 (7.7)
Sjogren	1 (1.2)
Lupus-Like Diagnosis	15 (18.5)

5.6 Other Medical Problems

Medical problems outside those already discussed were also recorded and are shown in Table 5-34.

Table 5-34: Other Medical Problems in XL-CGD Carriers

	Number of Carriers (%)
Hypothyroid	4 (5)
Diabetes Mellitus	1 Gestational 1 Type 2
Vitamin B12 Deficiency	2 (2.5)
Recurrent Blackouts/Faints	3 (3.6)

Two of the XL-CGD carriers reported suffering from seizures in the neonatal period relating to hypocalcaemia. It was not possible to find any further details about this.

Dental

Three of the XL-CGD carriers reported suffering from slightly unusual dental problems and these are shown in Table 5-35.

Table 5-35: Dental Complaints in XL-CGD Carriers

Carrier ID	Problem
60	All teeth came through at 18 months of age. The teeth were black and conical and required removal. Normal adult teeth
63	Extra teeth required removal
76	Lost all teeth at the age of 50 years. Her mother had lost all teeth at the age of 30 years

Gynaecological Problems

There were 9 XL-CGD carriers who reported suffering from at least one miscarriage. A comparison between those reporting miscarriage and those not is shown in Table 5-36.

Table 5-36: Recurrent Miscarriage and Associations in the XL-CGD Carrier Cohort

	Miscarriage	No Miscarriage	P value
Number	9	72	
Mean Age (years)	37.7	43.2	0.19
NOB (%)	48.4	46.9	0.56
Lupus-like Diagnosis (%)	11	24	0.17
ARA SLE Criteria (mean number)	2.2	2.7	0.78
Autoantibody (%+ve)	25	26	0.5

Gynaecological problems outside of miscarriage were also reported and these results are shown in Table 5-37. All problems were reported infrequently.

Table 5-37: Gynaecological Problems in the XL-CGD Carriers

Problem	Number of XL-CGD Carriers Affected (%)
Polycystic Ovaries	1
Chronic Endometritis	1
Breast Fibroadenoma	2
Ectopic Pregnancy	1
Menorrhagia	2

Ocular Problems

The ocular problems reported or volunteered by the XL-CGD carriers are shown in Table 5-38. They are all reported in low numbers. Coats disease is a rare, hereditary condition, which in this case resulted in the eye being removed.

Table 5-38: Ocular Manifestations in XL-CGD Carriers

Problem	Number of XL-CGD Carriers
Chorioretinitis	2
Retinal Infection (non specific)	1
Photopsia	1
Esotropia	1
Coats' Disease	1

Cardiovascular Disease

The XL-CGD carriers volunteered cardiovascular problems and these are shown in Table 5-39. Only four reported hypertension but Figure 5-10 shows that 9 XL-CGD carriers are prescribed anti-hypertensive medication suggesting that this number may be higher. The same is also true for high cholesterol, with two XL-CGD carriers reporting it as a problem but five XL-CGD carriers have been prescribed a statin as a cholesterol lowering agent.

Table 5-39: Cardiovascular Problems in XL-CGD Carriers

Problem	Number of XL-CGD Carriers Affected
Hypertension	4
High Cholesterol	2
Palpitations	2
Atrial Fibrillation	1
LBBB on ECG	2
Chest Pain requiring Investigation	2
Stroke	2
Death	1

5.7 Malignancies

There were 3 XL-CGD carriers who had been diagnosed with malignancy and these are shown in Table 5-40.

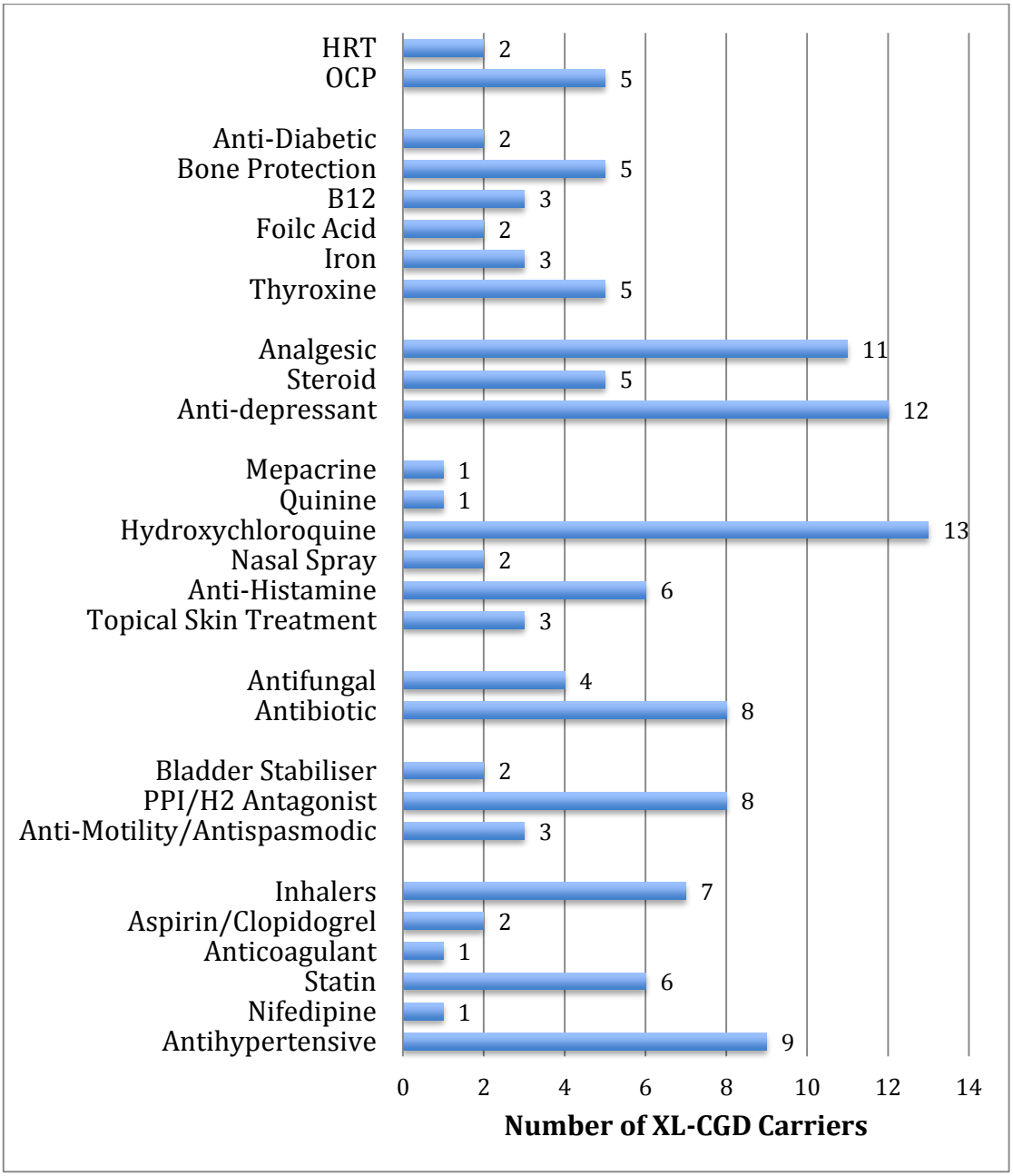
Table 5-40: Malignancy in XL-CGD Carriers

Type of Malignancy	Number of XL-CGD Carriers
Ovarian Cancer	1
Renal Cancer	1
Basal Cell Carcinoma	1

5.8 Medications

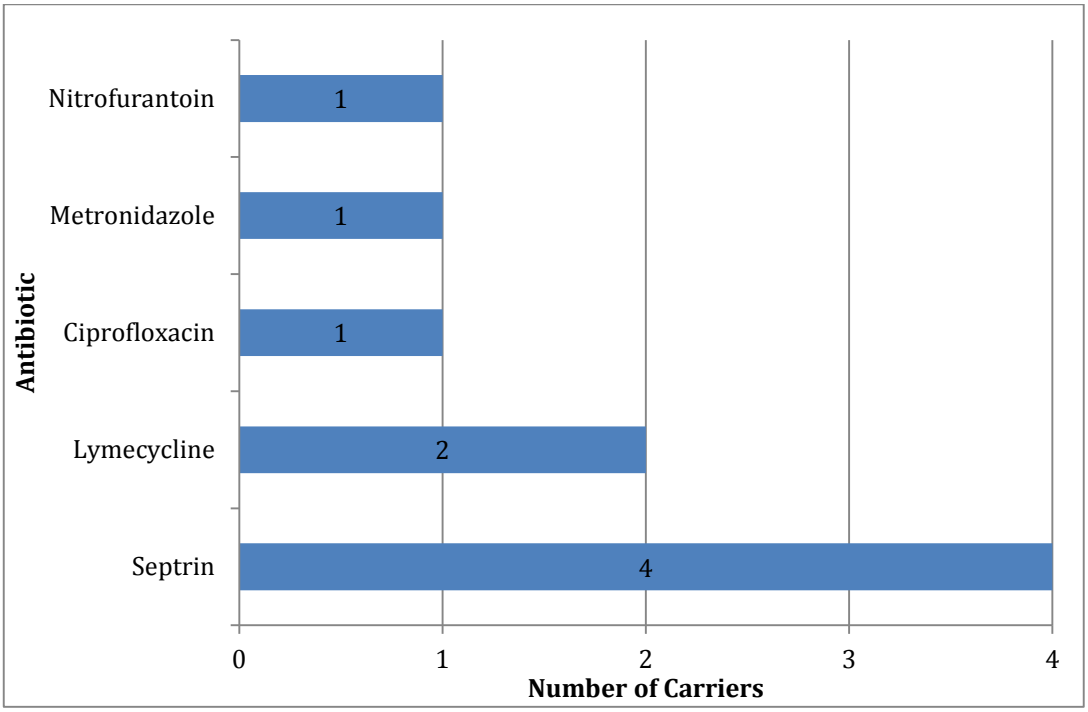
Prescribed medications were recorded from XL-CGD carriers and confirmed from GP records. The medications were then classified according to their class. 45 of the XL-CGD carriers were prescribed at least one medication including topical treatments. The frequency of the prescription of different classes of medications in XL-CGD carriers is shown in Figure 5-10 with analgesics, anti-depressants and hydroxychloroquine being the most frequently prescribed. Patients with CGD who are managed conservatively are on regular antibiotic and antifungal medication and frequently topical skin treatments. These were prescribed in a small number of the XL-CGD carriers. Of particular interest is the use of agents similar to those used in CGD patients including septrin and steroids.

Figure 5-10: Prescribed Medications in XL-CGD carriers



There were 4 XL-CGD carriers who were prescribed prophylactic antibiotics. The breakdown of prophylactic antibiotic use is shown in Figure 5-11, with septrin being the most frequently prescribed.

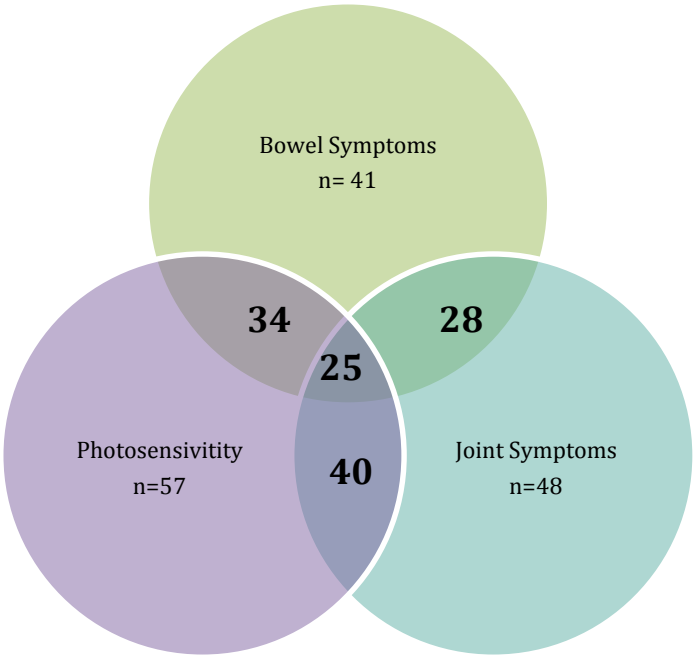
Figure 5-11: Prophylactic Antibiotic Use in XL-CGD carriers



5.9 Combination of Symptoms

We looked for symptom patterns. Figure 5-12 shows the number of XL-CGD carriers affected by joint symptoms, bowel symptoms and photosensitivity. Where the symptoms overlap the number affected by the combination is shown in bold. The number in the centre shows the number of XL-CGD carriers affected by all three.

Figure 5-12: Number of XL-CGD Carriers affected by Bowel and Joint Symptoms and Photosensitivity



A comparison in the NOB and age for the XL-CGD carriers affected by different combinations of symptoms is shown in Table 5-41. It can be seen that the most frequently occurring combination is photosensitivity and joint symptoms, and this combination has the youngest median age.

Table 5-41: Combination of Symptom Type in XL-CGD Carriers

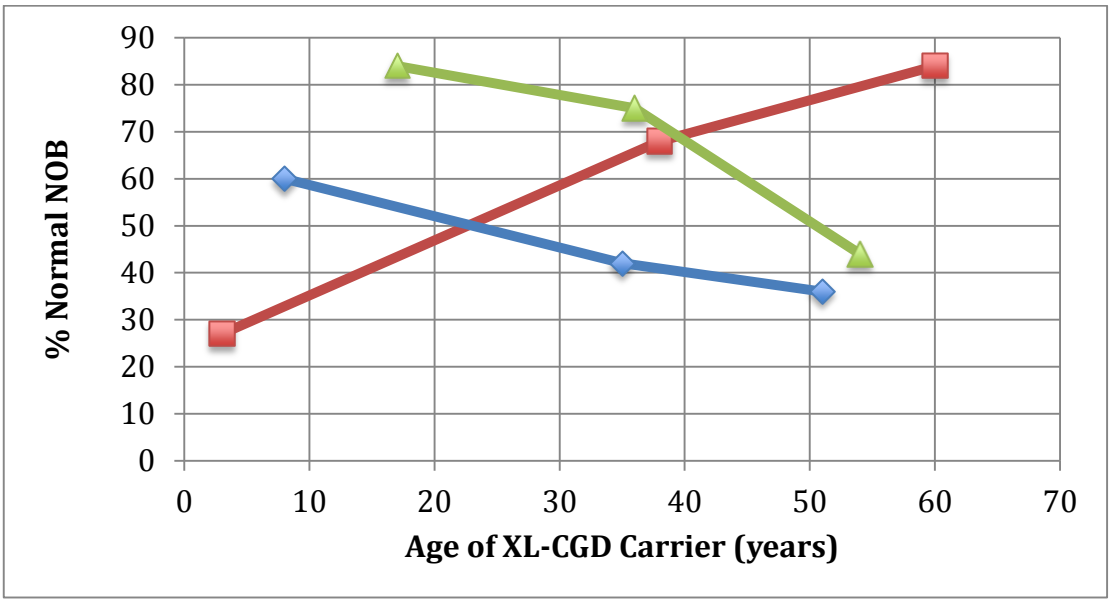
	Photosensitivity and Joints	**Photosensitivity and Bowel Symptoms**	**Joints and Bowel Symptoms and Photosensitivity**	**Joint and Bowel Symptoms**
Number Affected	40	34	25	28
Average NOB (% normal)	47.3	52.3	44.0	52.8
Mean Age (years)	37	42	42	41

5.10 Blood Results

5.10.1 Neutrophil Oxidative Bursts

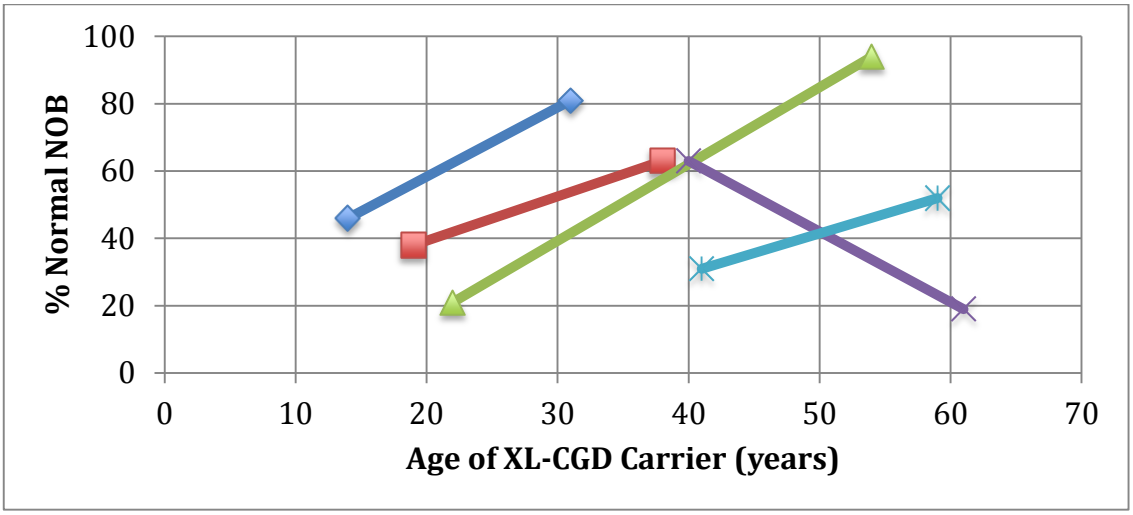
The NOB values for enrolled participants were shown in Table 5-11. Further analysis of NOB values was undertaken per kindred. Where there were three generations represented these NOB values against age are shown in Figure 5-13. Here it can be seen that in two of the families, the NOB was lower with increasing age but in one family this trend was reversed.

Figure 5-13: NOB and Age for 3 generations in 3 families



Where there were less than three generations represented, these results are shown Figure 5-14. Here it can be seen in four of the five families represented, the higher NOB was seen in the older generation.

Figure 5-14: NOB and Age for 2 generations in 5 families



NOB and Symptoms

Table 5-42 shows a comparison in NOB value in those affected and unaffected by symptom. It shows that there was a significant difference in NOB in those suffering from recurrent abscesses and those who were prescribed prophylactic antibiotics. There were no other statistically significant differences.

Table 5-42: NOB Values in affected and unaffected XL-CGD carriers by symptom

	Affected Mean NOB	Unaffected NOB	P Value
Raynaud's Phenomenon	48.68	45.7	0.68
Joint Symptoms	46.55	47.15	0.46
Ulcers	45.65	53.67	0.13
Photosensitivity	46.31	48.54	0.38
Skin Abscesses	30.13	49.73	0.0088
Miscarriage	47.00	48	0.54
Hydroxychloroquine	39.2	47.133	0.16
Antibiotic	49.03	23.167	0.0025
Antifungal	46.61	32	0.100
Infection	47.51	45.5	0.39
Fatigue	42.88	51.07	0.085

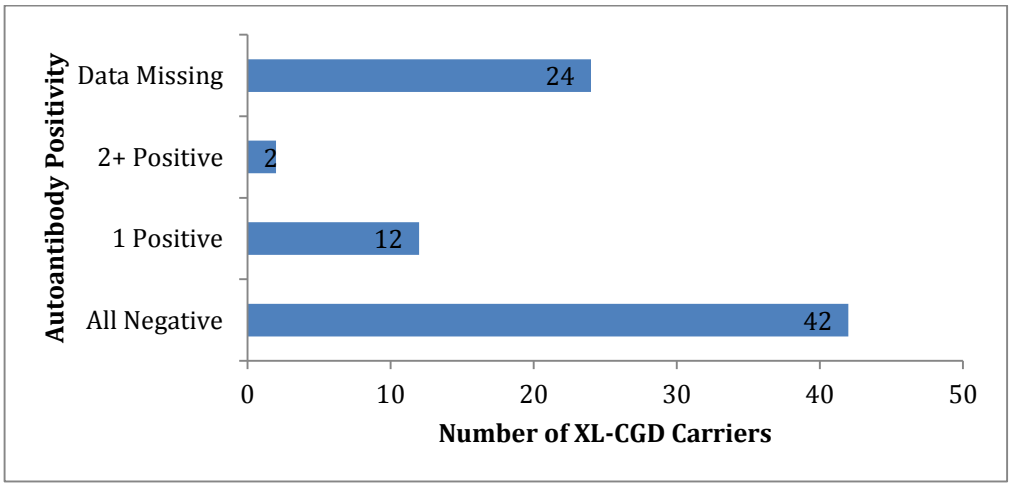
NOB and Number SLE Criteria

There was no significant difference between the number of SLE criteria met and the NOB result when compared using the one way test of ANOVA (p=0.58).

5.10.2 Autoantibodies Immunofluorescence

An autoantibody panel was performed by immunofluorescence in 56 XL-CGD carriers. The number of positive autoantibodies is shown in Figure 5-15. The majority of XL-CGD carriers had no positive autoantibodies, as measured by immunofluorescence, and only two XL-CGD carriers had more than one autoantibody positive.

Figure 5-15: Number of Autoantibodies Positive in an Individual XL-CGD Carrier as measured by Immunofluorescence



The type of autoantibody found in the XL-CGD carriers is shown in Table 5-43. ANA was the most frequently positive autoantibody, with anti-gastric parietal cell antibody the next most common.

Table 5-43: Type of Autoantibody Positive

	Positive (%)	**Negative (%)**
ANA	8 (14)	48 (86)
Anti Gastric Parietal Cell Antibody	4 (7)	52 (93)
Anti Mitochondrial Antibody	1 (1.8)	55 (98.2)
Anti Smooth Muscle Antibody	1 (1.8)	55 (98.2)

5.11 Summary of Clinical Results

- The mean NOB in the XL-CGD carriers was 47%, with the majority falling in the range of 21-60%
- Infective, inflammatory and autoimmune features were seen in the XL-CGD carriers
- 23% suffered recurrent or significant infection and overall this did not correlate significantly with NOB value

- Recurrent skin abscesses were seen in 17% and there was a significant correlation with lower NOB in those suffering abscesses
- 53% XL-CGD carriers in this study suffered gastrointestinal symptoms. Overall, there was no association with NOB value. However, those suffering from abdominal pain and diarrhoea had significantly lower NOB values
- 59% suffered recurrent joint symptoms but there was no correlation with NOB or autoantibodies
- Photosensitivity was seen in the majority of XL-CGD carriers with 74% affected and 40% suffered from DLE-type skin rashes. These did not correlate with NOB values.
- Features of SLE were common in the XL-CGD carriers with 30% of XL-CGD carriers meeting 3 criteria and 26% meeting 4 or more of the criteria
- Overall, there was poor correlation of medical symptoms with NOB with the exception of recurrent skin abscesses and the gastrointestinal symptoms

Chapter 6: Psychological Health Results

This chapter will present the results of the psychological assessment.

6.1 Anxiety and Depression

There were 61 XL-CGD carriers who completed the HADS and 7 controls (MD carriers).

6.1.1 Anxiety

Anxiety in XL-CGD Carriers

The frequency of a pre-existing diagnosis of anxiety or anxiety and depression is reported in Table 6-1. Only 1 XL-CGD carrier suffered from isolated anxiety, but a greater number had a diagnosis of mixed anxiety and depression. There were 12 XL-CGD carriers who had been prescribed antidepressants.

Table 6-1: Pre-existing Anxiety and Depression Diagnoses and Treatment

Diagnosis	Number of XL-CGD Carriers	% Affected
Anxiety	1	1.6
Depression	7	11.6
Mixed anxiety and depression	6	10
Prescribed antidepressants	12	20

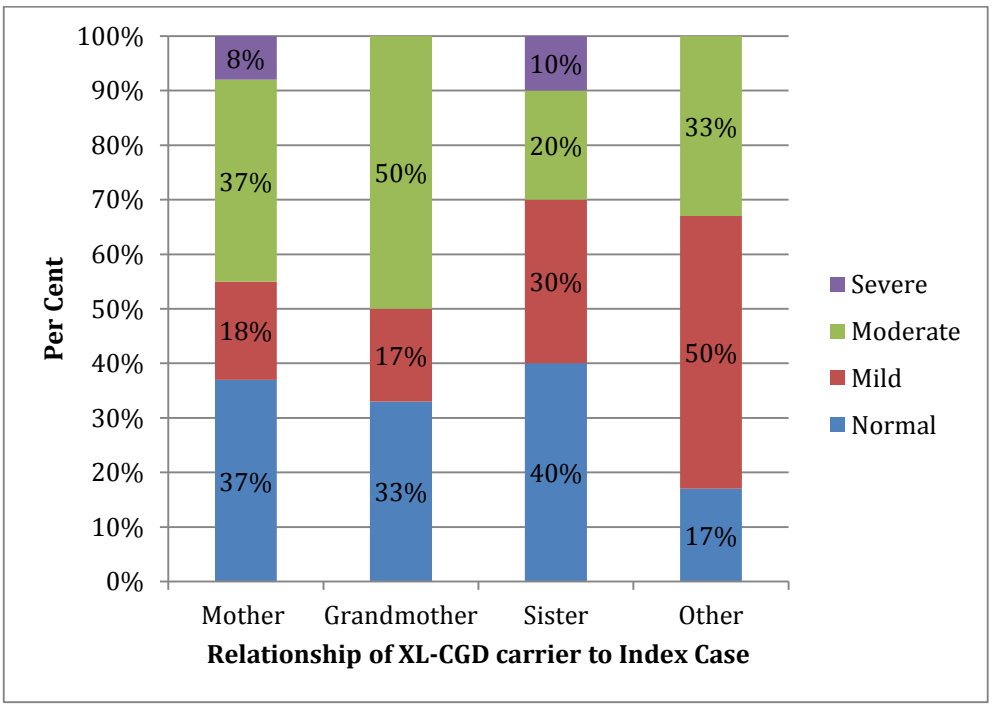
There were 61 XL-CGD carriers who completed the HADS. The mean score was 9.47 (SD 4.05). The distribution of XL-CGD carriers in the anxiety categories is shown in Table 6-2. Over 40% of XL-CGD carriers suffered from moderate or greater levels of anxiety with only one third being classified as normal.

Table 6-2: Anxiety Categories in XL-CGD Carriers

HAD Anxiety Category	Number of Carriers	%
Normal (0-7)	21	34.4
Mild (8-10)	14	23.0
Moderate (11-14)	22	36.1
Severe (>14)	4	6.5
Total	61	100

Anxiety scores were reviewed in the context of the relationship of the XL-CGD carrier to the index case and these results are shown in Figure 6-1. The most striking feature is that 50% of the grandmothers suffered moderate anxiety. The distribution across anxiety categories is otherwise similar, irrespective of the relationship to the index case.

Figure 6-1: Anxiety Categories by Relationship to Index Case in XL-CGD Carriers



The differences in HAD-A scores between the relationship groups did not reach statistical significance. Table 6-3 shows the mean scores.

Table 6-3: HAD-A Scores in XL-CGD carriers by relationship to index case

	Number of Carriers	HAD-A Mean (SD)	p-value
Mother	39	9.5 (4.05)	0.98
Grandmother	6	9.8 (3.76)	
Sister	10	9.5 (5.25)	
Other	6	8.8 (3.11)	

As there were small numbers in the groups, a comparison of mothers and all other relatives was also made. There was no significant difference when the mothers were compared to the rest of the group as a whole (p=0.56).

There was no significant difference in mean anxiety scores of the XL-CGD carriers when considered with regard to age categories of the index case (p=0.27).

The comparison in anxiety scores between participants where the index case had undergone HSCT and those where they had not is shown in Table 6-4. The anxiety scores were higher in the relatives where the index case had undergone HSCT, but they were not significantly higher.

Table 6-4: Anxiety Scores in XL-CGD Carriers where the index case had undergone HSCT compared with those where the index case had not undergone HSCT

	HSCT in index case	No HSCT in index case	p-value
Number	24	13	0.56
Mean	9.13	8.92	

Anxiety in XL-CGD Carriers Compared to Other Populations

The mean scores for HAD-A in the control group of MD carriers is shown in Table 6-5 and compared to the mean scores in XL-CGD carriers. It can be seen here that the mean score was lower, but that this did not reach statistical significance.

Table 6-5: HAD-A Scores in XL-CGD Carriers and the MD Control Group

	XL-CGD Carriers	MD Controls	p-value
Number	61	7	0.12
Mean	9.47	8.86	
SD	4.05	4.52	

The proportion of XL-CGD carriers affected by anxiety was compared to the proportion of CF parents in Besier et al's [251] published study. The comparison is shown in Table 6-6. A significantly higher proportion of XL-CGD carriers suffered from abnormal levels of anxiety compared with the parents of children with CF. The parents of children with CF were less likely to suffer from anxiety compared to the XL-CGD carriers.

Table 6-6: Frequency of Anxiety in XL-CGD Carriers and Other Published Groups

HAD-A Category (Score)	XL-CGD Carriers % (n)	CF Parents[251] % (n)	p-value
Normal (0-7)	34.4 (21)	62.7 (408)	<0.01
Borderline (8-10)	22.9 (14)	20.2 (131)	0.30
Abnormal (>10)	42.6 (26)	17 (111)	<0.001

Anxiety Scores in the XL-CGD carriers were compared with published scores from SLE patients [140, 229] and parents of children with CF [251]. The demographics and results are shown in Table 6-7. XL-CGD carriers suffered significantly greater levels of anxiety than the CF parents and also than SLE patients categorised as having low pain. However, there was no significant difference in anxiety scores when compared with the SLE patients in Tench et al's[140] study and the results were comparable with the SLE patients suffering high levels of pain.

Table 6-7: XL-CGD Carrier HAD-Anxiety Scores compared with published studies in other groups

	XL-CGD Carriers	SLE (High Pain)[229]	SLE (Low Pain)[229]	SLE Patients [140]	CF Parents[226]
Number	61	20	64	120	650
Median Age	42.5	45.9	45.9	38	40.35
Mean Anxiety Score	9.54	9	4	9	7.52
p-value		0.18	<0.01	0.18	0.0002

Factors Affecting Anxiety

Higher anxiety scores were significantly ($p<0.05$) correlated with higher depression scores, lower self-esteem and the presence of joint or bowel symptoms. Higher anxiety scores were also significantly correlated with higher levels of fatigue. There was no significant correlation of high anxiety scores with age, relationship to the index case or a diagnosis of SLE. These results are shown in Table 6-8. There was no difference in anxiety scores when the age of the index case was considered by age category ($p=0.268$).

Table 6-8: Relationship of Anxiety with other factors in XL-CGD Carriers

	Correlation Coefficient (rho)	p-value
Depression (HAD-D)	0.62	<0.0001
Self-esteem (Rosenberg)	-0.74	<0.0001
Total PIP	0.29	0.19
Fatigue (total)	0.50	0.0031
Bowel Symptoms	0.47	0.0059
Joint Symptoms	0.43	0.012
Lupus Diagnosis	0.21	0.24
Number of ARA Criteria	0.14	0.42
Age	0.20	0.26
Relationship to Index Case	0.23	0.20
HSCT in Index Case	0.003	0.98
MH (SF-36)	-0.6	<0.0001

6.1.2 Depression

Depression in XL-CGD Carriers

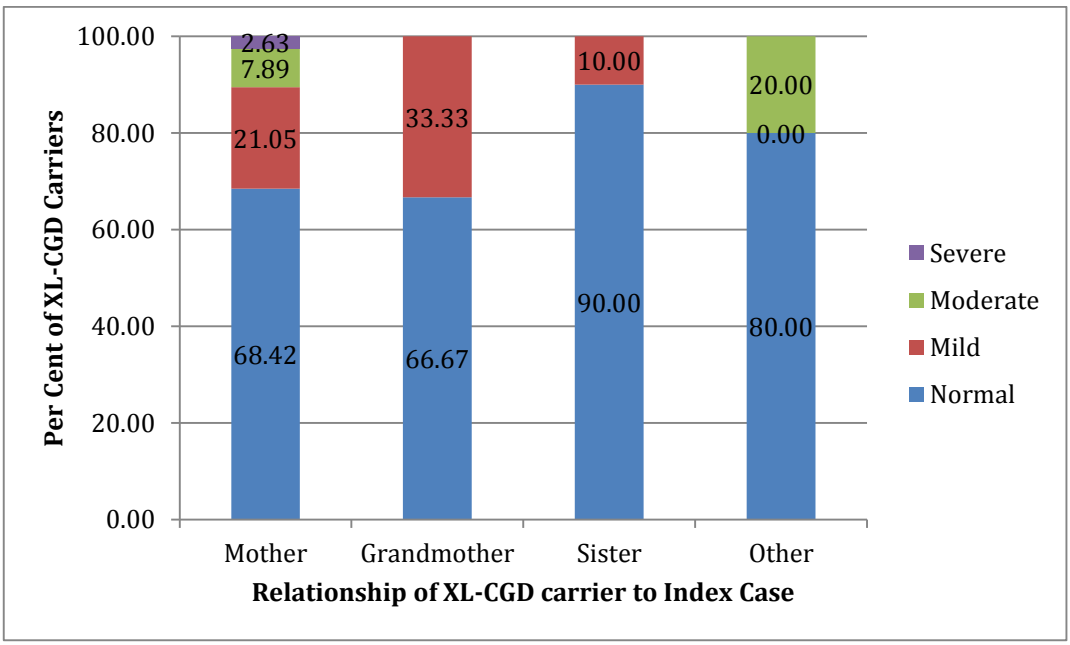
There were 61 XL-CGD carriers who completed the HAD (depression component). The mean score was 5.03 (SD 3.76). The distribution of XL-CGD carriers amongst the defined categories is shown in Table 6-9. Nearly three quarters of the cohort are categorised as normal, with only 1 XL-CGD carrier in the severe category. Depression was considerably less prevalent than anxiety in the XL-CGD carriers.

Table 6-9: Depression Categories in XL-CGD Carriers

HAD Depression Category (Score)	Number of XL-CGD Carriers	%
Normal (0-7)	45	73.8
Mild (8-10)	11	18.0
Moderate (11-14)	4	6.6
Severe (>14)	1	1.6
Total	61	100

The categorical distribution for depression is shown in relation to relationship to index case in Figure 6-2. Here it can be seen that the distribution was similar independent of the relationship to the index case.

Figure 6-2: Depression Categories by Relationship to Index Case in XL-CGD Carriers



The differences in HAD-D scores between the relationship groups did not reach statistical significance and Table 6-10 shows the mean scores.

Table 6-10: HAD-D Scores in XL-CGD Carriers by relationship to index case

	Number of XL-CGD Carriers	HAD-D Mean	p-value
Mother	38	5.26 (4.09)	0.14
Grandmother	6	6.33 (3.01)	
Sister	10	3.9 (2.42)	
Other	6	2.4 (2.7)	

As there were small numbers in the groups, a comparison of mothers and all other relatives was also made. There was no significant difference when the mothers were compared to the rest of the group as a whole (p=0.77).

There was no significant difference in the depression scores (p=0.36) when the XL-CGD carriers were considered between index case age categories.

The comparison in depression scores between participants where the index case had undergone HSCT and those where they had not is shown in Table 6-11. The scores were higher in the relatives of the XL-CGD patients who had undergone HSCT compared with those managed conservatively, but this did not reach statistical significance.

Table 6-11: Depression Scores in XL-CGD Carriers where the index case had undergone HSCT compared with those where the index case had not undergone HSCT

	HSCT in index case	No HSCT in index case	p-value
Number	24	13	0.65
Mean	5.75	5.23	

Depression in XL-CGD Carriers compared to Other Populations

The mean score for HAD-D in the control group of MD carriers is shown in Table 6-12. It can be seen here that the mean score was higher in the MD control group and that this reached statistical significance, meaning the MD carriers were, on average, more depressed than the XL-CGD carriers.

Table 6-12: HAD-D Scores in XL-CGD Carriers and MD Control Group

	XL-CGD Carriers	MD Controls	p-value
Number Completed	61	7	0.05
Mean	5.03	5.86	
SD	3.76	3.13	

The proportion of XL-CGD carriers with borderline or abnormal HAD-D scores was compared with the proportion seen in a published cohort of CF parents [251]. These results are shown in Table 6-13. There were no significant differences in the proportion in each category.

Table 6-13: Comparison of proportion of XL-CGD carriers with abnormal HAD-D scores with CF Parents

HAD-D Cat	XL-CGD Carriers % (n)	CF Parents [251] % (n)	Proportion Test p-value
Normal	73.7 (45)	72 (468)	0.62
Borderline	18 (11)	16.4 (107)	0.63
Abnormal	8.3 (6)	11.6 (75)	0.66

The mean scores for HAD-D were compared with published data in SLE patients with low and high pain scores and also with the CF parents. These results are shown in Table 6-14. XL-CGD carriers suffered significantly greater depression scores than the SLE low pain group. The scores were comparable to the CF parents and SLE patients in Tench et al's study[140].

Table 6-14: Comparison of mean HAD-D Scores in XL-CGD carriers and SLE patients and CF parents

	XL-CGD Carriers	SLE (High Pain)[229]	SLE (Low Pain)[229]	SLE [140]	CF Parents [226]
Number	60	20	64	120	650
Age (years)	42.5	45.9	45.9	38	40.35
Depression Score (Mean)	5.08	8	3	6	4.36
p-value		1.0	<0.01	0.98	0.084

Factors Affecting Depression

Correlation of HAD-D scores and potential contributing factors are shown in Table 6-15. Higher depression scores were significantly correlated with higher anxiety scores, lower self-esteem and higher fatigue scores. There was no significant correlation of HAD-D scores and age, relationship to index case and clinical symptoms (gastrointestinal and joint symptoms, diagnosis of SLE-like disorder and number of ARA SLE criteria met).

Table 6-15: Correlation of Associated Factors with HAD-D Scores

	Correlation Coefficient (rho)	p-value
Anxiety (HAD-A)	0.61	0.0001
Self-esteem (Rosenberg)	-0.74	<0.01
Total PIP	0.21	0.25
Fatigue (total)	0.54	0.0013
Bowel Symptoms	0.23	0.20
Joint Symptoms	0.31	0.080
Lupus Diagnosis	0.14	0.44
Number of ARA Criteria	0.20	0.25
Age	0.20	0.27
Relationship to Index Case	0.11	0.55
HSCT in index case	0.03	0.84
MH (SF36)	-0.67	<0.0001

6.2 Self-Esteem

There were 61 XL-CGD and 7 MD control carriers who completed the Rosenberg questionnaire to assess self-esteem. The median score for XL-CGD carriers was 19 (IQR 14-24). Table 6-16 shows the distribution of XL-CGD carriers in the categories for self-esteem. Nearly half of the XL-CGD carriers fell into the normal category, with one third being categorised as having low self-esteem.

Table 6-16: Categories of Self-Esteem in XL-CGD Carriers

Category	Number of XL-CGD Carriers	%
Low	19	31
Normal	28	46
High	14	23
Total	61	100

The median and IQRs of the Rosenberg Self-Esteem Scale in the different relationship categories are shown in Figure 6-3.

Figure 6-3: Median and IQR of Rosenberg Self-Esteem Scores by Relationship to Index Case

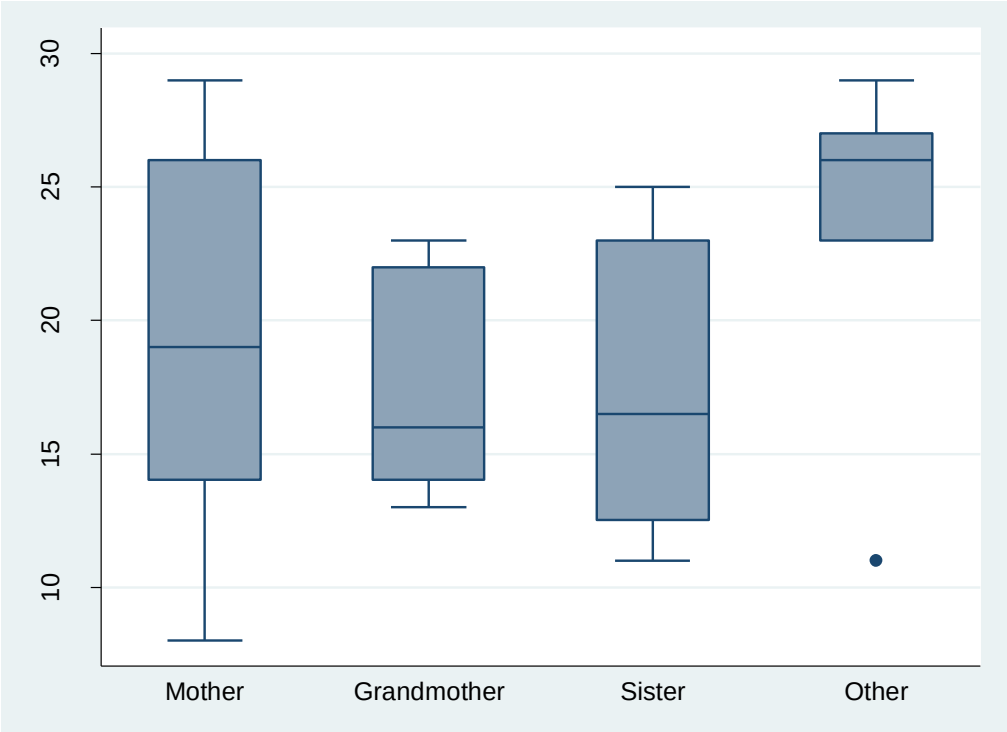


Table 6-17 shows a comparison of the Rosenberg scores in mothers of the index case compared to all other relatives. There was no significant difference when the two groups were compared.

Table 6-17: Comparison of Rosenberg Self-Esteem Scores in Mothers vs. Other Relatives

	Mothers	**Others**	**p-value**
Number Completed	39	22	0.46
Median	19	17	
Interquartile Range	14-26	12.5-23	

XL-CGD Carriers Self-Esteem compared with MD Controls

There were 7 MD carriers who completed the Rosenberg Self-Esteem Scale. A comparison between the median scores in the XL-CGD carriers and the MD control group is shown in Table 6-18. There was a significant difference between the groups, with a lower median score in the XL-CGD carrier group.

Table 6-18: Rosenberg Self-Esteem Scores in XL-CGD carriers and MD Control Group

	XL-CGD Carriers	MD Controls	p-value
Number Completed	61	7	<0.001
Median	19	23	
IQR	14-25	13-29	

Correlation in Self-Esteem

There was a significant correlation of anxiety and depression scores with self-esteem scores in the XL-CGD carriers, with higher scores in anxiety and depression correlating with lower self-esteem. Total and frequency scores from the PIP were approaching statistically significant correlation with self-esteem scores, with higher scores in these domains correlating with lower self-esteem. There were no other significant correlating factors. These results are shown in Table 6-19.

Table 6-19: Correlation of Associated Factors with Self-Esteem Scores in XL-CGD Carriers

	Correlation Co-efficient	p-value
Anxiety (HAD-A)	-0.74	<0.01
Depression (HAD-D)	-0.74	<0.01
PIP-T	-0.30	0.084
PIP-F	-0.29	0.095
PIP-S	-0.25	0.15
Skin Disease	-0.26	0.13
Joint Symptoms	-0.37	0.004
Bowel Symptoms	-0.23	0.19
Age of Participant	0.13	0.45
Index Case Age	0.091	0.60
Relationship to Index Case	-0.06	0.72

6.3 Caring for a Child with Chronic Illness

The Pediatric Inventory for Parents (PIP), which assesses the stresses associated for caring for a child with chronic illness, was completed by 36 XL-CGD carrier mothers and 7 of the control group (MD mothers). A numerical value is generated for PIP total (PIP-T), PIP Severity (PIP-S) and PIP frequency (PIP-F), with a higher score indicating higher levels of distress. Data were normally distributed. Table 6-20 shows the mean scores in each domain for the XL-CGD carriers.

Table 6-20: Summary Statistics for PIP in XL-CGD Carrier Mothers

	Mean	SD
PIP-Total	214.8	63.5
PIP-Frequency	112.3	30.3
PIP-Severity	103.0	35.3

Table 6-21 shows the breakdown of scores compared with the age of the index case. The highest scores were seen in the 7-12 year old group, with lower scores

seen in the older groups. The lowest scores were seen in the over 18 year old group. However, none of these differences reached statistical significance.

Table 6-21: PIP Scores in XL-CGD Carrier Mothers according to Age of the Index Case

Age of Index Case	Number XL-CGD Carriers	PIP Frequency	p-value	PIP Severity	p-value	PIP Total	p-value
Infant (<2 years)	6	112.2 (17.3)		96.7 (18.7)		208.0 (35.9)	
Young Child (3-6 years)	5	106.2 (15.9)		107.4 (39.3)		213.6 (54.9)	
Older Child (7-12 years)	10	128.2 (29.9)	0.094	117.4 (34.3)	0.36	245.6 (58.6)	0.166
Adolescent (12-18 years)	8	107.5 (20.3)		92.6 (27.1)		200.0 (38.9)	
Adult (>18 years)	5	86.4 (41.7)		84.0 (44.8)		167 (91.6)	

A comparison in PIP scores between those where the index case had undergone HSCT and those where they had not are shown in Table 6-22. The scores in the no HSCT group were higher than in those without, but no statistical significance was observed.

Table 6-22: Comparison of PIP Scores in XL-CGD Mothers of index case who had undergone HSCT with those who had not undergone HSCT

	Index Case undergone HSCT	No HSCT	p-value
Number	19	6	
Total PIP	210.6	241.3	0.14
PIP – F	112.0	123.0	0.19
PIP – S	97.9	118.0	0.12

Comparisons of XL-CGD Mothers with other Populations

Table 6-23 shows the mean scores for all three domains of the PIP in XL-CGD mothers and in the control group of MD carrier mothers. The MD group scored significantly higher in the severity domain, whilst the XL-CGD mothers scored significantly higher in the frequency domain. The overall scores were not significantly different. There were too few to compare across the age categories.

Table 6-23: PIP Scores in XL-CGD mothers compared to MD carriers

	XL-CGD Carriers (Mean + SD)	MD Carriers (Mean + SD)	p-value
Number Completed	36	7	
Frequency (PIP-F) Score	112.3 (30.3)	104.4 (41.1)	0.06
Severity (PIP-S) Score	103.0 (35.3)	115.6 (36.8)	0.02
Total (PIP-T)	214.8 (63.5)	220.0 (73.8)	0.31

Comparison with Published Data

The PIP scores were compared with published data from parents of children with an oncological diagnosis. These results are shown in Table 6-24. There was a significant difference seen in the frequency domain, with XL-CGD carrier mothers scoring more highly. There was no significant difference in total or severity scores.

Table 6-24: Mean PIP Scores in XL-CGD Mothers compared with published data from oncology parents

	XL-CGD Cohort Mean Scores ±SD	Oncology Parents [255] Mean Scores ±SD	*p*-value
PIP-T	214.8 ±63.5	206 ± 34.2	0.20
PIP-F	112.3 ±30.3	94.0 ±33.3	0.0005
PIP-S	103.0 ±35.3	112.4 ± 35.1	0.06

Correlation of PIP Total

There was no association between total PIP scores and the age of the XL-CGD carrier. The correlation between total PIP score and the number of children an XL-CGD carrier had approached statistical significance, as was the association with anxiety level. There were no other significant correlations found. The results are shown in Table 6-25.

Table 6-25: Correlation of Associated Factors with Total PIP Scores in XL-CGD Carriers

	Correlation Coefficient	*p*-value
Age of XL-CGD carrier	-0.073	0.69
HSCT	-0.13	0.56
Number of Children	0.32	0.067
Number of Affected Children	0.07	0.69
Age of Index Case	-0.19	0.30
Anxiety (HAD-A)	0.32	0.057
Depression (HAD-D)	0.27	0.11

6.4 IQ Assessment

The WAIS was completed by 9 XL-CGD carriers. The scores and per centile for each domain and the total for the XL-CGD carriers are shown in Table 6-26. For 6 of the XL-CGD carriers the lowest result (when considered by per centile) was seen in the working memory domain.

Table 6-26: IQ Scores (Total and Components) in XL-CGD Carriers

Carrier Number	FSIQ (Per centile)	VCI (per centile)	PRI (per centile)	WMI (per centile)	PSI (per centile)
1	109 (73)	108 (70)	123 (94)	89 (23)	102 (55)
5	123 (94)	114 (82)	119 (90)	128 (97)	114 (82)
8	97 (42)	98 (45)	104 (61)	95 (37)	92 (30)
11	102 (55)	91 (27)	111 (77)	95 (37)	114 (82)
17	98 (45)	98 (45)	98 (45)	92 (30)	105 (63)
18	81 (10)	72 (3)	90 (25)	83 (13)	97 (42)
20	104 (61)	107 (68)	111 (77)	86 (18)	102 (55)
21	94 (34)	83 (13)	94 (34)	102 (55)	105 (63)
43	110 (75)	110 (75)	96 (39)	128 (97)	102 (55)

6.5 Summary of Psychological Results

- High rates of anxiety seen in XL-CGD carriers with 40% of the XL-CGD carriers having moderate or greater anxiety symptoms, with lower rates of depression (74% no significant depressive symptoms)
- There was no difference in anxiety symptoms if the index case had undergone HSCT or when considering the relationship to the index case
- Anxiety symptoms in XL-CGD carriers did not significantly differ from the MD control group
- Anxiety more common in XL-CGD carriers than in other published parent groups and similar to that seen in SLE patients with high pain
- Anxiety and depression were more common in XL-CGD carriers suffering physical symptoms
- Depression symptoms were seen less frequently in XL-CGD carriers than in the MD control group
- When depression scores were compared with other published groups, the XL-CGD carriers suffered less depression than SLE patients and were similar to the parents of children with CF

- Depression symptoms did not differ depending on relationship to the index case
- Self-esteem well preserved in XL-CGD carriers

Chapter 7: Fatigue Results

This chapter will present the results of the fatigue component of the research including fatigue questionnaires and cytokine measurement.

There were 60 XL-CGD carriers who completed the MFSI-SF questionnaire along with 7 controls.

7.1 Fatigue in XL-CGD Carriers

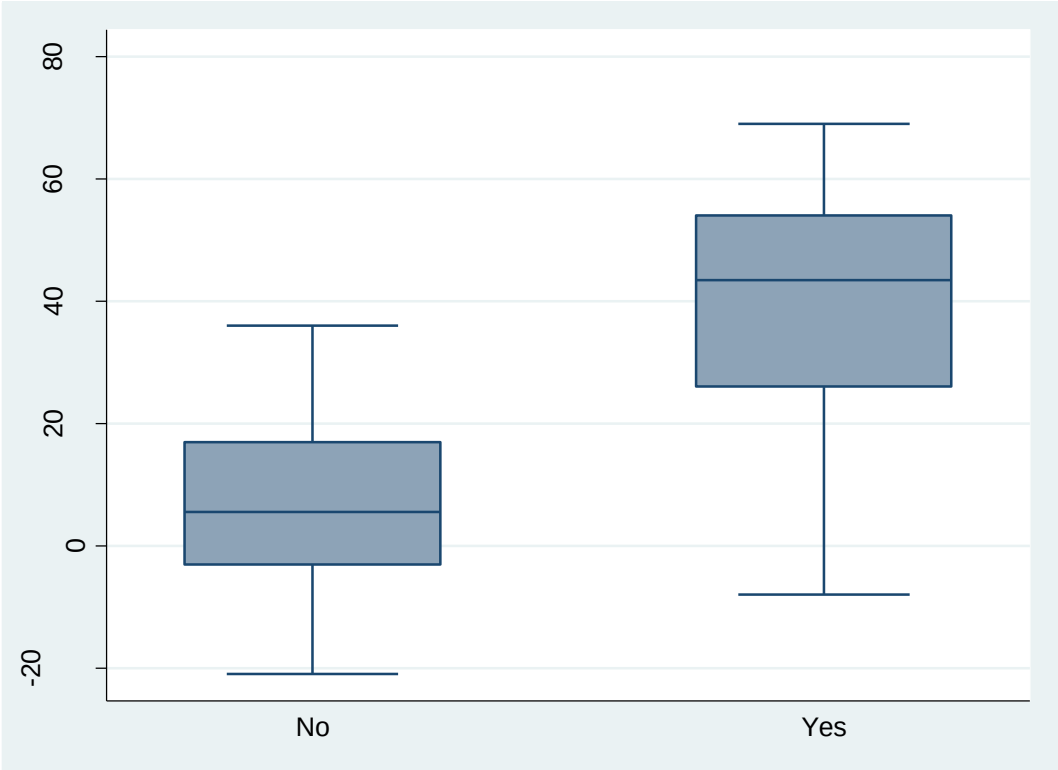
There were 37 XL-CGD carriers who reported that they felt they suffered from excessive levels of fatigue when asked about any medical problems. This was quantified using the MFSI-SF in all XL-CGD carriers and these results are shown in Table 7-1. In all domains except vigor, a higher score reflects greater fatigue. In the domain of vigor, a higher score reflects less fatigue. The highest, most significant, levels of fatigue are seen in the general domain with lowest scores seen in the physical domain. The vigor scores reflect protective features.

Table 7-1: XL-CGD Carrier MFSI Fatigue Scores in all domains

	XL-CGD Carriers Median (IQR)
Number Completed	60
Total	20 (2.5-43.5)
General	9 (5-20)
Physical	4 (1-12)
Emotional	6 (3-11)
Mental	6 (4-10)
Vigor	10 (7-13)

Scores for the MFSI-SF were compared between those that reported fatigue and those who did not and these are shown in Figure 7-1. The scores were significantly higher in those who reported fatigue ($p<0.0001$).

Figure 7-1: MFSI-SF Scores in XL-CGD Carriers reporting Fatigue vs. those who did not



MFSI Domains Correlation

The MFSI domains were significantly correlated with each other and with the total score showing that each domain contributed to the total score. The MFSI was also significantly inversely correlated with the VT domain of the SF-36. These results are shown in Table 7-2.

Table 7-2: Correlation of MFSI Domains (correlation coefficient and p-value)

	Total	**General**	**Physical**	**Emotional**
General	0.92 <0.0001			
Physical	0.83 <0.0001	0.79 <0.0001		
Emotional	0.67 <0.0001	0.54 0.0008	0.33 0.05	
Mental	0.75 <0.0001	0.66 <0.0001	0.48 0.003	0.47 0.005
VT (SF36)	-0.61 <0.0001	-0.61 <0.0001	-0.61 <0.0001	-0.32 0.06

Correlation of MFSI domains with other psychological and clinical factors are shown in Table 7-3. There was significant correlation between all of the domains

of the MFSI and the vitality component of the SF36. There was also a significant correlation between joint symptoms and fatigue.

Table 7-3: Fatigue Correlations in XL-CGD carriers (Correlation coefficient with associated p-value)

	HAD-A	HAD-D	Bowl Sx	Joint Sx	Number of Children	Age of Index Case	Relationship
Total	0.472 0.004	0.57 0.0004	0.326 0.056	0.58 0.0003	0.24 0.06	0.06 0.68	-0.007 0.96
General	0.32 0.060	0.52 0.0012	0.30 0.076	0.54 0.0009	0.19 0.14	0.05 0.72	0.004 0.97
Physical	0.37 0.028	0.37 0.03	0.37 0.03	0.64 <0.0001	0.26 0.04	-0.02 0.87	-0.08 0.52
Emotional	0.48 0.004	0.39 0.022	0.14 0.40	0.25 0.15	0.28 0.04	0.18 0.18	-0.04 0.77
Mental	0.35 0.042	0.52 0.0014	0.08 0.65	0.46 0.006	0.15 0.26	0.05 0.69	-0.03 0.79
VT (SF36)	-0.42 0.01	-0.58 0.0003	-0.468 0.005	-0.5 0.002	-0.04 0.74	0.09 0.50	-0.04 0.78
HAD-A		0.605 0.0001	0.47 0.005	0.42 0.011	0.12 0.35	0.11 0.39	-0.05 0.7
HAD-D	0.61 0.0001		0.273 0.11	0.33 0.055	0.24 0.06	-0.05 0.66	-0.12 0.34
Bowel Sx	0.47 0.005	0.273 0.11		0.22 0.21	0.16 0.16	-0.07 0.59	-0.07 0.52
Joint Sx	0.42 0.011	0.33 0.055	0.22 0.21		0.19 0.09	-0.03 0.83	-0.07 0.52
Total PIP	0.30 0.085	0.266 0.12	0.14 0.43	0.36 0.03	0.50 0.002	-0.11 0.50	0.29 0.09

The scores for MFSI in the XL-CGD carriers and the MD control group are shown in Table 7-4. The scores in the MD control group are higher than the XL-CGD carriers in all, domains except vigor, demonstrating that the MD controls suffered greater fatigue in all domains. The scores for vigor were very similar, but the XL-CGD carriers had a slightly higher score.

Table 7-4: Fatigue Scores in XL-CGD Carriers and MD Control Group

	XL-CGD Carrier Scores Median (IQR)	MD Carrier Scores Median (IQR)	p-value (Mann-Whitney)
Number Completed	60	7	
Total	20 (2.5-43.5)	34 (1-60)	0.56
General	9 (5-20)	16 (3-22)	0.92
Physical	4 (1-12)	9 (0-15)	0.81
Emotional	6 (3-11)	10 (4-16)	0.45
Mental	6 (4-10)	11 (0-17)	0.61
Vigor	10 (7-13)	11 (7-13)	0.70
SF36 VT	37.5 (25-62.5)	20(25-75)	0.57

7.2 Fatigue and Regression

In order to assess the factors affecting fatigue in the XL-CGD carrier cohort, a logistic regression analysis was undertaken, with fatigue caseness defined as excessive fatigue present. There were no significant associations and the results are shown in Table 7-5.

Table 7-5: Logistic Regression for Fatigue in XL-CGD Carriers

	Odds Ratio	95% CI	P-Value
Age	1.00	0.91, 1.10	0.977
Index Case Age	0.99	0.95, 1.03	0.654
Relationship to Index Case	0.79	0.25, 2.52	0.690
Number of Children	1.93	0.48, 7.70	0.352
Anxiety (HAD-A)	1.20	0.78, 1.83	0.406
Depression (HAD-D)	1.43	0.74, 2.75	0.284
Joint Pains	2.05	0.12, 34.60	0.618
SLE Criteria	1.05	0.44, 2.52	0.906
Bowel Sx	0.95	0.84, 1.07	0.418

A linear regression analysis, with total MFSI score as the outcome was undertaken and the results are shown in Table 7-6.

Table 7-6: Linear Regression of Total Fatigue in XL-CGD Carriers

	Coefficient	95% CI	p-value
Anxiety (HAD-A)	1.73	-0.83, 4.30	0.176
Depression (HAD-D)	0.89	-1.12, 2.90	0.368
Parental Distress (PIP)	0.07	-0.06, 0 .21	0.264
Age	-0.48	-1.58, 0 .62	0.376
Relationship	20.60	-8.36, 49.57	0.155
Number of Children	-3.15	-12.95, 6.65	0.512
Age of Index Case	0.367	-0.88, 1.62	0.549
Bowel Symptoms	1.62	-11.76, 15.00	0.804
Joint Symptoms	-0.41	-23.44, 22.62	0.971
Number of SLE Criteria	5.39	-0.26, 11.04	0.061

7.3 Fatigue and Neutrophil Oxidative Burst

The NOB values were compared between the fatigued and non-fatigued groups and the results are shown in Table 7-7. It can be seen that there is a lower NOB value in those who reported fatigue.

Table 7-7: NOB in Fatigued and Non-Fatigued Patients

	Fatigued	Non-Fatigued	p-value
Number	25	28	0.085
NOB (mean)	42.88	51.07	

7.4 Fatigue and Correlation with other symptoms

Fatigue and Physical Symptoms

Figure 7-2 compares the MFSI total scores between those XL-CGD carriers suffering from gastrointestinal symptoms with those XL-CGD carriers who suffered no joint symptoms. The XL-CGD carriers suffering from gastrointestinal symptoms scored significantly more highly in the fatigue questionnaire (p=0.011)

Figure 7-2: MFSI Total Scores in XL-CGD Carriers affected and unaffected by gastrointestinal symptoms

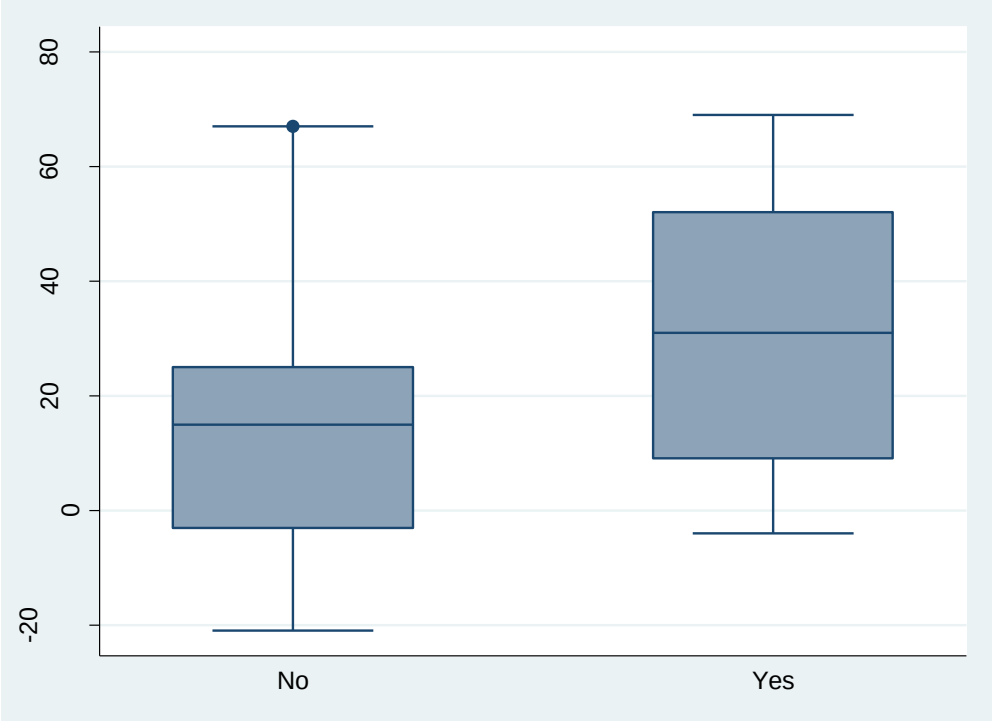
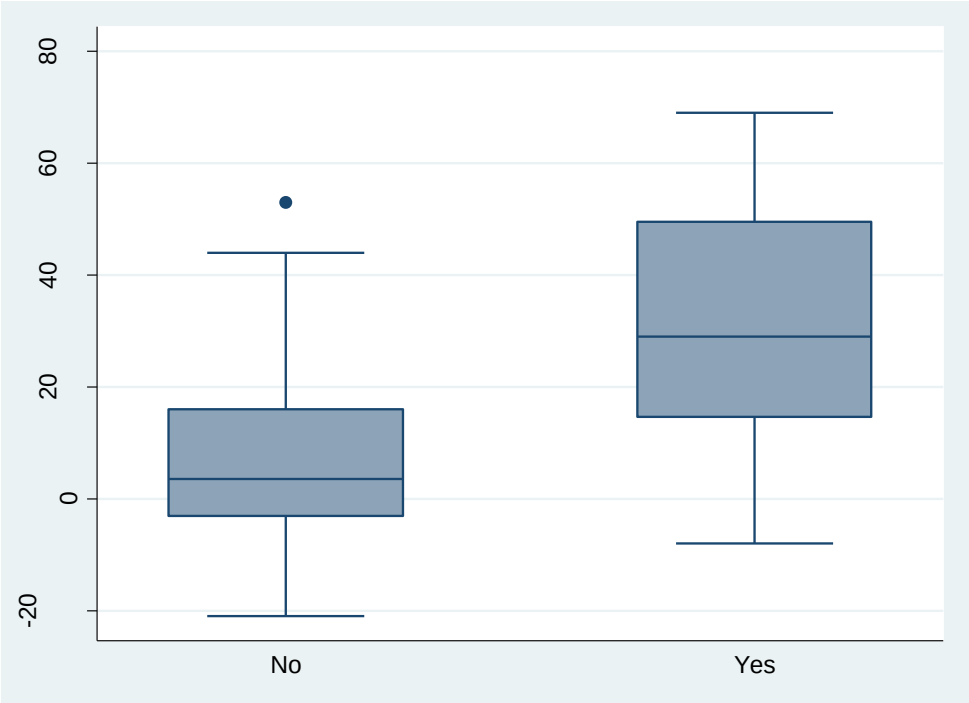


Figure 7-3 compares the MFSI total scores between those XL-CGD carriers suffering from joint symptoms with those XL-CGD carriers who suffered no joint symptoms. XL-CGD carriers suffering from joint symptoms, scored significantly higher in the fatigue questionnaire (p=0.003).

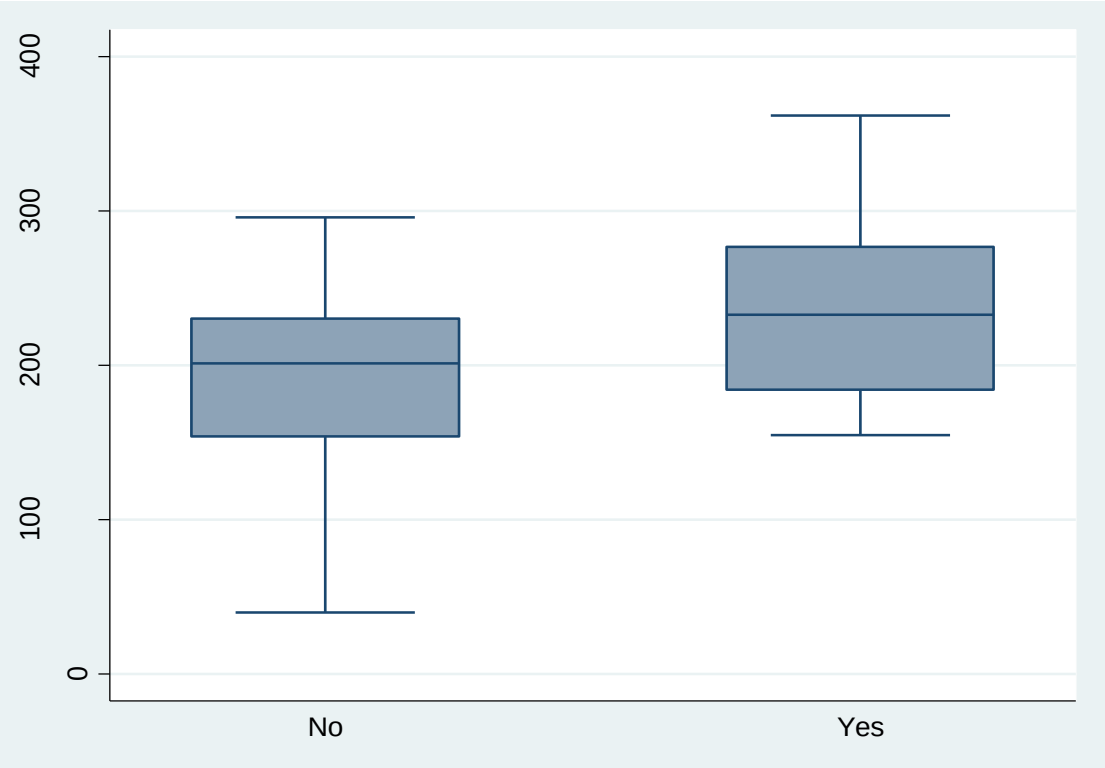
Figure 7-3: MFSI Total Scores in XL-CGD Carriers affected and unaffected by joint symptoms



Fatigue and PIP

Figure 7-4 compares total PIP scores in XL-CGD carriers who did and did not suffer from excessive fatigue. Those XL-CGD carriers who suffered excessive fatigue, scored more highly in the PIP (p=0.314).

Figure 7-4: Total PIP Scores in Fatigued and Non-Fatigued XL-CGD Carriers



Fatigue and Psychological Health

Figure 7-5 and Figure 7-6 show fatigue scores (total MFSI) compared with categories for anxiety and depression. The number of XL-CGD in each category for anxiety and depression is shown in Table 7-8, whilst the comparison of median scores and distribution is shown in Figure 7-5 and Figure 7-6.There were significant differences in the fatigue scores when they were compared by anxiety and depression category ($p<0.001$).

Table 7-8: Number of XL-CGD carriers in each category for Anxiety and Depression

	Anxiety	**Depression**
Normal	21	45
Mild	14	11
Moderate	22	4
Severe	4	1

Figure 7-5: MFSI Scores and HAD-A Categories in XL-CGD Carriers

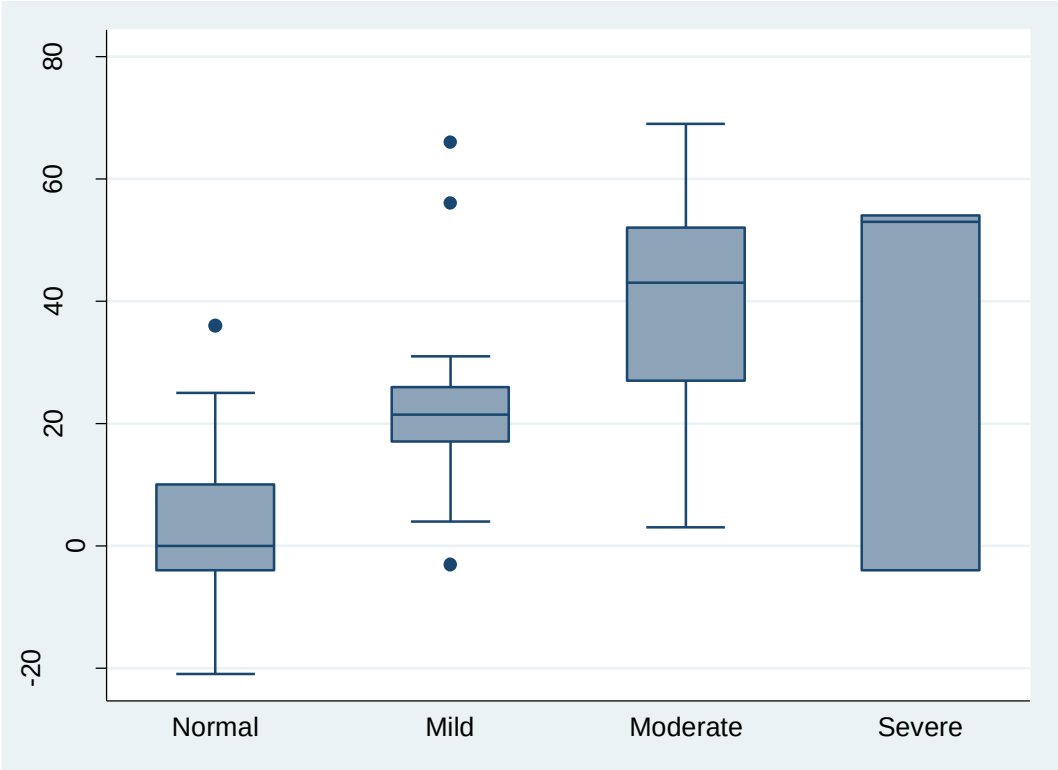
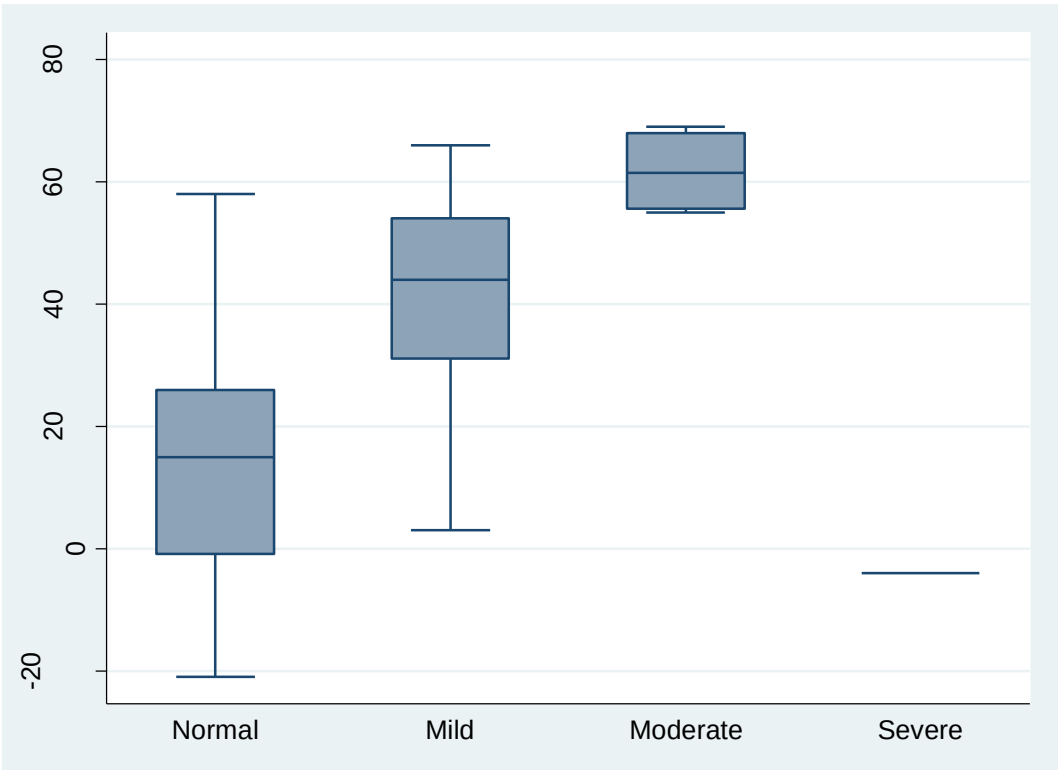


Figure 7-6: MFSI Scores and HAD-D Scores in XL-CGD Carriers



7.5 Fatigue and Cytokines

Table 7-9 shows the median values for serum cytokines in XL-CGD carriers compared with healthy controls and Sjögrens patients. The Sjögrens patients were divided into low and high fatigue. There was a significant difference in the IL8 measurements; with XL-CGD carriers demonstrating significantly higher values compared with healthy controls and Sjögrens patients.

Table 7-9: Serum Cytokine Values in XL-CGD Carriers compared with Healthy Controls and Sjögrens Patients (High and Low Fatigue)

	XL-CGD Carriers Median	Healthy Controls Median	p-value	Sjögrens (High Fatigue) Median	p-value	Sjögrens (Low Fatigue) Median	p-value
IL5	0.62	0.62	0.89	0.60	0.60	2.67	<0.001
IL8	110.8	28.2	0.02	15.18	0.039	20.48	0.031
IL17	4.71	4.71	0.84	7.99	0.35	63.29	0.016
IL10	1.66	1.66	0.63	2.34	0.067	7.585	<0.001
IFNα	2.9	2.46	0.49	6.66	0.14	14.21	0.0031
IL1α	4.71	4.71	0.78	7.99	0.15	63.29	<0.001
IFNγ	5.05	6.88	0.02	8.33	0.01	7.75	0.0012

7.6 Summary of Fatigue Results

- Excessive fatigue reported in 50% of XL-CGD carriers
- Higher levels of IL-8 seen in XL-CGD carriers when compared with healthy controls and Sjögrens patients
- Higher levels of serum IL-8 in XL-CGD carriers reporting fatigue than those who did not report fatigue

Chapter 8: Quality of Life Results

This chapter will present the findings from the assessment of quality of life (QoL).

There were 62 XL-CGD carriers who completed the SF-36 quality of life questionnaire along with 7 of the control group (MD carriers). The mean scores of the XL-CGD carriers for each domain are shown in Table 8-1. The highest score for each domain is 100 and it can be seen that the lowest scores are seen in GH, VT, MCS and PCS.

Table 8-1: QoL Scores in XL-CGD Carriers

Domain	XL-CGD Carriers Mean Scores (SD)
Physical Function (PF)	78.85 (28.3)
Role Physical (RP)	73.33 (32.6)
Bodily Pain (BP)	63.44 (30.6)
General Health (GH)	54.93 (28.7)
Vitality (VT)	44.23 (25.7)
Social Functioning (SF)	67.37 (30.1)
Role Emotional (RE)	70.14 (30.9)
Mental Health (MH)	62.70 (17.6)
Mental Component Score (MCS)	42.41 (10.8)
Physical Component Score (PCS)	49.05 (12.6)

8.1 Comparison of XL-CGD Carriers with other groups

Table 8-2 shows the QoL scores in the XL-CGD carriers compared with the MD control group. The XL-CGD carriers had significantly worse scores in GH, VT, RE and MCS. The MD control group scored significantly worse in BP.

Table 8-2: QoL Scores in XL-CGD Carriers and MD Control Group

Domain	XL-CGD Carrier Mean Scores (SD)	MD Control Group Mean Scores (D)	p-value
PF	78.85 (28.3)	76.43 (35.8)	0.234
RP	73.33 (32.6)	77.68 (30.4)	0.169
BP	63.44 (30.6)	54.14 (27.6)	0.0095
GH	54.93 (28.7)	65.71 (29.1)	0.002
VT	44.23 (25.7)	50 (23.7)	0.035
SF	67.37 (30.1)	60.71 (22.2)	0.508
RE	70.14 (30.6)	89.29 (21.4)	<0.0001
MH	62.70 (17.6)	63.57 (21.9)	0.342
MCS	42.41 (10.8)	46.27 (9.3)	0.0034
PCS	49.05 (12.6)	47.82 (15.9)	0.223

Table 8-3 shows a comparison of the XL-CGD carrier QoL scores in each domain compared with published UK population data of women aged 35 to 54 years[257]. The XL-CGD carriers scored significantly lower in all domains.

Table 8-3: QoL in XL-CGD Carriers compared with UK Population Data

Domain	XL-CGD Carriers Mean (SD)	UK Women (age 35-54) [257] Mean (SD)	p- value
Physical Function	78.85 (28.3)	89.4 (18.3)	0.0025
Role Physical	73.33 (32.6)	84.0 (32.0)	0.007
Bodily Pain	63.44 (30.6)	79.4 (22.0)	0.0001
General Health	54.93 (28.7)	74.1 (20.3)	0.0001
Vitality	44.23 (25.7)	58.2 (19.9)	0.002
Social Function	67.37 (30.1)	86.7 (20.5)	0.001
Role Emotional	70.14 (30.9)	80.3 (33.6)	0.0067
Mental Health	62.70 (17.6)	71.6 (17.8)	0.0001

Table 8-4 shows QoL scores in the XL-CGD carriers compared with adult CGD patients. The XL-CGD carriers scored significantly worse than the CGD patients, indicating poorer QoL in the BP, VT and SF domains. However, the XL-CGD carriers scored significantly better than the CGD patients in the PF and RP domains.

Table 8-4: QoL in XL-CGD Carriers compared with Adult CGD Patients

Domain	XL-CGD Carriers Mean Scores (SD)	CGD Adult Patients [258] Mean Scores (SD)	p- value
Physical Function	78.85 (28.3)	70.0 (34.4)	0.007
Role Physical	73.33 (32.6)	66.7 (41.9)	0.049
Bodily Pain	63.44 (30.6)	83.8 (26.4)	<0.001
General Health	54.93 (28.7)	52.4 (26.5)	0.23
Vitality	44.23 (25.7)	58.3 (26.1)	<0.001
Social Function	67.37 (30.1)	77.8 (33.5)	0.004
Role Emotional	70.14 (30.9)	75.0 (34.8)	0.11
Mental Health	62.70 (17.6)	65.0 (27.5)	0.15

Table 8-5 shows a comparison between QoL scores in XL-CGD carriers and adult carers of patients with brain tumours. The XL-CGD carriers scored significantly worse in PF, BP, VT and GH, but scored significantly better in RE, MH and SF.

Table 8-5: QoL in XL-CGD Carriers compared to Carers of Patients with brain tumours

Domain	XL-CGD Carriers Mean Score (SD)	Brain Tumour Carers [211] Mean Score (SD)	p-value
Physical Function	78.85 (28.3)	92 (14.39)	0.0003
Role Physical	73.33 (32.6)	76.76 (33.19)	0.23
Bodily Pain	63.44 (30.6)	74.43 (25.06)	0.0029
General Health	54.93 (28.7)	68.81 (17.29)	0.0002
Vitality	44.23 (25.7)	50.55 (19.9)	0.024
Social Function	67.37 (30.1)	58.66 (26.97)	0.014
Role Emotional	70.14 (30.9)	48.09 (36.79)	<0.001
Mental Health	62.70 (17.6)	48.32 (21.56)	<0.001

Table 8-6 shows a comparison in QoL scores in the XL-CGD carriers compared with published research about SLE patients[140]. Overall, the scores in the SLE

patients are worse in the SLE group, although XL-CGD carriers scored lower in the vitality (fatigue) domain.

Table 8-6: QoL in XL-CGD Carriers compared with SLE Patients

	SLE Patient Scores [140] Median (IQR)	**XL-CGD Carrier Scores Median (IQR)**
Physical Functioning	63 (40-85)	90 (75-100)
Role – physical	25 (0-75)	93.75 (50-100)
Bodily Pain	51(22-74)	72 (41-84)
General Health	42 (25-57)	60 (30-82)
Vitality	40 (25-55)	37.5 (25-62.5)
Social Functioning	50 (38-75)	75 (50-100)
Role – emotional	33(0-100)	75 (50-100)
Mental Health	60 (48-76)	65 (55-70)

HSCT in the Index Case

Information about HSCT in the index case was available for those XL-CGD carriers who completed the SF36. There were 30 XL-CGD carriers where the index cases had undergone HSCT. QoL scores in where the index case had undergone HSCT were compared with those who had not and these results are shown in Table 8-7. There were no significant differences, but BP and MH were approaching statistical significance with worse scores in the XL-CGD carriers where the index case had undergone HSCT.

Table 8-7: Comparison of QoL Scores in XL-CGD Carriers where the index case has and has not undergone HSCT

Domain	Index Case HSCT Mean Scores (SD)	Index Case No HSCT Mean Scores (SD)	p-value
Physical Function	80.5 (29.0)	78 (28.0)	0.73
Role Physical	77.0 (33.6)	71.5 (32.0)	0.52
Bodily Pain	70.4 (25.6)	58.4 (33.0)	0.12
General Health	58.2 (27.2)	53.4 (28.8)	0.54
Vitality	44.0 (27.3)	45.2 (23.8)	0.87
Social Function	68.0 (30.5)	68.0 (29.3)	0.99
Role Emotional	66.7 (32.2)	74.0 (29.6)	0.36
Mental Health	59.0 (17.5)	66.9 (16.3)	0.07

There were 40 of the participants who completed the SF36 who were mothers of the index case and 22 who were other relatives. Table 8-8 shows a comparison in means QoL scores between these groups. There were no significant differences found.

Table 8-8: QoL Scores in XL-CGD carrier Mothers compared with XL-CGD carrier other relatives

Domain	XL-CGD Carrier Mothers Mean Scores (SD)	XL-CGD Carrier Other Relatives Mean Scores (SD)	p-value
Physical Function	80 (28.0)	77.3 (28.8)	0.64
Role Physical	73.7 (33.8)	73.6 (30.7)	0.50
Bodily Pain	62.6 (32.2)	65.0 (27.2)	0.61
General Health	57.5 (28.8)	50.6 (28.0)	0.18
Vitality	44.7 (26.2)	42.7 (25.0)	0.62
Social Function	68.4 (32.2)	65.3 (25.9)	0.65
Role Emotional	70.3 (31.2)	70.1 (30.3)	0.98
Mental Health	62.6 (19.4)	62.7 (13.4)	0.98

-

8.2 Correlation with Psychological Health

Table 8-9 shows the correlation coefficient and p values for the association of mental health and physical health component scores of the QoL questionnaire in XL-CGD carriers with psychological health assessments. It can be seen there is a significant correlation for the MCS with anxiety, depression, self-esteem and fatigue but no significant correlation with the PIP scores. The PCS was significantly correlated with fatigue and the other domains were significant, but not highly significant. The correlation between mental health and physical health was not significant.

Table 8-9: Correlation Coefficient and P Value for MCS and PCS Domains of QoL with Psychological Factors

	MCS	**PCS**
MCS		0.21 0.1
PCS	0.21 0.1	
HAD-A	-0.55 <0.0001	-0.29 0.026
HAD-D	-0.72 <0.0001	-0.29 0.025
MF Total	-0.64 <0.0001	-0.53 <0.0001
Total PIP	-0.19 0.28	-0.39 0.022
Self-esteem	0.65 <0.0001	0.15 0.25

8.3 Correlation with Physical Health

Table 8-10 shows the correlation of MCS and PCS with physical factors. It can be seen that there are significant associations between PCS and all the physical factors except the skin manifestations of photosensitivity and recurrent abscesses. The are significant associations between MCS and joint pains, gastrointestinal and respiratory symptoms, number of ARA criteria fulfilled and recurrent skin abscesses.

Table 8-10: Correlation Coefficient and P value for MCS and PCS Scores of QoL with Physical Factors

	MCS	PCS
Joint Symptoms	-0.33 0.0085	-0.42 0.0009
IBD Score (bowel symptoms)	-0.69 <0.0001	-0.50 <0.0001
Respiratory Score	-0.32 0.016	-0.56 <0.0001
Photosensitivity	-0.12 0.36	-0.18 0.17
Recurrent Abscesses	-0.34 0.0074	-0.14 0.27
Ulcers	-0.14 0.30	-0.39 0.002
Number of ARA Criteria	-0.31 0.016	-0.39 0.002

8.4 Correlation with Social Factors

Table 8-11 shows the correlation of social factors with mental and physical component health scores in QoL assessment. It can be seen that there were no statistically significant associations, although the age of participant was approaching significance with the PCS scores.

Table 8-11: Correlation Coefficient and P value for MCS and PCS Scores of QoL with Social Factors

	MCS	PCS
Age of Participant	-0.55 0.67	-0.23 0.07
Relationship to Index Case	-0.049 0.71	-0.046 0.73
Age of Index Case	0.12 0.36	0.098 0.46
Number of Children	-0.13 0.33	-0.074 0.57
Index Case undergone HSCT	-0.13 0.45	0.17 0.32

8.5 Regression

A logistic regression analysis to evaluate the factors affecting QoL (physical function) in XL-CGD carriers was undertaken. The results are shown in Table

8-12, which shows that depression was significantly associated with increasing odds of poor physical function QoL. The presence of fatigue and gastrointestinal symptoms were approaching statistical significance.

Table 8-12: Logistic Regression for Factors affecting Physical Function domain of Quality of Life in XL-CGD Carriers

	Odds Ratio	95% CI	p-value
Anxiety (HAD-A)	0.91	0.65, 1.27	0.59
Depression (HAD-D)	0.62	0.39, 0.99	0.05
Self-esteem	1.05	0.83, 1.34	0.67
Fatigue	0.87	0.74, 1.03	0.11
GI Symptoms (IBD Score)	0.87	0.73, 1.02	0.089
Respiratory Symptoms	0.96	0.91, 1.01	0.17
SLE Criteria	0.70	0.29, 1.71	0.44
Relationship to index case	1.25	0.28, 5.67	0.77
Age	0.98	0.89, 1.08	0.66

8.6 Summary of QoL Results

- QoL is reduced in XL-CGD carriers overall
- Compared with data from women in the UK, XL-CGD carriers reported significantly poorer QoL in all domains
- QoL of XL-CGD carriers was poorer than that reported in adult CGD patients in several domains
- The presence of physical symptoms correlated with poorer QoL
- Anxiety and depression correlated with poorer QoL
- Fatigue correlated with poorer QoL

Chapter 9: Discussion

This study has generated a considerable amount of data on a wide range of issues. In this chapter, I will discuss the findings of the study, initially as separate sections; clinical findings and investigations, fatigue, psychological and quality of life. Finally, in chapter 10, I will draw together the discussion as a whole.

The principal finding of this study is that XL-CGD carriers suffer from more medical problems than previously described. The majority of the XL-CGD carriers suffered from at least one significant medical complaint.

Broadly, the medical problems may be categorised in a similar manner to those experienced by CGD patients, namely, infective, inflammatory, autoimmune and miscellaneous features. I will discuss each in turn.

9.1 Infective Manifestations

Infection is one of the hallmarks of CGD and, as outlined in chapter two, patients suffer recurrent, severe infection with characteristic organisms, including fungi and catalase positive organisms [28]. Significant or recurrent infections in XL-CGD carriers have been only sporadically reported in the literature, as discussed [74, 80]. Although it is likely that only significant, unusual or life threatening infections would be reported in the literature, and subsequently, there may be a greater burden of infection in XL-CGD carriers which has not yet been described.

This study demonstrated that 19 (23.5%) XL-CGD carriers suffered some form of significant or recurrent infection. The causative organism was not known in the majority of cases, but of particular interest was one case of fungal pneumonia, which is unusual in an otherwise immunocompetant individual, and whilst this is an individual case, it can be hypothesised that it is related to the XL-CGD carrier status and inability to handle fungus. Other significant infections included meningitis and pneumonia, which do occur in the general population, but may relate to the XL-CGD carrier status. However, there was no association with degree of reduction in NOB in those XL-CGD carriers affected by significant infection.

Recurrent infection was seen in the form of recurrent urinary tract infection (UTI) or recurrent skin abscesses. Six (7%) XL-CGD carriers suffered from recurrent urinary tract infection. The symptoms of this may be similar in nature to genitourinary obstructive symptoms. Whilst in the general population, infection is more common, obstructive symptoms may be present in the XL-CGD carriers. As the participants had not been investigated, this is speculation only.

Recurrent skin abscesses have been described in the literature in XL-CGD carriers and have also been problematic, requiring antibiotics and, at times, surgical drainage [70, 76, 77, 81]. They have been shown to isolate CGD typical organisms including *Staphlyococcus Aureus* [82].

This study confirmed this finding and demonstrated that it was a frequently occurring problem. Recurrent skin abscesses were reported in 14 (17%) of the XL-CGD carriers and all reported requiring antibiotic treatment.

Published literature considers an NOB value greater than 5% sufficient to prevent significant infection [58, 260]. This study evaluated whether there was an association between per cent functioning neutrophils and susceptibility to infection. Due to the small numbers, infections were evaluated as either systemic or skin and an association with NOB sought.

There was a significant association between the presence of recurrent skin abscesses and a lower NOB value. This was not found when infections outside of skin abscesses were considered. In all other infections there was no significant difference in NOB value between affected and unaffected. The mean NOB values were lower in the affected group, but not significantly. The lack of significance may relate to the relatively small numbers. However, given that CGD is a rare disease and the coverage of this cohort study, it is unlikely that the UK would be able to generate a larger study cohort of XL-CGD carriers.

Salmonella infection was reported in the European registry [10] as a significant problem in CGD patients. This was not replicated in the XL-CGD carrier population as only one XL-CGD carrier reported suffering from salmonella infection.

The reports of abnormal reaction to vaccination with BCG by 3 (4%) of the XL-CGD carriers demonstrates a striking resemblance to the report by Lee et al [33] of a similar finding in the CGD patients, supported by the large European registry report [10]. It is possible that the reactions reported from the XL-CGD carriers are due to abnormal response to the BCG as a result of their carrier status due to the similarities with the reports from CGD patients, but with such small numbers it is difficult to draw definitive conclusions. A documented complication of BCG vaccination is abscess, particularly if given incorrectly by the intramuscular rather than intradermal route [261], and this may be a factor in this case. However, the similarities of the descriptions suggest that there may be an association and further observations should be noted.

The lack of published literature about infection in XL-CGD carriers perhaps indicates a lack of significant infection in XL-CGD carriers and this study corroborates this. Infection was not reported as a significant problem in the majority of carriers and there were no reported deaths from sepsis or severe infection in the XL-CGD carrier population.

There were few infections reported in this cohort of XL-CGD carriers. Whilst this is the largest number of XL-CGD carriers studied, the rates of infection are still low. However, there were reports of some XL-CGD carriers suffering from CGD-like infections including lymphadenitis and fungal pneumonia. It has not been possible to determine why some suffer from infection and others do not. It would appear that there is no correlation with NOB value and risk of recurrent or significant infection with the exception of recurrent skin abscesses.

There were eight XL-CGD carriers on prophylactic antibiotics and four on prophylactic antifungal. This may have affected the infection rate in the XL-CGD carriers, but it cannot be certain. The lack of severe infections found in this cohort would not support routine prophylactic antibiotics or antifungal agents in XL-CGD carriers.

9.2 Inflammatory Manifestations

9.2.1 Skin Disease

Skin disease is the most commonly described problem in XL-CGD carriers with an association with discoid lupus erythematosis (DLE) well described [69, 72, 84, 85]. It was also the first reported medical problem in XL-CGD carriers as it was first described in 1970 [6]. Unsurprisingly, therefore this study has found significant skin disease in the XL-CGD carriers.

The majority (74%) of the XL-CGD carriers suffered from at least one skin manifestation. The most common problem was photosensitivity, which was seen in a large majority of the XL-CGD carriers (74%). This is in keeping with previously published work, although at a higher rate. In the largest published study to date, photosensitivity occurred in 58% of their 19 XL-CGD carriers [69].The higher rate reported in this study compared to previous studies may relate to the methodology used. All recruited participants were directly asked how they reacted to sunlight, rather than waiting for them to volunteer this information. XL-CGD carriers of all ages reported photosensitivity in this study, which was persistent through life.

After photosensitivity the most common skin complaints were a malar rash (resembling or confirmed as DLE) and eczema. Other frequently reported complaints were rosacea and adult acne, although it is possible that these diagnoses were not accurate as there was little evidence of investigation or expert opinion. The skin manifestations demonstrated in the XL-CGD carriers may reflect cutaneous inflammation. A high number of XL-CGD carriers also described recurrent episodes of hives, which may also reflect an inflammatory process. Without examining XL-CGD carriers during an outbreak or without photographic evidence it is difficult to be clear exactly what this represents.

Further to the diagnosed skin complaints described, two of the XL-CGD carriers complained of poor wound healing. It is difficult to be certain of how to interpret this information. The information was volunteered by the XL-CGD carriers and described as a significant problem, but it was not actively asked about. Therefore, this may represent an underestimate of the problem. CGD patients

may suffer from poor wound healing particularly after surgery [28]. It may be that this manifestation is related to carrier status, but in this study it is too infrequently described to be certain of the association. It is difficult to quantify this problem due to normal variations in wound healing.

There was one case of skin malignancy, which although noteworthy, as an isolated case does not advance our understanding about the health of XL-CGD carriers, but given the photosensitivity prevalence it should not be ignored.

Mechanism

The mechanism for the skin manifestations in XL-CGD carriers is unclear. The significant number of XL-CGD carriers who suffered from dermatitis may suggest that there is an inflammatory component.

There was no association between NOB value and photosensitivity or other skin manifestation seen. Additionally, there was no association between skin manifestations and autoantibody positivity when measured by immunofluorescence.

Freemer et al [134]found that there were higher rates of cutaneous lupus features in SLE patients who smoked. However, there was no association between smoking status in XL-CGD carriers and photosensitivity or other skin manifestations.

Strengths and Weaknesses

The main strength in this study is in the direct questioning of all XL-CGD carriers about photosensitivity. Responses to initial open questions frequently did not yield comments about photosensitivity, but when asked how they reacted to being in the sun, many more reported photosensitive eruptions.

A weakness of this study is the lack of involvement of a dermatologist. All skin manifestations were reliant upon self-reporting and pre-existing diagnoses. For conditions such as photosensitivity, history alone is sufficient, but for the more complicated or less well-defined diagnoses, history alone is less reliable. Very few of the XL-CGD carriers with skin complaints had undergone skin biopsy. Skin biopsy, whilst not essential, is helpful in confirmation of the diagnosis and in

increasing our understanding of the causation. The other limitation is perhaps unavoidable; the nature of many of the skin complaints is that they are transient, meaning that they were frequently not present at the time of enrolment and history taking. Time did not allow for frequent follow up with every individual participant. Medical photography may have been a useful adjunct for this aspect of the study.

Skin Implications

There are clinical implications from the findings of this study. XL-CGD carriers should be counselled that they are likely to suffer from photosensitivity. They should be given advice about sun protection; high factor sun screen, remain covered and ideally sun avoidance. What is unclear is whether the presence of photosensitivity puts the XL-CGD carriers at increased risk of skin malignancy. This was a predominantly middle aged cohort with a mean age of 42 years. Longer-term follow-up is required to see if there is an increased risk of developing skin malignancy in this cohort.

Uncertain dermatological manifestations in women who are XL-CGD carriers should be referred for accurate diagnosis (potentially including histopathological diagnosis) in order to focus treatment. From a research perspective this would also ensure a better understanding of the on-going pathological process.

Skin Future Work

The photosensitivity seen in the XL-CGD carrier cohort is now well described and this study has added considerable weight to the evidence that it is highly prevalent. What is unclear is the mechanism by which this occurs. Future work could involve photo testing the XL-CGD carriers which would determine the exact sensitivity e.g. UVA, UVB which may aid understanding about causation.

Biopsies were not available in the majority of XL-CGD carriers. Histological findings in the past from XL-CGD carriers manifesting DLE rashes have been similar to typical DLE [84]. However, all studies evaluating the DLE-like manifestations have been small scale making conclusions not well supported. Therefore, a large-scale study with skin biopsies from all symptomatic XL-CGD

carriers would be useful to further understand the causation of this manifestation.

Treatment of skin disease has not been well studied in the XL-CGD carrier population and for some it may be particularly problematic. Hydroxychloroquine has been trialled in the carrier population, but not in a systematic manner. A randomised control trial or at least an observational study of the use of hydroxychloroquine or similar agent would be helpful to advance treatment options.

9.2.2 Gastrointestinal

This study has found that XL-CGD carriers suffer from significant gastrointestinal (GI) symptoms, with over 50% suffering at least one symptom.

As discussed in chapters one and two, patients with CGD, and particularly XL disease [37], suffer from gastrointestinal disease, particularly colitis.

Children with CGD may present with poor growth or failure to thrive [28]. This was not seen in the XL-CGD carrier cohort where BMI was used to assess nutritional status. Less than 10% of the XL-CGD carriers fell into the underweight category with the majority falling into the healthy BMI range. When compared with female UK population data, the XL-CGD carriers are similar although the proportion in the underweight and overweight categories was significantly higher. This may reflect the small numbers and the inclusion in the XL-CGD carrier group of participants who were under 18 years, which may have skewed the results. Overall, the XL-CGD carriers have similar BMIs to the UK female population suggesting that gastrointestinal symptoms do not impact significantly upon weight in XL-CGD carriers.

This study found GI symptoms were present in over 50% of the XL-CGD carriers recruited, with abdominal pain and diarrhoea the most frequently reported. This has not been previously demonstrated with only sporadic cases reported [69, 90]as discussed in the literature review. In this study, one third suffered from frequent abdominal pain, one-third recurrent diarrhoea; one quarter regular rectal bleeding and just over ten per cent suffered constipation. Other symptoms including urgency and vomiting were infrequently described.

The types of gastrointestinal symptoms suffered in the XL-CGD carrier cohort were similar to that seen in the CGD patients, with abdominal pain and diarrhoea the most frequent. When compared with published data from CGD patients, the pattern of symptoms was slightly different in the XL-CGD carriers. Rectal bleeding was significantly more frequent in the XL-CGD carrier cohort and fewer suffering from abdominal pain, although it remained the most common symptom in both groups. Constipation was significantly more common in the XL-CGD carriers. It is possible these symptoms are unrelated to their XL-CGD carrier status and is an incidental finding, which may be reproduced in the general population, but the similar presentation and symptoms in carriers and patients suggests that there may be a unifying underlying pathological process.

The severity of symptoms and their impact can be assessed using the scores from the IBD disability index. Those who were affected by GI symptoms scored significantly higher than those who were unaffected. The high scores reflect the impact the GI symptoms have upon quality of life. Therefore, irrespective of the pathogenesis of the gastrointestinal symptoms, this study shows that they are of clinical importance to the XL-CGD carriers themselves.

Unfortunately, the majority of the XL-CGD carriers who were found to have GI symptoms had not undergone investigation, making drawing conclusions about the cause of their symptoms difficult, as there is limited information about the histopathology.

The reasons for lack of investigation may be multiple. It may represent under reporting of their symptoms to medical practitioners outside of the study. Alternatively, in those who had reported their symptoms, failure to undergo investigation may be due a presumptive diagnosis of an IBS-like diagnosis being made. The XL-CGD carriers frequently volunteered IBS as an explanation for their symptoms and, whilst we can only speculate as to the reason, it may be that the XL-CGD carriers underplayed the significance of their own symptoms.

Those who were investigated, and where results were available, showed a range of findings. The most interesting finding is in the two XL-CGD carriers who were diagnosed with Crohn's disease. As discussed in chapters one and two, the histopathological appearance of IBD and particularly Crohn's is very similar to

the findings in CGD colitis[15, 38] and it may not be possible to confidently distinguish between them, especially if the individual examining the tissue is not aware of CGD carrier status. One XL-CGD carrier, after investigation, was diagnosed with CGD colitis and had required extensive surgery.

In one symptomatic XL-CGD carrier, minor, non-specific inflammation was found on colonoscopy and was reported in the pathology sample as non-significant. However, it would be interesting to compare samples from other symptomatic XL-CGD carriers to see if they had any degree of inflammation and whether this finding was reproducible.

From those XL-CGD carriers who had undergone investigation, it can be hypothesised that their GI involvement is on a spectrum with those suffering from IBD and CGD colitis. It seems likely that there is a unifying pathology, although it has not been possible to prove this.

Appendicitis was reported in the European cohort [10] as occurring at similar rates to the population as a whole. In the XL-CGD carrier cohort, four had undergone appendicectomy. Histopathology was not available, therefore it is not possible to comment if this represents granulomatous disease.

Associations of GI Symptoms

As not all XL-CGD carriers suffered from gastrointestinal symptoms, and of those who did not all suffered to the same degree, associations were evaluated to see if there were tools, which could be used to differentiate the symptomatic from the asymptomatic and aid in the understanding of the underlying pathogenesis.

NOB and GI Symptoms

Neutrophil Oxidative Burst values were significantly lower in those suffering from abdominal pain and in those reporting diarrhoea. However, there was no significant difference when considering gastrointestinal symptoms as a whole, suggesting, firstly, that NOB will not help as a screening tool to identify those XL-CGD carriers at risk and, secondly, that it may not be the only factor in the pathogenesis of GI disease.

Kuhns et al [14] found no correlation between residual NADPH oxidase function and the presence of colitis, despite the association between residual NADPH oxidase and survival. There was an association between specific mutation and residual NADPH oxidase function.

In XL-CGD carriers the degree of residual NADPH oxidase function is, in part, related to the degree of skewing, reflected by the per cent NOB, and, in part, due to the specific genetic mutation. Therefore, if an individual is an XL-CGD carrier of a mutation conferring high residual NADPH oxidase function, they could have near normal NADPH oxidase function, whereas an XL-CGD carrier with a mutation conferring minimal residual function, who demonstrates extreme skewing would have virtually no NADPH oxidase function.

As the exact mutation was not known in the majority of the XL-CGD carriers in this study, it is not possible to make the same evaluations about XL-CGD carriers as Kuhns et al made in the study of CGD patients. All that can be said is that the degree of reduction in NOB does not correlate with GI symptoms as a whole. However, correlation with the mutation may be evaluated by using the index case as a surrogate marker for mutation.

Further associations of NADPH oxidase function and IBD have been demonstrated in a recent study by Dhillon et al[262] and specifically very early onset disease. Dhillon et al [262]demonstrated that patients suffering from very early onset IBD carried functional hypomorphic variants of NADPH oxidase components. Whilst this study of XL-CGD carriers has not been able to confidently conclude that the GI symptoms are associated with XL-CGD carrier status, the increasing understanding of NADPH oxidase suggest that there is likely to be a link, and that XL-CGD carriers are a further part of this spectrum of inflammatory bowel diseases.

The per cent of functioning neutrophils was derived from peripheral blood. It is not known if there is tissue-to-tissue variability in the number of functioning neutrophils and if this may account for why some are affected and others remain unaffected. The per cent of normally functioning neutrophils within the GI tract may differ to that found in peripheral blood and this may account for why some XL-CGD carriers are affected and not others. Sharp et al [263] found that in older

women, there was a significant difference in the degree of severe skewing between tissue types when they evaluated urine, buccal swab and blood DNA. This was less pronounced in younger women, however, and they suggested that this might be the result of secondary selection. It is, therefore, possible that there is a different degree of skewing in the gastrointestinal tract of those XL-CGD carriers who are affected by GI symptoms, compare with those who are not, particularly with increasing age.

It may also be that there is failure of clearance of an infective agent, which subsequently stimulates excessive inflammation. As outlined in chapter 2, in CD it is thought that underlying inflammation is caused by a high bacterial load triggering chronic inflammation as a result of patients being unable to clear the infective organism. Defective neutrophil recruitment has been implicated in CD. In XL-CGD carriers, there may be failure of clearance of organisms within the gut due to a similar mechanism. The reduced number of functioning neutrophils may be unable to clear the infective agent and thus result in on-going inflammatory changes.

Index Case Colitis and GI Symptoms

As discussed in chapters one and two, not all CGD patients suffer from colitis or GI manifestations of disease. Large studies support the higher prevalence of GI manifestations in XL disease, compared with AR disease[11].

The exact mutation was not confirmed in all of the XL-CGD carriers and it was assumed to be the same as the index case from which they were identified. As a surrogate marker for mutation, the index case was used and we looked for correlation between the presence of GI symptoms in the XL-CGD carrier and colitis in the index case to consider if there was an association with the mutation.

There was a significant association found, with XL-CGD carriers suffering from GI symptoms were more likely to have an index case suffering colitis. This suggests that there may be mutations more associated with GI disease than others in the XL-CGD carrier cohort.

Autoantibodies and Gastrointestinal Symptoms

There was no association found between the presence of GI symptoms and positive autoantibodies by immunofluorescence. However, the autoantibody panel performed was a baseline screening assessment only. It was not designed to be specific for gastrointestinal disease and as such, different autoantibody testing may have yielded a different result. For example, work by Lodes et al [264]in 2004 identified bacterial flagellin as a potent antigenic stimulant in patients with Crohns disease. Broader evaluation of potential antigens may have yielded better understanding of the mechanism of causation for GI disease in XL-CGD carriers.

Other Factors Affecting GI Symptoms

Numerous factors, including smoking, anxiety and age, have been shown to exacerbate underlying bowel inflammation particularly in the presence of inflammatory bowel disease (IBD) both at the onset of symptoms and in the presence of an established diagnosis [265] and these factors were studied in the XL-CGD carrier cohort.

There was a significant correlation between increasing age and gastrointestinal symptoms. No correlation was found with smoking.

As highlighted in the literature review, psychological factors may impact upon gastrointestinal symptoms, both in exacerbating existing conditions, including IBD, and in precipitating symptoms. We, therefore, looked for an association between anxiety and depression symptoms and the presence of gastrointestinal disease. A significant correlation was found between anxiety and the presence of gastrointestinal symptoms. However, what it is not possible to extrapolate the direction of the relationship between psychological distress and GI disease. This association has important treatment implications. It may be that those with gastrointestinal disturbance become anxious in part as a result of these symptoms, or it may be that there is a predisposition to anxiety, which precipitates GI disease or increases symptoms of underlying disease.

There was a significant correlation between fatigue as scored by the MFSI and the presence of gastrointestinal symptoms. As with the psychological correlation,

it is not possible to determine the direction of this relationship, but it would be useful to monitor fatigue levels after optimising gastrointestinal treatment.

The association of fatigue and anxiety with the gastrointestinal symptoms in XL-CGD carriers are similar to that described in the IBD patients[183]. XL-CGD carriers have not been shown to be significantly unwell prior to this study, but the findings of the gastrointestinal disease in XL-CGD carriers and the similar associations to those seen in IBD patients, show that not only are the XL-CGD carriers unwell, but they have similar factors exacerbating their symptoms as inflammatory bowel disease of other aetiology.

Strengths and Weaknesses of Gastrointestinal Research

Previous studies of XL-CGD carriers have not asked about gastrointestinal disease. This study actively asked about gastrointestinal symptoms and assessed their impact on quality of life using the IBD disability index. On direct questioning, many XL-CGD carriers admitted that they suffer from symptoms, which they had not previously divulged. Had the XL-CGD carriers not been questioned about gastrointestinal symptoms, this study would not have discovered the extent of the problem in the cohort.

Conversely, it is possible that by actively seeking out symptoms of gastrointestinal disease, this study has overestimated the severity of the problem with individuals reporting symptoms that may not be problematic. Gastrointestinal symptoms are subjective and this may be reflected in the individual reporting.

Additionally, formal results of GI investigations undergone by XL-CGD carriers were not always available despite being actively sought. Where possible, investigation reports were collated by the researcher to gain as much information as possible. This was due to the investigations being undertaken at a high number of centres and a lack of response to written requests for information. Informal results from the XL-CGD carriers themselves lacked the detailed information required for a study such as this.

BMI was used as a surrogate marker of nutrition. Poor growth is seen in the boys with CGD as already discussed and as such height and weight recorded in the XL-

CGD carriers to see if this was an important manifestation of disease. However, a more detailed assessment of nutrition may have been appropriate including more detailed measurements such as midarm circumference and triceps thickness, to accurately assess nutritional status. However, BMI was used as a crude assessment to look for hidden disease and as such is likely to be an acceptable screening tool.

Not all of the symptomatic XL-CGD carriers were investigated, making it difficult to draw conclusions about the pathogenesis. In those who were investigated, not all results were available despite being actively sought. This was due to the number of centres involved in the investigation and management of the XL-CGD carriers. A further limitation to this study is that not all of the specimens were examined at a specialist centre. As discussed in chapters one and two, differentiating between IBD and CGD specimens is difficult, particularly if the assessments are infrequent and this may be of particular importance in the XL-CGD carriers.

The specific mutation was not known at the time of analysis in a high number of families. The index case was used as a surrogate marker of mutation, but it would have been preferable to confirm the exact mutation in each XL-CGD carrier.

Implications and Meaning

This study has demonstrated significant GI symptoms in XL-CGD carriers, but no clear pathology and this has several clinical implications.

Firstly, the identification of gastrointestinal symptoms as a significant problem in the XL-CGD carriers means that appropriate clinical follow up and referral should be organised. As highlighted, there are many XL-CGD carriers who have not been investigated and as such, do not have a formal diagnosis and subsequently are not receiving optimal treatment. XL-CGD carriers should be specifically asked about gastrointestinal symptoms with an appreciation that they may not volunteer them otherwise.

Secondly, this greater awareness of gastrointestinal symptoms demands that there should be greater consideration of how these XL-CGD carriers should be

treated. Currently, treatment of gastrointestinal symptoms in XL-CGD carriers is erratic. Those with a formal diagnosis, such as Crohn's or ulcerative colitis, are managed as IBD patients. These patients may respond to conventional IBD therapy but may need to be treated as CGD patients. They should perhaps be considered along the spectrum of CGD colitis rather than IBD. Whilst there is considerable overlap in therapy, there may be differences to consider. Those without a diagnosis, have a wide array of treatments with steroids, anti-motility drugs, protein pump inhibitors and anti-spasmodic agents all reported. That symptoms are still described, along with an apparent impact upon quality of life, shows that there is considerable under or ineffective treatment.

A standardised approach to investigation and treatment of XL-CGD carriers with GI symptoms needs to be formulated. As more is learned about the pathology associated with these symptoms treatment options can be considered in greater depth. Closer liaison with gastroenterology services may improve the management options of all XL-CGD carriers and subsequently optimal treatment of these symptoms may improve QoL in XL-CGD carriers.

Gastrointestinal Future Work

This study has clearly demonstrated that there are significant gastrointestinal symptoms in the XL-CGD carrier population. What is not clear is the underlying pathology and mechanism of these symptoms. The significant correlation with the index case disease (and therefore specific mutation), the similarity in symptoms with the CGD patients and the findings in those investigated, implies there is a unifying pathology related to their XL-CGD carrier status. Future work needs to be directed at identifying the cause and pathogenesis of these symptoms.

Ideally, a future study would involve thorough investigation, including endoscopy, colonoscopy and biopsies, in both symptomatic and asymptomatic XL-CGD carriers for comparison. Ethically and practically, this may not be possible, but at the very least it would be beneficially to fully investigate all those who are symptomatic. It has not been possible to gather all histopathological results in this study. In future, it would be preferable to have all biopsy samples

evaluated at one of the specialised centres, where there is considerable experience of both CGD and IBD and their differentiation.

Further to microscopic examination of samples from the GI tract, an evaluation of tissue specific neutrophil function would aid the investigation of causation. It can be hypothesised that in XL-CGD carriers with GI symptoms there may be a lower number of functioning neutrophils in affected gastrointestinal tissue.

This study has not gathered information about the gut microbiota in XL-CGD carriers. This may be an important consideration in the pathogenesis. There is a growing body of evidence about the differences between the gut microbiota in healthy and affected individuals in conditions outside of CGD, including IBD [266] and necrotising enterocolitis in premature infants[267], amongst others. This may be of particular relevance in XL-CGD carriers who may be more susceptible to infection due to their reduced NOB and may be unable to clear GI infection. Analysis of the microbiota of XL-CGD carriers may aid this understanding.

9.2.3 Respiratory Disease

There is no published literature about respiratory disease in XL-CGD carriers, despite it being one of the commonest sites of disease in studies of CGD patients [8, 10, 11].

In this study, respiratory complaints were described in 15 participants. The most common problem was a diagnosis of asthma. Asthma is a common medical condition, and may frequently be over diagnosed when there is an alternative explanation for respiratory symptoms [268], perhaps suggesting some of the asthma diagnoses may not be accurate.

Cough was reported in three of the XL-CGD carriers as a significant problem, and all those reporting cough as a problematic symptom were non-smokers.

The results from the SGRQ when compared to the population data show that there was no significant difference between scores. This means that the XL-CGD carriers did not suffer respiratory symptoms significant enough to impact upon their quality of life. Those who did score highly on the SGRQ had an alternative diagnosis such as alpha-1-antitrypsin deficiency, which was more likely to

account for their symptoms and higher score. Co-existing XL-CGD carrier status and alpha-1-antitrypsin deficiency is previously unreported.

A limitation of this study is that no formal assessment of respiratory function or chest imaging was undertaken. It would have been useful to assess respiratory function in order to be confident that there were no respiratory manifestations of disease in the XL-CGD carrier cohort. Imaging in the symptomatic group would also have been a useful addition as this may have clarified the cause and elucidated more about the pathological process.

Despite the limitations, from this study, it is reasonable to conclude that XL-CGD carriers do not suffer from respiratory disease at a rate greater than that expected in the general population. However, further information about those with a diagnosis of asthma would be useful to explore that potential association.

9.2.4 Other Inflammatory Manifestations

This study did not examine for evidence of chorioretinitis as this has been previously well studied and XL-CGD carriers found to be at risk of chorioretinitis [34].

Inflammation of the urinary tract in CGD patients was discussed in chapters 1 and 2. There were 2 XL-CGD carriers who were prescribed bladder stabiliser medication and 6 XL-CGD carriers reporting recurrent UTI. Stress incontinence was also reported. There were no associated investigations documented in these women and these symptoms, may in fact represent inflammation within the genitourinary tract. In future, it may be prudent to undertake investigation in XL-CGD carriers presenting with urinary symptoms.

9.3 Autoimmunity and Autoimmune Symptoms

9.3.1 Mouth Ulcers

Recurrent, painful mouth ulcers have been previously described in XL-CGD carriers [69]. Sillevis Smitt's study found 70% of the 16 XL-CGD carriers surveyed suffered recurrent apthous ulcers [72].

This study confirmed this with 55 (75%) of the XL-CGD carriers suffering from painful, recurrent mouth ulcers, which is a slightly higher rate than described in earlier studies.

The presence of mouth ulcers did not correlate with the NOB value. However, there was an association between the presence of mouth ulcers and the presence of gastrointestinal symptoms. Oral ulcers are frequently seen in gastrointestinal disease including, but not exclusively, IBD [158]. Oral ulceration also form part of the ARA diagnostic criteria for SLE and will, therefore, also be considered in this context. Subsequently, there may be two mechanisms resulting in such a high rate of mouth ulcers in this cohort; a lupus like illness and inflammation of the gastrointestinal tract.

It would have been useful to photograph these mouth ulcers in order to provide a more accurate description and to demonstrate the similarities with those seen in the CGD patients. Biopsy results may improve our understanding of the cause.

Several of the XL-CGD carriers reported particularly problematic oral ulceration, which is difficult to quantify. Additionally, treatment of these oral ulcers was erratic. During the study, two XL-CGD carriers commenced hydroxychloroquine specifically for treatment of oral ulcers, but the response is not clear at the time of writing. Further evaluation of the use of hydroxychloroquine is required before it may be recommended for all XL-CGD carriers with problematic ulceration.

9.3.2 Joint Symptoms

Joint symptoms were reported in 47 (61%) of the XL-CGD carriers. Whilst they have been occasionally described in XL-CGD carriers previously [69] they have not been found at such high rates. All those in whom joint symptoms were a significant problem, reported episodic inflammation, pain and redness, often associated with a sensation of extreme fatigue, suggesting an inflammatory rather than degenerative pattern.

The most commonly affected joints were the hips and knees, and the small joints of the hands and fingers. The affected joint pattern may aid in the diagnosis of many rheumatological conditions, for example the small joints of the hand are

commonly affected in rheumatoid arthritis. Within the XL-CGD carrier cohort, there was an equal distribution of affected joints between lower and upper limbs.

Rheumatological conditions may also be categorised according to the presence or absence of autoantibodies, seronegative or seropositive. An association between autoantibody positivity and the presence of joint symptoms was investigated. More affected XL-CGD carriers were autoantibody positive then in the unaffected group. However, those suffering from joint problems were not universally autoantibody positive.

Increasing age may also affect the presence of non-inflammatory joint complaints such as osteoarthritis. The median age in the affected group was significantly higher and this may suggest that some of those affected by joint problems were suffering from age associated degenerative arthritis. More detailed information, including imaging, would improve the distinction between the different joint problems and allow for a comparison of factors affecting the development of joint problems in the different groups.

There was no significant difference between the NOB values in the affected and unaffected groups, suggesting that percent functioning neutrophils does not impact upon the presence of joint symptoms and does not predict which XL-CGD carriers are likely to be at risk of joint symptoms.

Non-erosive arthritis forms part of the ARA diagnostic criteria of SLE. The joint symptoms in the XL-CGD carriers will now be considered in the context of lupus criteria.

9.3.3 Systemic Lupus Erythematosus

As discussed in the section about skin disease, this study confirmed the findings from early literature that XL-CGD carriers have a high rate of discoid lupus manifestations. However, this study has found other features of SLE to be prevalent in the XL-CGD carriers.

The most striking finding in this study is that 26% of the XL-CGD carriers met 4 or more of the ARA criteria for a diagnosis of SLE and 30% met 3 of the criteria. A diagnosis of SLE is usually made when 4 or more ARA criteria are met. However,

despite this, only 18% had been diagnosed with a lupus-like disorder and the majority affected had not been diagnosed with SLE. Anecdotally, the XL-CGD carriers with SLE-like symptoms had been told that they did not have SLE as they had negative autoantibodies and some had not reported their symptoms prior to this study, which may account for the lack of SLE diagnoses.

Compared with patients with a diagnosis of SLE, XL-CGD carriers have similar manifestations. However, the pattern of disease is different, and therefore perhaps Lupus-like is a more appropriate diagnosis than SLE. A comparison of proportions was made between a large European study of SLE patients [129] and this cohort of XL-CGD carriers. The XL-CGD carriers were significantly more likely to suffer from photosensitivity, oral ulcers and Raynaud's phenomenon than the SLE patients. They also had significantly higher rates of arthritis and death. Nephropathy, serositis and neurological manifestations were more common in typical SLE than in the XL-CGD carrier cohort.

Previous reports have referred to a 'lupus-like' disease in XL-CGD carriers due to the lack of positive 'lupus autoantibodies' in these patients[69, 100]. However, the different pattern of symptoms may suggest that the process seen in XL-CGD carriers is a different process and forms part of the spectrum of disease of SLE, rather than is simply SLE. This is in keeping with what has been written about SLE, where authors have questioned if SLE, as it is currently defined, is one disease or many [137].

Neuropsychiatric lupus, as already discussed, includes manifestations, which are non-specific including anxiety and depressive disorders. Outside of mood disorder, the only neurological manifestations of SLE seen in the XL-CGD carrier cohort were headache, which is again non-specific, and cerebrovascular disease. Mood disorder will be considered at a later stage as it may or may not be part of a lupus pathogenesis.

The findings from this study are much more extensive than previously seen. Arthritis, photosensitivity and oral ulcers have already been discussed and shown in previous studies. This study, however, is the first to demonstrate the extent to which XL-CGD carriers may be affected by lupus like disease, and the first to note the different pattern. This study has also demonstrated that a

significant number of the XL-CGD carriers are suffering from sufficient numbers of the ARA criteria to be diagnosed with SLE, but a diagnosis has not been made. The importance of this is that it means many XL-CGD carriers are currently undiagnosed and untreated. SLE has a better outcome when treatment is initiated early [127] and SLE may impact upon quality of life (QoL), with patients with SLE reporting poorer QoL [140]. QoL in the XL-CGD carriers will be discussed later in this chapter and comparisons with SLE patients made.

9.3.4 Miscarriage

Recurrent miscarriage has been shown to be associated with SLE and specifically with APS [145]. There have also been reports, as highlighted in the literature review, that any extreme skewing of the X-chromosome may result in recurrent miscarriage[269]. Additionally, Haidar et al[270] reported one XL-CGD carrier with a poor obstetric outcome.

Nine XL-CGD carriers suffered at least one miscarriage, with one XL-CGD carrier suffering from more than one miscarriage. Miscarriage was not actively asked about, but was documented if it was volunteered or if it was evident in the GP medical records. Therefore, the rate of miscarriage and even recurrent miscarriage may have been higher than actually reported.

In this cohort of XL-CGD carriers, there was no association of miscarriage with NOB value. The assumption made is that NOB is an accurate surrogate marker for percentage skewing of the X-chromosome. This may be an accurate measurement but it will not reflect tissue to tissue variability, as already discussed, which may be an important consideration. Additionally, it is possible that the per cent of functioning neutrophils and NOB value may alter over time. In this study, NOB was only available at enrolment and in 33% of cases an historical value but not at the time of pregnancy or miscarriage which may be important.

There was no difference in NOB, number of ARA SLE criteria met or those diagnosed with lupus-like disease, between those who reported miscarriage and those who did not. Those who had suffered a miscarriage were younger in mean age, but this did not reach statistical significance. The XL-CGD carriers who

reported suffering miscarriage were not more likely to have positive autoantibodies, but specific autoantibodies associated with antiphospholipid syndrome were not tested.

Additionally two XL-CGD carriers described 'fertility' or 'reproductive' issues but did not elaborate. Fertility is a sensitive subject and women are frequently reluctant to discuss this in detail. It is also difficult to quantify what an individual means when they describe difficulties with conception. It is therefore, difficult to draw conclusions about this sub-group and know how to interpret this.

At least one of the XL-CGD carriers had received steroids during early pregnancy due to previous miscarriage. She delivered a full term baby without any obstetric complications. However, this is a simple case report. In order to determine if there is a benefit to steroids in early pregnancy a more structured approach is required and ideally a randomised, control trial. However, due to the small numbers of XL-CGD carriers it is unlikely that such a trial could be conducted in a useful time period. Therefore, more information is required about obstetric complications in XL-CGD carriers and miscarriage.

It is plausible that the reproductive issues, including miscarriage, described are related to being an XL-CGD carrier, but there is insufficient evidence from this study to support this. However, if an XL-CGD carrier suffers from lupus-like features, even in the absence of positive autoantibodies, and presents with fertility difficulties or miscarriage it may be reasonable to manage them as an SLE patient and a trial of steroids. This information should be collated centrally in order that the effect may be seen and further conclusions drawn.

9.3.5 Other Autoimmune Phenomena

Raynaud's phenomenon was seen in 27 (35.5%) of the XL-CGD carriers and was seen across all age groups. The prevalence of Raynaud's phenomenon in the general population is quoted as between 6-21%[271] in women. Therefore, the rate found in the XL-CGD carrier population is surprisingly high. Furthermore, in the XL-CGD carriers, Raynaud's phenomenon was seen in all age groups. Whilst Raynaud's phenomenon alone is not a life threatening medical complaint, it may cause significant discomfort and thereby potentially impact upon quality of life.

It also has associations with autoimmune conditions such as scleroderma [271], which may be more significant and further highlight an autoimmune pathogenesis in the XL-CGD carriers.

Alopecia was seen in 6 (7.7%) XL-CGD carriers. Alopecia may also have an association with autoimmunity[272]. It is difficult to be certain as to whether this is a significant finding in the XL-CGD carriers given the small number affected. As a finding alone it is unlikely to be relevant, but viewed in the context of the other autoimmune features, it adds weight to the hypothesis that XL-CGD carriers are at risk of autoimmunity.

SLE was the most commonly seen autoimmune disorder in the XL-CGD carriers. However, one XL-CGD carrier suffered from Sjögren's disease.

9.4 Other Medical Problems

9.4.1 Malignancy

The literature review highlighted case reports of increased risk of malignancy in CGD patients[50-52], although this was not replicated in the large registries [8, 10].This study of XL-CGD carriers did not report high rates of malignancy with only three XL-CGD carriers reporting any form of malignancy.

9.4.2 Dental

There are several examples of dental abnormalities associated with primary immunodeficiencies, for example Hyper IgE syndrome with the persistence of baby teeth and NEMO defects with conical incisors [273]. Delayed primary tooth loss has been described in patients with XL-CGD[274]

There have been few reports of XL-CGD carriers with dental problems and as such, recruited participants were not actively asked about dental problems. However, three XL-CGD carriers volunteered that they had unusual dental complaints.

The findings are of interest but given their low frequency and lack of detailed information or photographic evidence, it cannot be immediately assumed that these dental manifestations are part of the spectrum of problems from which XL-

CGD carriers suffer. However, as XL-CGD carriers suffer from recurrent mouth ulcers, there is already sufficient reason to encourage enhance oral health care to include regular dental follow up. Highlighting this as a potential problem may enable further evaluation of this as a potential are affecting XL-CGD carriers.

9.4.3 Ocular

Chorioretinitis was demonstrated to be prevalent in the XL-CGD carriers in Goldblatt et al's study [34] in 1999. This study did not aim to replicate this and chorioretinitis was not screened for. However, many of the XL-CGD carriers will have participated in both studies. Two of the XL-CGD carriers volunteered that they had chorioretinitis and this information is shown for completeness, but this low frequency is explained by the lack of screening.

Other ocular problems, photopsia and esotropia are reported in this cohort but at low frequencies. It is difficult to assess the significance of these findings and it seems likely that they are not particularly associated with XL-CGD carrier status.

However, the one case of retinal infection is of particular interest. In this case the retinal infection resulted in the XL-CGD carrier becoming partially sighted. There is a clear potential mechanism to associate risk of infection with the outcome, and this has been described in CGD patients[275]. There was limited information available in this case and more information may have provided better understanding of the relevance of this finding.

9.4.4 Cardiovascular

Increased cardiovascular risk has been found in chronic inflammatory conditions such as rheumatoid arthritis [276]. Subsequently, cardiovascular disease may be particularly pertinent in the XL-CGD carrier cohort. One of the two deaths in this cohort was as a result of coronary heart disease in an individual known to suffer from SLE. It important that cardiovascular risk is further studied in order to be considered in the context of other modifiable risk factors and primary prevention of cardiovascular disease in XL-CGD carriers.

Only four XL-CGD carriers volunteered that they were hypertensive, despite a greater number being prescribed anti-hypertensive medications. This highlights

the drawbacks to interviewing individuals about their medical health; there may be a discrepancy between what the patient believes to be wrong with them and what their physician considers to be their main medical complaints.

9.5 Patterns of Clinical Symptoms

A wide range of medical symptoms were described, therefore, patterns of clinical symptoms were looked for. The first observation is that it was rare to have significant physical symptoms and not suffer from photosensitivity, although photosensitivity as a solitary symptom was not infrequent. This suggests that if photosensitivity is not present, other symptoms are less likely, although possible.

Secondly, the most frequently occurring combination of symptoms was photosensitivity with joint symptoms affecting almost half of the XL-CGD carriers. This combination of joint symptoms and photosensitivity is suggestive of an autoimmune or SLE-type disease, which is in keeping with what has been described in earlier sections. The mean age in those affected by joint symptoms and photosensitivity was lower than in the other groups, highlighting that symptoms were not confined to the older XL-CGD carriers.

Thirdly, a quarter of the XL-CGD carriers suffered from joint and bowel symptoms and photosensitivity. Those XL-CGD carriers suffering from this trio of symptoms had a lower mean NOB than the other combination of symptoms. This may reflect a further symptom cluster and further work on evaluation of the different groups may improve understanding of aetiology. It is also important to observe that this was not an uncommon pattern, occurring in a quarter of XL-CGD carriers, despite it being the least common combination.

In summary, analysis of the combination of symptoms highlights that there may be different patterns of disease amongst the XL-CGD carriers, with those suffering from autoimmune or SLE-like diseases representing just one pattern. It also highlights that the majority of XL-CGD carriers suffer from more than one medical problem and that symptoms other than the presenting problem should be actively asked about. The analysis of NOB values in the patterns will be discussed in more detail in section 9.7.1.

9.6 Current Management of XL-CGD Carriers

Current management of XL-CGD carriers in the UK is erratic. The majority are not seen in a hospital setting, but those who are, are seen by a number of specialities with gastroenterology, immunology and rheumatology all involved.

45 of the XL-CGD carriers were on at least one prescribed medication. The broad spectrum of prescribed medications reflects the diversity of symptoms seen in the XL-CGD carriers. The commonest medications were hydroxychloroquine, antidepressants and analgesic agents other than paracetamol.

The large number of different classes of medications prescribed highlights two important features of the current management of XL-CGD carriers. Firstly, it shows a lack of uniform approach reflecting the poor understanding of the medical health of these women. Secondly, it reflects the diverse range of symptoms suffered. The medications are treating symptoms rather than an underlying pathology, which may be particularly important when considering psychological health and anxiety or depressive symptoms.

Due to the relatively small number of patients and the large number of medications, it is difficult to ascertain the impact of medications upon symptoms. Not all those who were symptomatic with lupus-like symptoms had been prescribed hydroxychloroquine. In SLE, early intervention is important to improve outcome [127]. The use of hydroxychloroquine has not been evaluated in XL-CGD carriers and further research is required to see if this may improve symptoms.

Prophylactic antibiotics were prescribed in eight (10%) of the XL-CGD carriers with 4 co-trimoxazole and 4 an alternative agent. Significant or recurrent infection occurred in 25% of the XL-CGD carriers. These infections were not associated with the degree of reduction in NOB. However, XL-CGD carriers were more likely to be prescribed prophylactic antibiotics with a lower NOB. It is unclear from this study whether prophylactic antibiotics should be recommended in XL-CGD carriers. The low rate of significant infection would suggest that overall there is no clear indication for prophylactic antibiotics. However, if an XL-CGD carrier suffers a significant infective episode or had

troublesome recurrent bacterial infections then a trial of co-trimoxazole may be appropriate.

The fact that recurrent skin abscesses were associated with a lower NOB may suggest these individuals would benefit from prophylactic antibiotics. During the course of the study, at least one XL-carrier suffering from recurrent skin abscesses commenced prophylactic co-trimoxazole. Further long-term follow up in this group of carriers is needed to determine the benefit of prophylactic antibiotics.

Four carriers were on prophylactic anti-fungal agents. However, fungal infection was not reported as a significant problem with only one episodes described. Therefore, it cannot be recommended from this study that prophylactic antifungal agents be part of standard care.

Steroids had been prescribed in five XL-CGD carriers. The reason for steroid prescription was varied. Steroids have been used with good effect in inflammatory rheumatological conditions including SLE. There may be a role for the use of steroids in XL-CGD carriers who suffer from joint symptoms but more information about the pathogenesis of symptoms is required and steroids should be used in a more formal trial setting to ascertain if there is a role for their use. At least one XL-CGD carrier had been prescribed steroids due to concerns about recurrent miscarriage.

9.7 Blood Investigations

9.7.1 Neutrophil Oxidative Bursts

The mean NOB in this cohort was 47% with a range of 7 to 94%. Published data prior to this study revealed a slightly smaller range of 20 to 80%[69]. This study is the largest available of XL-CGD carriers and this is likely to account for the wider range seen compared with previous studies. NOB was performed at enrolment, but historical data were available regarding neutrophil oxidative burst on 27 (33%) of the XL-CGD carriers.

Comparison of enrolment NOB results with historical NOB results revealed that there was a significant decrease in value. Whilst this is an interesting finding, it cannot be assumed that this reflects what happens to NOB with age.

Subsequently, NOB values were studied in comparison with the age of the individual. No significant correlation was found. However, this was comparing NOB value against age on a population (cohort) level rather than an individual level. There may be more subtle or significant changes when examined on an individual basis, but this would require a larger population-based sample.

This study was not designed to assess longitudinal NOB values and the methods used in historical and enrolment NOB tests may have differed. However, it does raise the question of whether NOB values change with increasing age. In order to look at the effect of ageing on change in per cent functioning neutrophils a longitudinal study is required to look at individuals at regular intervals over a prolonged study period. This work is currently underway as a joint project with GOSH, GNCH and Amsterdam.

Koker et al [59] reviewed three generations of XL-CGD carriers and demonstrated that there was a reduction in the per cent of normal neutrophils with increasing age over the three generations thus suggesting that as age increased, the per cent of functioning neutrophils reduced. In the current study, three families had three generations represented allowing evaluation of the different NOB values over the generations. Two of the families concurred with Koker's finding with lower NOB values with increasing age and generation. However, one family did not follow this pattern and in fact reversed the trend with the lowest value in the youngest generation and the highest value in the oldest generation.

Five families had two generations represented. In all five of these families, the lower NOB values were seen in the younger generation. One family had 2 generations, but 2 branches represented. In this family, there were higher values seen in the younger generation.

The lack of pattern through the generations in this study demonstrates that factors other than age are clearly important in the percentage of functioning neutrophils.

As highlighted in chapter 2, there are differing opinions over how lyonisation is governed, with increasing interest directed towards the genetics of controlling this apparently random process of X inactivation. Studying different generations of XL-CGD carriers gives us a small insight into how this process may manifest. Our observation is that there is no consistent pattern, with different generations showing highly different levels of skewing across different families, highlighting the need to investigate this further. Age alone cannot be the only factor determining the degree of skewing. As XL-CGD is a rare disease, research into more common XL genetic conditions may yield answers more quickly as to what governs this process.

NOB as a predictor of which XL-CGD carriers are likely to suffer from symptoms is not straightforward. Some correlation of NOB and symptoms was seen, as discussed earlier, with recurrent skin infection and specific gastrointestinal symptoms associated with lower values, but this did not correlate with all clinical symptoms. Additionally, some significantly affected XL-CGD carriers had greater than average NOB function.

However, when patterns of clinical symptoms were considered NOB values did differ between groups. The lowest NOB values were seen in the group where photosensitivity, joint symptoms and bowel symptoms were all present, with the next lowest seen in those affected by photosensitivity and joint symptoms.

It is difficult to be certain of the exact significance of this finding but nevertheless it is an important observation. The combination of photosensitivity and joint symptoms appears to be the connecting factor associated with lower NOB values suggesting it is those with autoimmune and SLE-type features who have lower NOB values, rather than simply those who are most affected.

9.7.2 Autoantibodies

Where it was possible to test for autoantibodies, the majority of XL-CGD carriers had negative autoantibodies. This is in keeping with previous research[69, 100].

In those who did have positive autoantibodies, it was most frequently that there was a positive ANA pattern.

Where autoantibodies were positive symptoms were present. However, not all XL-CGD carriers with physical symptoms, even in those with significant disease, had positive autoantibodies. Therefore, the absence of autoantibodies does not equate to the absence of disease. Anecdotally, this has led to XL-CGD carriers not having their lupus-like symptoms managed as they would be for SLE patients as it has led to uncertainty of diagnosis.

The lack of positive autoantibodies and correlation with clinical symptoms suggests one of two possibilities. Either autoantibodies are not present and another underlying mechanism is involved in the pathogenesis of disease in XL-CGD carriers or they are present but in insufficient quantities to detect by conventional methods. Measurement of autoantibodies was by immunofluorescence, and it may be that using more sophisticated techniques further autoantibodies would be detected.

At present, using conventional testing, autoantibodies cannot be used as a screening tool for symptoms of disease in XL-CGD carriers.

9.8 Clinical Summary

In summary, from this study it can be said that XL-CGD carriers are at risk of infective and inflammatory manifestations of CGD which, on the whole, do not correlate with the degree of reduction in NOB. XL-CGD carriers also suffer from a range of autoimmune phenomena.

It is unclear how affected individuals can be predicted with current standard non-invasive investigations. There are no clear guidelines about how symptoms in XL-CGD carriers should be managed.

9.9 Fatigue

Excessive fatigue was reported unprompted in over half of the XL-CGD carriers. Subsequently, fatigue was evaluated using the fatigue questionnaires, the MFSI and the vitality (VT) domain of the QoL SF36.

As outlined in chapter two, fatigue as an independent symptom has generated increasing interest over the past decade as it is recognised as an important feature of inflammatory conditions. However, it has also been described in the general population. Cale et al [69] commented in their study of 19 XL-CGD carriers, that the XL-CGD carriers reported fatigue, but it was not quantified or assessed using a validated tool. There is no other published literature about fatigue in XL-CGD carriers.

In this study, validated assessment tools supported the reporting of fatigue by the XL-CGD carriers, with those reporting excessive fatigue scoring higher in the MFSI than those who did not report fatigue as a significant problem. Fatigue was a consistent finding in the XL-CGD carriers irrespective of which tool was used to assess fatigue, as there was a significant correlation between the VT scores and all of the domains in the MFSI, thus demonstrating a degree of internal consistency. The VT domain has been shown to correlate with other fatigue questionnaires [179] and suggests that, however fatigue is measured, it was present as a problem in this cohort of XL-CGD carriers.

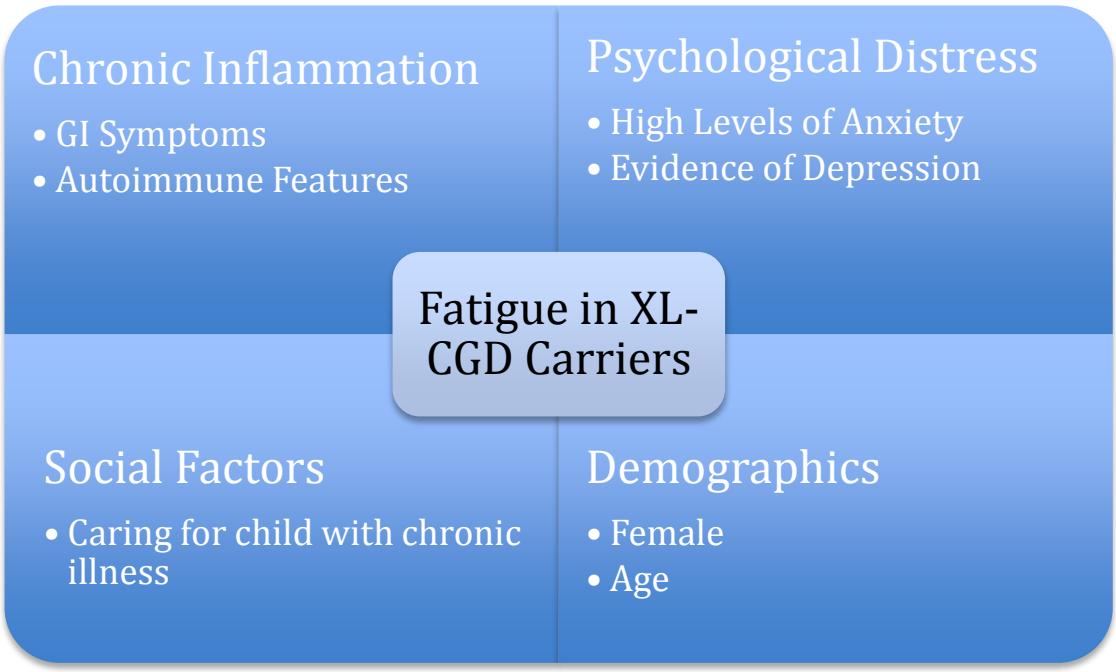
As already highlighted, fatigue is multidimensional and the MFSI allows for comparison between categories of fatigue. In the XL-CGD carriers, the highest scores were seen in the general category with mean scores in all other domains similar. This suggests an equal contribution of each domain to the overall high scores and subsequently, does not help to ascertain the cause of the fatigue.

The MFSI in XL-CGD carriers was compared with the scores from the MD carrier control group. The scores in the XL-CGD carriers did not demonstrate significantly higher levels of fatigue. Higher scores were seen in the MD carrier group. However, only 7 MD carriers completed the questionnaire compared with 60 in the XL-CGD carrier group. This makes a direct comparison unreliable. Additionally, one MD carrier reported suffering from Chronic Fatigue Syndrome, which in a small sample may skew the results. It may be that this woman was suffering as a result of being a carer of a child with a chronic illness, but it may be that she represents an outlier in this group. In a small cohort one individual may significantly affect the results. The MD carriers scored higher in the general domain than in any other domain. The MD and XL-CGD carriers had very similar

scores in the vigor domain. However, the MD carriers may also suffer from fatigue as a result of their own carrier status and disease.

Figure 9-1 shows the possible contributing factors to the aetiology of fatigue in XL-CGD carriers and each will now be considered in turn.

Figure 9-1: Potential Contributors to Fatigue in XL-CGD Carriers



Firstly physical symptoms were considered. Conditions outside of CGD, where there is chronic inflammation have been associated with fatigue as a significant problem. Literature about SLE, Sjögrens, IBD, sarcoid and primary biliary cirrhosis all report fatigue as a predominant feature, and frequently the most significant problem for patients [141, 214, 277]. As discussed in chapter two, a logical hypothesis may be that XL-CGD carriers suffer similar chronic inflammation resulting in the symptom of fatigue. Supporting this hypothesis is the association of the physical symptoms in the XL-CGD carriers with the presence of fatigue.

There was a significant correlation between level of fatigue and the presence of joint symptoms. XL-CGD carriers frequently described episodes of significant joint pain and swelling along with overwhelming fatigue. Those patients with joint pain scored higher on the MFSI than those without joint pain. Similarly, those with autoimmune features scored higher on the MFSI. It also further

highlights the similarities between SLE and the symptoms seen in XL-CGD carriers, as fatigue has been reported as an overwhelming feature in SLE [214].

The joint and bowel symptoms correlating with fatigue scores reflect the physical component of fatigue and may reflect a chronic, inflammatory process on going in the XL-CGD carriers. Due to this association, we hypothesised that the fatigue seen in the XL-CGD carriers could be the result of ongoing chronic inflammation and thus may be driven by pro-inflammatory cytokines.

The findings of raised IL-8 in the XL-CGD carriers support this hypothesis. There were significantly higher levels of IL-8 in the XL-CGD carriers compared with the healthy control group. When the XL-CGD carrier groups were divided into those who reported excessive fatigue and those who did not, there was a significant difference with fatigued XL-CGD carriers having higher IL-8 levels than the non-fatigued. A further comparison strengthened this hypothesis. IL-8 levels in the XL-CGD carriers were higher than Sjögrens patients who also suffered fatigue. The number of samples in this aspect of the study was small and the study was not statistically powered to look for differences and it demonstrates an association, but not direct causation.

However, the association of IL-8 with fatigue in XL-CGD carriers supports the concept of a biological component to the fatigue described. It is conceivable that XL-CGD carriers suffer fatigue due to a similar mechanism as that seen in the patients with chronic inflammatory conditions such as SLE, IBD and Sjögrens and may in fact be more pronounced. The overlap of fatigue in XL-CGD carriers with the symptoms seen in these chronic inflammatory conditions, in addition to the raised IL-8 levels support the hypothesis of chronic inflammation in XL-CGD carriers.

Secondly, psychological factors were considered. Fatigue may be a manifestation of depression and it could therefore be expected that if the fatigue seen was associated with depression, XL-CGD carriers with high fatigue scores would have higher depression scores and that there would be a correlation between fatigue and depression. Significant correlation was seen between HAD-D scores and fatigue levels; however, the HAD-D scores fell within the normal range suggesting that the depression levels were not significant. As the depression

scores were within normal limits, it would be surprising if they were significant enough to cause fatigue, but it cannot be ignored as a potential contributing factor.

Conversely, the anxiety scores were significantly higher than the general population and fell outside of the normal range. There was a significant correlation between anxiety scores (HAD-A) and level of fatigue. Correlation does not explain the relationship between the factors examined, but it could be hypothesised that persistent anxiety may result in fatigue. However, as there was also an association with joint pains, it may be that anxiety is the result of physical symptoms and the fatigue is a primary symptom from which anxiety develops.

Thirdly, social factors were considered. The number of children, age of the index case and relationship to the index case were considered as potential contributors to the fatigue scores. The impact of caring for a child with a chronic illness was also considered. There was a significant correlation seen between the number of children a participant had and level of fatigue. This is not surprising. It is logical that an individual may be more fatigued with a higher number of dependents and this may not be specific to being an XL-CGD carrier and may be reproducible in the general population. The age of the index case was not significantly correlated with fatigue level, suggesting that the age of a child does not impact upon fatigue levels.

The PIP was used to assess the impact of caring for a child with chronic illness. The scores were not significantly different between the fatigued and non-fatigued group. The lack of correlation suggests that whilst the role of carer may contribute to fatigue levels, it is not the sole contributor.

There have been mixed reports of the impact of age on fatigue levels, with younger age being more associated with fatigue in conditions such as sarcoid [182]. There was no association between age and fatigue levels in XL-CGD carriers meaning age cannot account for the fatigue seen.

There are limitations in the assessment of fatigue in this study. Firstly, the participants only completed the assessment at one time point. This, therefore, does not take into account the variations, which may occur over time. The

questionnaire specifically asks the participant to consider the past 7 days, which may not be truly representative of an individual's fatigue levels. This study would have been improved by assessing fatigue at multiple time points to gain a better understanding of fatigue in the XL-CGD carriers. Secondly, there are no population data with which to compare the XL-CGD carrier responses. Thirdly, the difficulties in recruitment of the control group meant that there was no direct sizeable comparable group.

This study confirms the informal results found in the CGD Society survey that fatigue is a problem in XL-CGD carriers, as yet not widely recognised. Whilst the causation of the fatigue remains unclear, the importance should not be unstated. Physical, psychological and social factors all affect the degree of fatigue seen in XL-CGD carriers. The fatigue reported in the MD control group suggests that there is an inherent fatigue associated with caring for an unwell child. However, the physical factors are more specific to XL-CGD carriers and were also significant, demonstrating that the aetiology of fatigue in XL-CGD carriers is not limited to the social and psychological factors, a hypothesis supported by the association of raised IL8 seen in the XL-CGD carriers. From this study, it is not possible to determine which factors are the most important.

As previously discussed, physicians do not always consider fatigue as an important symptom. However, it is frequently one of the most important complaints considered by patients and impacts significantly on QoL.

In the XL-CGD carriers, fatigue may be improved by optimal treatment of the newly identified physical complaints. Additionally there may be benefits in offering treatment of anxiety both for the anxiety and in order to improve fatigue levels. The social factors, whilst not modifiable, may be improved by further support. In fatigue that is not improved by treatment of the physical and psychological symptoms alternative therapies need to be considered. In order to direct therapies appropriately, further understanding of the mechanism of the aetiology of fatigue is required.

9.10 Psychological

9.10.1 Anxiety

Symptoms of anxiety were found in a high number of the XL-CGD carriers with 40% suffering mild or greater anxiety symptoms when screened using the HADS. The mean value for anxiety in XL-CGD carriers as an overall group was 9.47, which is above the accepted symptomatic cut off and is on the 75th per centile for UK women [248], demonstrating that the group, as a whole, suffer more anxiety than would be expected in the general population.

Only one XL-CGD carrier had a pre-existing diagnosis of anxiety, whilst six had a diagnosis of mixed anxiety and depression. By screening for anxiety, this study found that there were more than these pre-defined 7 XL-CGD carriers suffering significant levels of anxiety. This suggests that anxiety is currently under-recognised and under-diagnosed in the XL-CGD carrier cohort. There is no published literature about XL-CGD carriers and anxiety and therefore, the findings from this study are novel and direct comparisons cannot be made.

XL-CGD carriers with a HADS-A score greater than 8 would warrant consideration of treatment independent of the cause of the anxiety. As only 20% of the XL-CGD carriers were prescribed antidepressants, which are the mainstay of medical treatment of anxiety, this leaves a considerable number (at least 20%) who were suffering symptoms but not receiving treatment.

Not all individuals with anxiety symptoms choose to take medication, but this study suggests that the medical team were not aware of the presence of anxiety symptoms in the XL-CGD carriers, meaning that they would not offer treatment. Identification of anxiety symptoms is the first step in the management of anxiety.

Having established that anxiety is a problem amongst this cohort of XL-CGD carriers, further evaluation of causation was undertaken. The mean scores for anxiety were considered by relationship to the index case. There was no significant difference across the categories, suggesting that anxiety is present independent of the relationship to the index case, and that other factors may contribute to the development of anxiety. This was a surprising finding as it may be expected that those most closely related, particularly mothers, to the index

case would suffer the greatest levels of anxiety. However, the highest scores were seen in the grandmothers. Although this was not statistically significant, this could suggest that those who are less closely involved are more anxious. The lack of association between relationship and anxiety suggests that there are other important factors in the aetiology of anxiety or that it may be intrinsic to being a carrier rather than external factors. Alternatively it may also suggest that the wider family may be at risk for the development of anxiety. However, the numbers in the study are relatively small and may lack statistical power, particularly when considering across more than one category.

As psychological health may change with age[212], anxiety scores were evaluated in respect to this. This study did not find a significant association with increasing age and anxiety; therefore, this is unlikely to account for the small differences seen between mothers and other relationships, specifically grandmothers.

A proportion of the index cases had undergone HSCT. The stresses for parents of the HSCT process were discussed in chapter two. A comparison of anxiety levels of the relatives where the index case had and had not undergone HSCT showed that the anxiety scores were higher where HSCT had been undertaken. The difference did not reach statistical significance, but this does demonstrate that anxiety persists despite the cure of the index case. The importance of this finding is twofold. Firstly, clinicians must be aware that psychological distress may persist even after the index case has undergone definitive treatment. Secondly, it suggests that there may be factors other than caring for a child with a chronic illness that are important in the development of anxiety in this group, including intrinsic factors of being a carrier. Alternatively, it may also reflect that parents, do not have the same confidence that physicians have that HSCT is curative.

How does anxiety in XL-CGD carriers compare to other groups?

As the anxiety scores were significantly higher than the UK norms and there are no previously published studies in the XL-CGD carriers, comparisons were made with published data from groups who may have similar factors affecting them.

Firstly, anxiety scores were compared with published data from parents of children with other chronic medical conditions to evaluate if a similar picture was seen in conditions outside of CGD.

Published data were available from CF parents[251]. The studies used the same assessment tool and were both one-off assessments of anxiety, with similar study designs allowing for comparisons to be made. CF is a chronic condition with a reduced life expectancy and requires parental involvement in medical care. Thus, there are considerable similarities with CGD.

Significantly more XL-CGD carriers suffered greater than normal anxiety symptoms compared to CF parents. The mean anxiety score was also higher in the XL-CGD carriers. Although this was not part of the same study, the use of the same tool allows for this direct comparison. The significantly higher scores in the XL-CGD carrier group, suggest that either diagnosis and living with CGD is associated with greater anxiety than CF or that factors outside of parenting a chronically unwell child play an important role in the causation of anxiety symptoms. This is further supported by the lack of correlation of anxiety scores with the perceived stresses of parenting shown in the PIP, which will be discussed later. One further consideration is that the CF study included fathers as well as mothers, which may have impacted upon their findings. Women are more likely to suffer from anxiety than men [212] and the lower scores in the CF group may reflect the lack of inclusion of fathers.

The anxiety scores were compared with a second group of parents, the MD control group. The findings were similar. The XL-CGD carriers had higher anxiety scores, although this did not reach statistical significance. The control group recruited was considerably smaller, but support the findings from the comparison with the CF parents, and a larger cohort of MD carriers may have validated this.

Secondly, as reasons outside of parenting a chronically unwell child may have impacted upon the high anxiety rates in the XL-CGD carrier group, anxiety scores were also compared to published data from SLE patients [140, 229]. As SLE symptoms have been described in the XL-CGD carrier cohort, anxiety in SLE is of

particular interest as there may be a common pathogenesis, particularly as anxiety is one of the possible diagnostic criteria for neuropsychiatric SLE[138].

The data published about SLE patients were divided into those with high and low levels of pain. Waldheim et al's[229] study found significantly higher levels of both anxiety and depression in the high pain group and concluded that pain was an important contributing factor. Anxiety scores in the XL-CGD carriers were most similar to the SLE patients with high pain (non significantly higher mean) and significantly higher than SLE patients with low pain. It is difficult to draw conclusions from this comparison but it furthers our appreciation that XL-CGD carriers are more comparable to SLE patients than the general population with regards to both physical and psychological symptoms, and suggests physical symptoms may contribute significantly to psychological health.

A study by Tench et al[140] also assessed anxiety in SLE patients and found similar results with a mean of 9 for anxiety as well. There was no significant difference between the XL-CGD carriers and the SLE patients in Tench's study, supporting the similarities between these groups.

Causation and Association

The presence of high rates of anxiety in XL-CGD carriers has been well demonstrated in this study, but the reason for the anxiety is less clear. As outlined in chapter 2 there are several possible contributing factors.

As already discussed, despite that it might be expected that being a mother of a case may be associated with greater anxiety levels, there was no significant increase in anxiety score for mothers of the index case when compared to non-mother relatives. This suggests that it is not solely the relationship with the index case that determines anxiety. It may be that mothers have more involvement in daily care and feel more in control, thus lessening their anxiety symptoms, whilst grandmothers are more on the periphery although still affected by genetic guilt and concern for their grandson. However, this is speculation and was not the primary aim of the study. A qualitative study would be required to establish the answer to this question.

There was no significant association between anxiety scores and the assessment of caring for a child with chronic illness (PIP). This concurs with the lack of association between anxiety and relationship to the index case and is further evidence that the anxiety present in XL-CGD carriers is not solely related to parenting a child with chronic illness.

The similar levels of anxiety to SLE patients, and particularly with the high pain group, suggest that physical symptoms may be an important contributor and therefore, these were evaluated. There was a significant correlation between anxiety score and the presence of both bowel and joint symptoms in the XL-CGD carriers. Due to the cross-sectional nature of this study, it is unclear in which direction this association is. It may be that those with symptoms of urgency or diarrhoea are anxious as a direct result of this, or, as previously highlighted; there may be more gastrointestinal disturbance in those with an underlying anxiety. The correlation of anxiety scores with the presence of joint symptoms also reached statistical significance. It is less likely that anxiety may predispose to joint pain and stiffness. However, the association of anxiety with high pain in the SLE study suggests that this may be a significant factor, which has been replicated in the XL-CGD carrier cohort.

As neuropsychiatric lupus may present with mood disorder and may be associated with other features of SLE, correlation with other SLE symptoms was evaluated. There was no significant association with either an established diagnosis of lupus-like disorder or the number of the ARA SLE diagnostic criteria met in the XL-CGD carriers. Whilst neuropsychiatric SLE may present without other systemic manifestations, the lack of correlation with these criteria may also reflect a different underlying mechanism in the development of anxiety.

Anxiety has been associated with chronic inflammation as discussed in chapter 2, and this may be an important component of the anxiety seen in XL-CGD carriers. As was demonstrated with the raised IL-8 and fatigue in XL-CGD carriers, an inflammatory component may be important in psychological health.

There was a significant correlation between scores for anxiety and the total fatigue score. Fatigue is a well-recognised symptom of depression[212], but it is less well described in isolated anxiety.

Impact of Anxiety on Other Psychological Factors

A significant association was seen between anxiety scores and depression scores, despite the fact that very few XL-CGD carriers suffered significant depression. An association was also seen between anxiety and self-esteem with those who were most anxious suffering the lowest scores in self-esteem. This may suggest that the presence of anxiety impacts upon other aspects of psychological health, reiterating the importance of identifying anxiety and managing it appropriately.

Anxiety also appeared to impact upon QoL, with those who were most anxious suffering from lower QoL. This is further evidence of the importance of identifying and managing anxiety in XL-CGD carriers.

Anxiety Summary

In summary, the XL-CGD carriers suffer high rates of anxiety, which is significant in several cases. This has not been previously demonstrated. The anxiety does not correlate with relationship to the index case, age or the presence of SLE-type symptoms, but does appear to correlate with the presence of bowel and joint symptoms, fatigue and higher depression scores. It is not more significant in the mothers than non-mothers and is more prevalent than in parents of children with other chronic conditions.

The identification of high rates and degrees of anxiety which were previously undiagnosed, highlight the need for screening tools to be introduced into clinical practice. All XL-CGD carriers should be screened regularly for the presence of anxiety symptoms and managed appropriately.

9.10.2 Depression

Depressive symptoms were less frequent than anxiety in the XL-CGD carriers with only 27% suffering from depressive symptoms. The mean HAD-D score was 5.08 in the overall XL-CGD carrier group. Whilst this mean score falls into the normal category, when compared to UK female norms this falls on the 66th per centile [248] suggesting there are slightly more symptoms than in the average population. Similarly to anxiety, there appears to be a greater burden of depression than previously appreciated, as only 13 (16%) XL-CGD carriers had a

pre-existing diagnosis of depression or combined anxiety and depression rather than the 27% found on screening. This may reflect under diagnosis or lack of reporting of symptoms.

There was no significant difference in depression scores when the relationship to the index case was considered. Slighter higher scores were seen in the cohort of grandmothers, but this did not reach statistical significance.

How does this compare to other groups?

The depression scores from this study were compared to results from other published studies.

The proportion of XL-CGD carriers suffering from depressive symptoms was very similar to the published work by Besier et al[251] about CF parents and there was no significant difference. In both the CF group and the XL-CGD carrier group, very few individuals suffered from significant depression. This suggests that the stresses of having a child with chronic illness do not manifest as depression. This is further supported by the lack of correlation between the total PIP scores and depression scores. Those with higher PIP scores did not have higher HAD-D scores.

The depression scores in the XL-CGD carriers were similar to those seen in the control group of MD carriers and were, in fact, slightly better. There are many factors, which may explain this. Firstly, it may relate to the lack of a definitive curative treatment for MD, whereas HSCT remains a curative treatment option for CGD. Secondly, it may reflect that other factors, outside of chronic illness impact upon depression. This supports the findings from the comparison with the MD carrier group.

When compared with the SLE patients, the results were mixed. XL-CGD carriers suffered greater depression scores than SLE patients classified as low pain from Waldheim et al's study [229], but were not as significant as the SLE patients with high levels of pain. This may reflect the importance of physical symptoms to the psychological health.

Factors affecting depression

Correlation between physical symptoms and depression scores were examined. Unlike anxiety, there was no significant association between the depression score and the presence of gastrointestinal and joint symptoms, and similarly there was no association with a diagnosis of lupus-like disease or the number of SLE ARA criteria met. Previously, in the general population depression has been associated with increasing age [251], but this was not found in the XL-CGD carriers where there was no correlation between age and depression score.

An association was seen between depression and anxiety as previously outlined, and the impact upon self-esteem by depression was also significant. There was a significant association between depression scores and overall fatigue scores. Fatigue has been described as a biological symptom of depression. However, the relationship between fatigue and depression in XL-CGD carriers may be bi-directional. There was no difference when HSCT in the index case was considered.

Depression Summary

In summary, depression scores were slightly higher in the XL-CGD carrier cohort than population data would predict, however, the majority of XL-CGD carriers did not suffer significant depression symptoms. There was no association with physical symptoms, with the exception of fatigue, age or caring for a child with chronic illness. Depression scores correlated with self-esteem and anxiety.

Implications of Anxiety and Depression Findings

The presence of anxiety within the XL-CGD carriers, irrespective of the cause, has important clinical implications.

All XL-CGD carriers should be screened for the presence of anxiety and depression symptoms given the high rates uncovered in this study, which were previously undiagnosed. Clinicians caring for children and families with CGD need to be aware of the high rates of anxiety in the XL-CGD carriers. Both anxiety and depression may present with non-specific symptoms and, as this study has shown, may not be overtly evident unless actively sought. Severe anxiety or

depression may impact upon the ability to manage complex medical needs without support. Anxiety and depression may also impact upon an individual's quality of life, which with treatment may be improved.

Those who are detected to suffer from anxiety or depression should be managed appropriately and offered medical or psychological therapy.

The findings from this study may be transferrable to conditions outside of CGD and particularly other primary immunodeficiencies and further studies should investigate this.

This study supports the development of psychological support services for the families and specifically the carriers.

9.10.3 Self-Esteem

Self-esteem was assessed in both the XL-CGD carriers and the control group.

Normal self-esteem was largely preserved in the XL-CGD carriers with 46% falling in the normal range. However, one third did suffer from low self-esteem and this was associated with higher scores in both anxiety and depression domains suggesting that anxiety and depression may impact upon self-esteem and highlighting the need for appropriate management.

There was no significant difference in self-esteem scores across the relationship groups. However, XL-CGD carriers scored significantly lower in self-esteem than the MD control group. Given the small numbers in the MD control group, it is difficult to be certain as to the significance of this difference. All that can be said is that there is reduced self-esteem in the XL-CGD carriers, which is out of keeping with that seen in another cohort of carriers of another chronic disease.

9.10.4 Psychology Summary

In summary, this study has demonstrated high rates of anxiety in the XL-CGD carriers and moderate rates of depressive symptoms, irrespective of age, relationship to index case and assessment of stresses associated with caring for a child with chronic illness. Screening for anxiety and depression should be implemented. Clinicians should be aware that these problems may impact upon self-esteem and quality of life.

9.11 Caring for a child with chronic illness

Unsurprisingly, this study has found that there are significant stresses associated with caring for a child with a chronic, life limiting condition, which is evidenced by the high scores seen in the PIP questionnaire. The scores were higher in frequency than severity.

There may be many factors affecting the difficulties of caring for a child with a chronic illness. The age of the child may impact upon caring; older children may be more challenging, as they will be required to cooperate with treatments whilst younger children have little autonomy. The older child may already have developed more significant complications of CGD or had several prolonged hospital stays. Despite these possibilities, there was no significant difference in the PIP scores when age of the index case was accounted for. This was unexpected but may be accounted for by the small numbers within each age category explaining why the differences did not reach statistical significance. The highest scores were seen where the children were aged 7-12 years. There were 5 mothers of index cases over the age of 18 who completed the assessment and this group showed the lowest scores in all aspects of the PIP (total, frequency and severity). However, it should be noted that several of the mothers of older index cases declined to complete the PIP assessment as they felt it was not appropriate as they were not involved in their son's medical care. Therefore, these scores would be even lower if these individuals were included.

The number of children a participant had correlated with higher PIP scores, but did not reach statistical significance, although it was approaching significance. Again, it is not surprising that having more children creates more distress, but this study has demonstrated that those with more children may require greater support.

The scores in the XL-CGD carrier mothers were compared to Streisand et al's [255] study in parents of children with an oncological diagnosis. The scores were overall similar, but the XL-CGD mothers scored significantly higher in the frequency domain, meaning that the regularity with which they were having to attend to their child's medical needs was more of a concern. These similarities

suggest that the condition per se is not the determining factor and raise the possibility that these findings would be transferrable to other conditions including other PIDs. This is further evidence of the need to support the mothers (and families) of children with CGD.

The XL-CGD mothers were also compared to the MD control group. The XL-CGD mothers score significantly higher in the frequency domain, whilst the MD carriers scored more highly in the severity domain. This comparison further supports the comparison with the oncology patients in demonstrating that the disease itself is not the determining factor.

The impact of the stresses of caring for a child with a chronic illness was evaluated by looking at the effect on psychological health and quality of life. As discussed earlier, the PIP scores did not correlate with anxiety and depression, suggesting that the stresses of caring for a child with a chronic illness were not the significant contributor to impaired psychological health.

Cole et al [278] demonstrated that quality of life in children with CGD was better having undergone HSCT, but there has been no formal assessment of how this impacts upon the mothers. A comparison was made between mothers of those who had undergone HSCT and those whose children were managed conservatively. This demonstrated that the PIP scores were lower where the child had undergone HSCT. However, these results did not reach statistical significance, but suggest that after a successful HSCT, improvements are seen in the mothers as well as children with lower levels of distress, although anxiety symptoms persisted.

The findings from this study are not surprising, but are important, as they have not previously been demonstrated on this scale. Within the field of PID, there has been limited research into the impact upon families. This study clearly shows that there are significant stresses associated with caring for a child with CGD. This may be more widespread within the family, but this was not assessed in this study. It highlights the need for support for the carers, as well as the patients.

Parental distress may not only impact upon their own mental health, but may impact upon their child. A recent study in parents of children with IBD[279]

demonstrated that high levels of parenting stress predicted depression in their adolescent children. This has not been evaluated in the XL-CGD children but highlights the importance of parental distress for the entire family as well as the individual parent.

This study was designed to evaluate the health of the female XL-CGD carriers and subsequently, we did not evaluate the impact upon fathers or male relatives. It may be useful to study this group in the future in order that services may be provided to best support the families as a whole.

In summary, this study has shown there are significant difficulties in caring for a child with CGD, but that these are at similar levels to published work in other conditions. There was no association with the degree of perceived stress and the presence of depression in the XL-CGD carriers, although levels of anxiety appeared to correlate. The stresses of caring were not significantly associated with the age of the child, but this may be due to the small numbers within each category.

9.12 IQ

At the conception of this study, evidence from Pao et al's[230] study suggested that CGD patients may have significantly more learning difficulties than the general population. Hence, IQ assessment in XL-CGD carriers was included in the study. However, as outlined in chapter two there were several flaws with their study and subsequently Cole et al[232] demonstrated no such deficit in CGD patients, albeit with a briefer IQ assessment.

IQ assessment was undertaken in only a small number (9) of the XL-CGD carriers. The main reason for the small number completing this component of the study was the time consuming nature of the assessment, as each assessment takes an hour to complete. The small number of participants completing the WAIS makes it impossible to draw conclusions. Additionally, there may have been an unintentional selection bias, as more educated individuals may have been more likely to agree to IQ assessment, or those not in full time employment may have been more likely to be able to spare the time.

Despite the small numbers, it can be observed that in six of the nine XL-CGD carriers, their lowest scores compared with national per centiles, were seen in the working memory domain. IQ is affected by a number of factors with genetic and environmental factors all thought to play a role [280, 281]. Working memory has been of particular interest as it has been demonstrated to be reduced in chronic inflammatory conditions [282]. Further evaluation of IQ on a larger scale is needed before conclusions can be drawn as to whether this finding is significant.

9.13 Quality of Life

Quality of life (QoL) is reduced in patients with CGD [278] but there has been no assessment of QoL in XL-CGD carriers prior to this study.

The most striking finding from this study is that quality of life was significantly worse in XL-CGD carriers than in the UK population. XL-CGD carriers have not been previously considered to be unwell or have poor QoL.

This study found that XL-CGD carriers scored poorly overall in the QoL questionnaires. XL-CGD carriers scored particularly poorly in the vitality, bodily pain, general health and social function domains. The vitality domain reflects energy levels and correlates with the fatigue reported in the XL-CGD carriers. They also had low overall scores for both physical and mental health component scores.

9.13.1 Comparison with other groups

In order to interpret these findings and to view them in context the scores from the XL-CGD carriers were compared to population data and other affected groups.

The QoL in the XL-CGD carriers was significantly lower than published UK norms when considered both as an overall score and when each domain was considered individually. XL-CGD carriers were significantly worse in all domains when compared to UK population data of a female cohort of similar age. This comparison confirms that the finding of reduced QoL in the XL-CGD carriers is significant and different to that found in the general population. It is of particular

importance that the comparison was made with population data from women of the same age and UK resident. As outlined in chapter two, gender may contribute to QoL and there may be variations between countries. The limitation to this assessment is that the data were not collected at the same time. It is possible that QoL at a population level is different at different time points due to the variability of factors affecting QoL.

The particularly low scores in the general health and bodily pain domains highlight that the XL-CGD carriers rate their physical health poorly. The poor scores in the social function domain demonstrate the effect of the reduced QoL on daily functioning.

QoL data were also compared with QoL data from adult CGD patients [258]. Interestingly, QoL was significantly worse in the XL-CGD carriers than in the CGD patient cohort in the bodily pain, vitality and social functioning domains. This was an unexpected finding, but we can hypothesise many reasons for this. It may be that the impact on QoL of caring for a relative with CGD, as evidenced by the high PIP scores, is greater than being unwell. Alternatively, the XL-CGD carriers own unmet health problems may have impacted upon their QoL. The psychological distress as evidenced by anxiety and depression symptoms may also have played an important contribution.

To explore the relationship between QoL and the burden of caring, the XL-CGD carrier QoL data were compared with published data from other carers. A study of carers of adult brain tumour patients was used [211]. This study was performed in a similar manner and used the same QoL assessment tool allowing for direct comparisons of scores to be made. In all domains the XL-CGD carriers scored lower than the published data. The XL-CGD carriers were significantly lower in four of the domains; vitality, general health, bodily pain and physical function.

The significant difference suggests that caring for an unwell relative is not the sole contributory factor to poor QoL. It is of particular interest that the domains in which there was a significant difference were those in which physical health was likely to contribute, suggesting that whilst the psychological effects of caring may be constant, the physical domains of QoL are affected by other factors.

However, it may be that the major contributor is the unmet physical problems found in the XL-CGD carriers.

In the brain tumour group, the majority of patients being cared for were adult patients. It is possible that there are differences between caring for an adult patient and a paediatric patient and that this may account for some of the differences. It is possible that whilst there are similarities in caring, whomever the patient, that caring for a child is more physically demanding than caring for an adult as evidenced by the worse scores in the physical domains for the XL-CGD carrier cohort. However, the XL-CGD carriers scored better than the brain tumour carers in the mental health, including social functioning domains. This may suggest that caring for a child with a chronic illness is less socially isolating than caring for an adult and this may be protective with regards to the mental health components of the QoL assessment.

The QoL scores in the XL-CGD carriers were compared with the MD control group of carriers. There were significant differences found between the groups. The XL-CGD carriers scored significantly worse in the general health, vitality and role emotional domains. The MD carriers were significantly worse in the bodily pain domain.

The major limitation with the control group is the size. As only 7 MD carriers completed the SF36 compared with the 62 XL-CGD carriers who completed it, the control group may not be reflective of MD carriers as a cohort. This may account for the lack of pattern in the differences and makes it difficult to draw firm conclusions.

However, the significantly lower scores in QoL in the XL-CGD carriers suggest that caring for a child with a chronic disease is not the only important factor in determining QoL. In the context of the other comparisons discussed, this further supports the hypothesis that intrinsic rather than extrinsic factors are important in QoL in XL-CGD carriers. MD carriers were chosen due to the similarities in the demands of caring for children with CGD and MD. Therefore, the lower scores in the XL-CGD carriers suggest that factors outside of caring for a child with chronic illness are important. The worse scores in the physical health domains reiterate the clinical findings of poor health in the XL-CGD carriers. The lower scores in

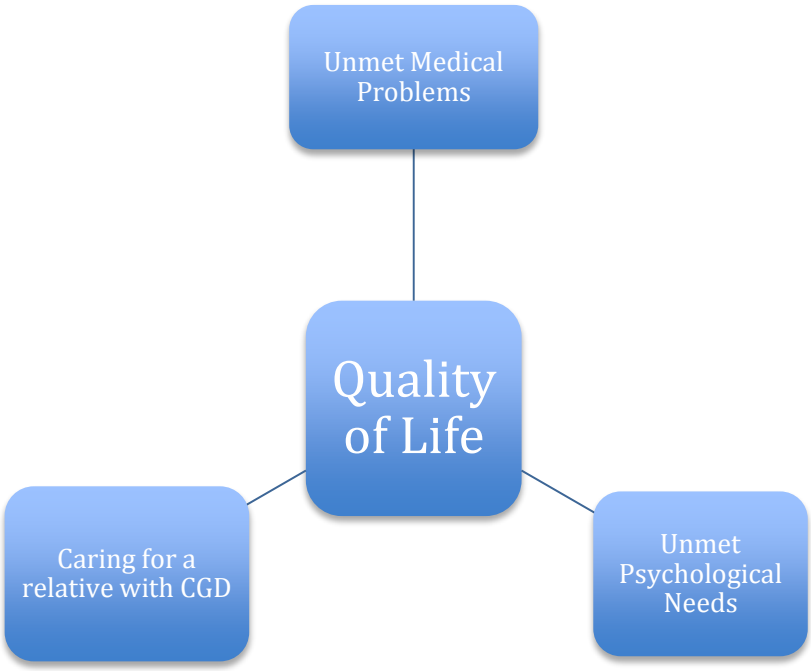
the vitality domain in the XL-CGD carrier cohort confirm the high levels of fatigue reported by the XL-CGD carriers and support the hypothesis that it is not related to caring, but that fatigue is associated with XL-CGD carrier status.

As the general health scores were low in the XL-CGD carrier cohort, it may be that this was a significant contributor to the poor QoL. SLE is the most similar disease process or symptom pattern to the problems manifesting in the XL-CGD carriers. Therefore, QoL data were compared with published studies of QoL in SLE patients. The published data from SLE patients demonstrated that QoL in SLE patients was significantly worse in all domains[140].

9.13.2 Factors Affecting QoL

There are many factors which a have contributed to the pooor QoL scores seen in the XL-CGD carriers. Figure 9-2 outlines potential contributing factors to the QoL in the XL-CGD carriers, which will now be discussed.

Figure 9-2: Potential contributing factors in quality of life in XL-CGD carriers



Medical Health

There was a significant association between QoL, in both physical and mental health component scores, with the presence of medical problems in XL-CGD

carriers. Joint and bowel symptoms, poor scores on the respiratory health questionnaire and the presence of recurrent mouth ulcers all significantly correlated with poor QoL. There was also a significant association of the number of ARA SLE criteria met and QoL. More minor medical problems such as photosensitivity, did not correlate with poor QoL suggesting that the other medical problems suffered by the XL-CGD carriers were more significant.

The importance of this association is twofold. Firstly, it demonstrates that impaired QoL is likely to be intrinsic to being an XL-CGD carrier and that their own medical problems are important factors, rather than solely being the relative of an unwell individual determines QoL. Secondly, there may be modifiable factors, which mean QoL can be improved for XL-CGD carriers.

Psychological Health

Psychological health may logically impact upon QoL and this was demonstrated in the XL-CGD carriers. Anxiety and depression scores correlated with QoL with both mental health and physical health component scores, although the correlation was more statistically significant for the mental health component scores. This is not surprising, as physical health problems were more significantly correlated with the physical health component score but highlights the overlap for both.

Again, this demonstrates the importance of identifying any unmet psychological health problems in the XL-CGD carriers in order to improve their QoL as well as anxiety and depression symptoms.

Social Factors

There will be factors relating to caring for a family that may impact upon QoL.

The XL-CGD carriers related to an index CGD case who had undergone HSCT were compared with those related to an index CGD case where HSCT had not been performed. The QoL scores in all domains were similar. The only significant differences were seen in the bodily pain and mental health domains. The mental health scores were lower in the HSCT group whilst bodily pain scores were worse in the non HSCT group. It is difficult to account for these differences

except that the amount of time elapsed since the HSCT was variable and whilst QoL assessment was deferred for at least a year after HSCT it may be that in some of the relatives these experiences remained heightened and contributed to mental distress. The lack of difference between the scores in the majority of domains may suggest that it is being an XL-CGD carrier that contributes to the poor scores rather than the impact of external influences such as a relative with CGD.

To evaluate the impact of relationship to the index case upon QoL scores, the XL-CGD carrier mothers were compared with the XL-CGD carrier other relatives. There were no significant differences found between the scores in each group. This highlights the need to address QoL in all family members and not solely the mothers and may reflect intrinsic issues for XL-CGD carriers rather than external factors, which is supported by the differences with the brain tumour carers.

Caring for a child with a chronic illness was significantly correlated with the physical health component score but not with mental health component score. This perhaps supports the need for practical help for caring for a child, as the impact appears to be greatest in the physical domains.

Implications

This is the first study to have examined the QoL in XL-CGD carriers. It has not been previously recognised that XL-CGD carriers have significantly reduced QoL. As discussed, the reasons for this poor QoL are complex and multifaceted. Some of the factors affecting QoL are modifiable and therefore, improvements in these may result in an overall improvement in QoL. For example, it can be hypothesised that improving pain or managing bowel or other medical symptoms may improve overall QoL. Unrecognised, and therefore, untreated anxiety and depression may also be treated and an improvement in symptoms may alter perceived QoL.

The impact of having a chronically ill child on maternal QoL may not be alterable, but an increased awareness of its impact may aid clinicians in caring for the entire family. It would be interesting to evaluate QoL in the fathers and also in the non-carrier relatives to see how they compare to the XL-CGD carrier

relatives. This would allow us to evaluate the direct impact of carrier status when other factors are the same.

The effect of any treatments may be monitored using the SF-36, the results from which are reproducible.

9.14 Strengths and Limitations

9.14.1 Recruitment

The major strength of this study is the recruitment number and approach. All known XL-CGD families were approached either in person or by post making it the most comprehensive study in the UK and worldwide. There were 11 XL-CGD families who did not respond to the invitation to participate in the study when contacted by post despite repeated attempts being made and there are several possible reasons for this. It is possible that those who did not reply differed in symptom burden to those recruited; affected individuals are more likely to be motivated to participate in the study and to complete the required questionnaires. However, it may also be that those who did not participate suffered from significant psychological symptoms and felt unable to participate. It is also possible that the addresses provided to the researcher were incorrect and the non-responding XL-CGD carriers simply did not receive the invitation. There may also have been reasons not considered here.

Approaching families in person at clinic appointments reduced the potential for selection bias as even if XL-CGD carriers felt they did not have any medical problems they were invited to participate.

The potential reasons for not returning completed questionnaires are similar to those for the non-responders. The questionnaires were kept as brief as possible to minimise the time required by each participant to complete the questionnaires, but lack of time have played an important role in those who failed to return questionnaires.

The advertisement of the study via the CGD Society allowed the project to be advertised to families who were not known to the research team and also to families who would not have been approached due to the death of an index case.

This was advantageous to the study as allowed for wider recruitment. There were XL-CGD carriers recruited through this method in which the index case was deceased. However, it is likely that those recruited via this method are more likely to be symptomatic, as they will have proactively sought out information about XL-CGD carriers.

There will also be unknown XL-CGD families and carriers in the general population in whom the defect has not been diagnosed. It is not possible to quantify this number. However, one such XL-CGD carrier was recruited to the study via the CGD clinic at the Royal Free Hospital when she presented to the immunology department with symptoms and was subsequently discovered to be an XL-CGD carrier.

This was an observational study of a rare disease. The study recruited the majority of known XL-CGD carriers in the UK but, despite this, the number in the study remains small. This may have led to some associations not being observed due to a lack of power. However, as this is a rare condition and this study was looking at rare complications, it is unlikely that it would ever be possible to recruit a suitably powered cohort within the UK. This study recruited as many XL-CGD carriers as was possible.

The recruited control group was considerably smaller than anticipated and there were several reasons for this. MD carrier controls were recruited from the MD clinic at the GNCH, Newcastle upon Tyne. Attendance at the MD clinic was poor and identified potential carriers frequently did not attend. Additionally, there is a high new mutation rate in MD with a new mutation occurring in one third of patients[237]. Unpredictably, there were three families in which the patient no longer lived with their biological parents as they had been adopted or placed in foster care and subsequently the research team did not have access to these potential carriers. Motivation to participate as a control participant is less than for those in the study group and as a result there were a greater number of control carriers who declined to participate.

9.14.2 Medical History

I extracted all data and took all medical histories. This provided consistency and ensured that questions were posed in the same manner throughout.

Previous case reports have focussed on isolated symptoms or presentations in single cases or families. This study collected information about a broad range of symptoms and actively looked for symptoms, which may not have otherwise been revealed, but have proven to be important and prevalent in the XL-CGD carrier population as a whole.

The assessments of non-specific symptoms, including those of fatigue are inherently difficult. This study attempted to account for this by using standardised questionnaires where possible. Specific interest was in symptoms seen in the CGD boys and therefore, questionnaires were chosen to look for these symptoms. The fatigue questionnaire was added at a later stage when it became clear that this was a problem volunteered by XL-CGD carriers and a method to quantify the symptom was required. This study also attempted to verify symptoms and diagnoses by accessing medical records in both primary and secondary care. However, due to the geographical distribution of the recruited individuals, it was not possible for the researcher to visit all general practices individual. Subsequently the information returned from the GP was determined not by the researcher but by the individual GP. This was therefore not standardised as it was not possible given time and staff limitations. However, the majority of the GPs sent the same documentation.

9.14.3 General

A limitation in this study is the lack of objective investigations to confirm the presence or absence of medical symptoms. However, at the outset it was not clear that the XL-CGD carriers would exhibit so many medical problems and therefore, it would have been impossible to predict which investigations would be appropriate. As the study progressed it became increasingly clear that there were significant medical problems. Furthermore, as this was a national study, the participants were located all over the UK making it difficult to perform

investigations in a timely and efficient manner, particularly as a single researcher performed the research.

The exact mutation was only known in 20 of the XL-CGD carriers. It would have been useful if the exact mutation was known and the degree of residual function could have been correlated with the presence of symptoms in the XL-CGD carriers.

9.14.4 Psychological Health

There are limitations to the assessment of anxiety and depression in this study. Anxiety and depression were assessed at one point in time and may not be reflective of that individual at another time. This may, therefore, represent either an over or an under representation of anxiety symptoms. It would have been beneficial to repeat the assessment to allow for a more accurate picture. However, GP records were also accessed which allowed for pre-existing diagnoses to be noted and this was compared to the presence found in the study.

Recruited XL-CGD carriers were put into a relationship category determined by the index case from which they were identified. However, in some cases this was artificial as there was more than one CGD patient within the family and therefore, each enrolled participant could be considered in more than one category. This may have affected the evaluation.

This is the first study to examine in depth and in a large cohort, the psychological health of XL-CGD carriers. Whilst the data collection is limited to one time point, the strength is the inclusivity with a range of relatives and ages represented, along with both before and after HSCT. Psychological health concerns are under reported. The strength of this study is that by screening for anxiety and depression, previously unidentified distress will be captured.

QoL assessment was delayed if the child had undergone HSCT to 1 year after this. It was felt that this would allow for a return to normality. However, McDowell et al's[204] study suggests that this may not be sufficient time. On a practical level, this study was time limited and a pragmatic approach was therefore, adopted. The strengths and limitations for QoL assessment are similar to those discussed for anxiety and depression. Additionally, QoL may be more greatly affected by

physical symptoms. As many of the clinical symptoms appear to be episodic, the assessment of QoL may have been affected by whether or not the physical symptoms were active or quiescent at the time of completion of QoL assessment.

9.14.5 Blood Tests

Neutrophil Oxidative bursts were all performed at the local recruiting centre owing to the time sensitive nature of the investigation. All laboratories used DHR and flow cytometry to assess the per cent of neutrophils producing an oxidative burst. As the investigations were performed at different centres it is possible that there were subtle differences in the techniques used and there may be variability. However, as outlined earlier, the most important consideration when assessing oxidative burst is that the sample is processed promptly and ideally within 12 hours. Therefore, on balance this was the most appropriate method but it should be acknowledged that there might be small differences.

All autoantibody samples were performed at the Royal Victoria Infirmary, Newcastle upon Tyne as they do not require the same time-dependant processing. By performing them at a single centre this ensured that they were processed in a standardised manner thereby minimising differences between the handling of samples. This is of particular importance when considering reference ranges which may vary between laboratories.

The ethnicity of the XL-CGD carriers is typical of that seen in CGD families in the UK, predominantly white British. As the majority of XL-CGD carriers will be diagnosed following the diagnosis of an index case, predominantly a son, the age range of this cohort is reflective of this with a median age of 41 years. The majority of the identified and recruited XL-CGD carriers were mothers, with the next most common being sisters and then grandmothers. The relative lack of other relatives reflects current attitudes toward carrier testing. The median age of the index cases was 12 years and as such their siblings are likely to be of similar ages.

Chapter 10: Conclusions and Clinical Recommendations

10.1 General

This study has demonstrated that XL-CGD carriers suffer a wide range of medical problems to varying degrees of severity. Subsequently, the first recommendation is that all XL-CGD carriers should be seen and assessed by a specialist in primary immunodeficiency in the first instance.

10.2 Clinical

Clinical symptoms should be actively asked about at an initial consultation and the lead clinician should not wait for the XL-CGD carriers to volunteer them.

Specifically, XL-CGD carriers should be actively asked about symptoms of gastrointestinal disease including abdominal pain and urgency, diarrhoea and rectal bleeding. They should also be asked about previous gastrointestinal infections.

XL-CGD carriers should be asked directly about episodes of joint pain, redness and swelling, which may be associated with fatigue. Ideally, XL-CGD carriers should see a rheumatologist when joint symptoms are active for assessment and consideration of treatment beyond simple analgesia.

Dermatology referrals should be made where there is significant skin involvement or where there are rashes of uncertain aetiology. All XL-CGD carriers should be advised about the need for sun avoidance and high factor sun block.

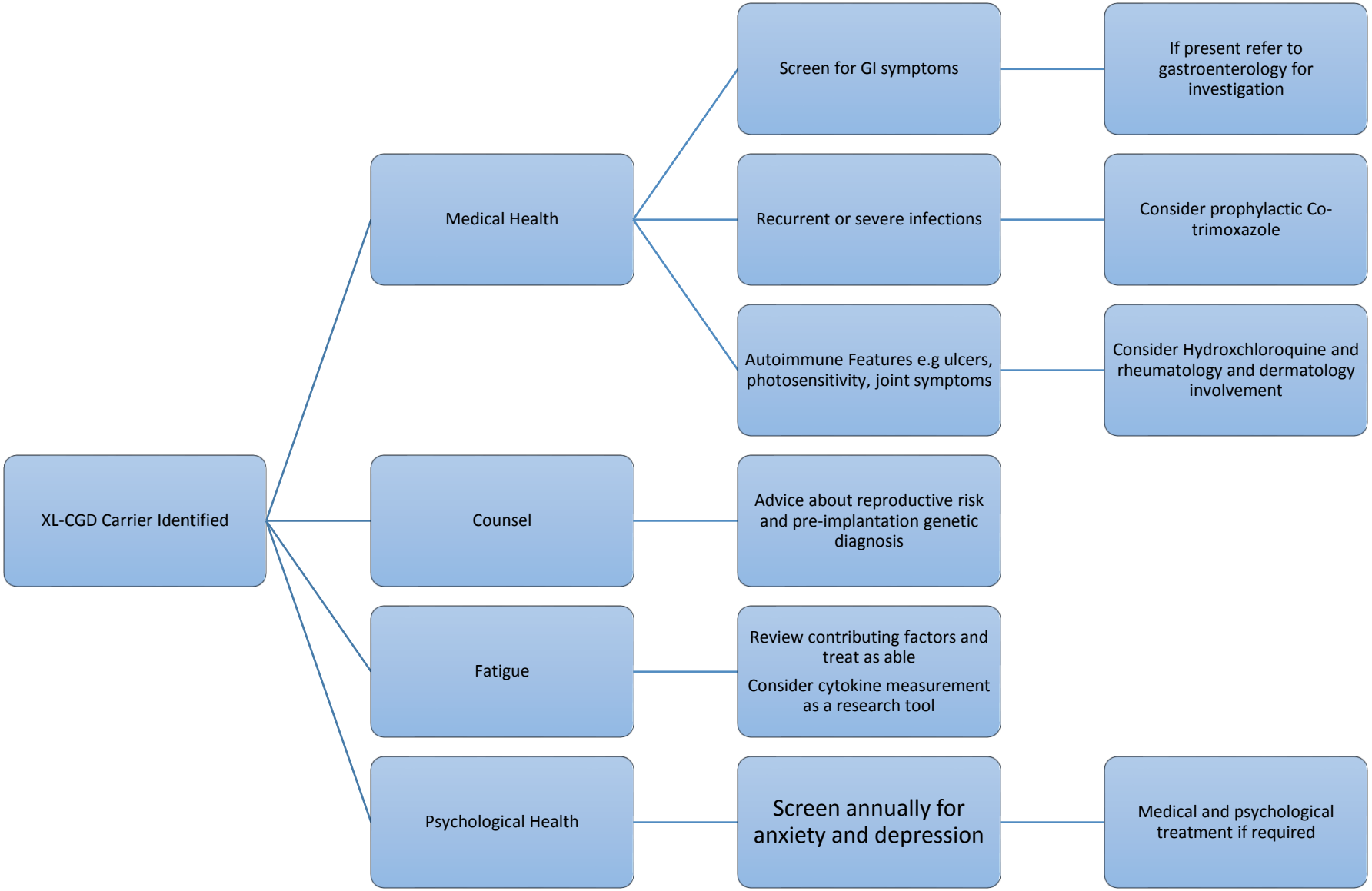
The role of, and need for, antibiotic prophylaxis in XL-CGD carriers remains unclear from this study. It should be considered where the XL-CGD carrier has suffered a significant infection or where they have recurrent, problematic infections such as skin abscesses. Co-trimoxazole would appear to be the logical choice, due to its proven efficacy in CGD patients [11, 60].

10.3 Clinical Follow Up

For some XL-CGD carriers, a single consultation will be sufficient and no further investigations or assessment required. However, for those where symptoms are present referral should be made to the appropriate speciality but long term follow up coordinated by the primary immunodeficiency team in a specialised centre. This will allow an overview of the care for the XL-CGD carriers but also enable more to be learned about the course of their symptoms.

A summary of the proposed management of XL-CGD carriers is shown in Figure 10-1.

Figure 10-1: Proposed Management of XL-CGD Carriers



10.4 Psychological

Anxiety is highly prevalent and under-diagnosed in XL-CGD carriers of all ages. This persists even after an index case has undergone HSCT and appears to be independent of the relationship to the index case. Anxiety may be exacerbated by physical symptoms and is more prevalent in those suffering from gastrointestinal disease or joint symptoms. Conversely, very few XL-CGD carriers suffer from significant depression, although the scores are higher than the general population.

XL-CGD carriers should be screened for anxiety and depression regularly and particularly where physical symptoms are present. The HADS appears to be a useful screening tool for anxiety and depression and can be recommended as the screening tool of choice.

Those who are found to score highly should be offered treatment, both pharmacological and psychological. Support for the family, as a whole should be offered as a standard part of care. Psychology services may need to be increased to meet these needs and good relationships with primary care should be established to facilitate optimal treatment.

10.5 Implications on Carrier Testing

Current guidance is that children under 16 years should only be tested for a genetic disease if it is of clinical benefit to the individual, and as such many of the siblings of children with XL-CGD are unaware of their carrier status. However, this study has demonstrated that there are significant clinical manifestations within the XL-CGD carriers and it may, therefore, be beneficial to test for carrier status at an earlier age. Unlike other genetic conditions where carrier status does not have clinical manifestations, this study has shown that XL-CGD carriers have clinical manifestations of disease, which may be improved by treatment, and diagnosis of carrier status has greater clinical implications than solely for reproductive risk. Therefore, with expert counselling, and psychological support, it may be appropriate to consider testing both younger children and wider family

members than is currently routine practice in order to facilitate earlier treatment.

10.6 Summary of Future Research

This study has highlighted a number of issues requiring further research. The main areas requiring further investigation are the identification of affected XL-CGD carriers, the underlying mechanism of disease process in the XL-CGD carriers and the treatment of XL-CGD carriers.

Pre-emptive identification of XL-CGD carriers may be beneficial in order to improve outcome and would aid counselling of XL-CGD carriers. Further understanding of the mechanism of the disease process would identify potential treatment targets and further understanding about possible manifestations of disease.

Treatment of symptoms is an important aspect of future research. This study has demonstrated significant medical problems and reduced quality of life. There are currently no satisfactory treatment options for XL-CGD carriers.

Chapter 11: Appendices

Appendix 1: Information Leaflets

Adult

Adult information sheet – version 2 – 02.04.12

Great Ormond Street Hospital for Children NHS Trust

The Newcastle upon Tyne Hospitals NHS NHS Foundation Trust

A collaborative study

4th Floor
Sir James Spence Institute
Royal Victoria Infirmary
Newcastle NE1 4LP

An Investigation into the General and Psychological health of Carriers of X-Linked Chronic Granulomatous Disease

Information Sheet for Patients

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it involves. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask if anything is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1 tells you the purpose of this study and what will happen if you take part.
Part 2 gives you more detailed information about the conduct of the study.

Part 1:
What is the purpose of this study?
The purpose of this study is to look at the health of carriers of the gene for Chronic Granulomatous Disease (CGD) in the United Kingdom. We know that sometimes these women have problems with their skin, and sometimes other medical problems. We don't know how often this happens and if there are other symptoms that these women suffer more frequently than people without this gene. We would like to know if these carriers suffer similar symptoms to the boys with CGD.

Why have I been invited?
You have been invited as either, you are known to be a carrier of CGD, or you have a close relative with CGD and are potentially a carrier.

What happens if I do not know my carrier status?
If this is the case and you want to be included in the study you will be tested for your carrier status. This will be done by the clinical team looking after your relative and will involve a blood test. This will tell you if you carry the gene or not. Your doctor will talk to you about this and what it means.

Do I have to take part?
No, it is up to you to decide. We will give you time to read and consider this information sheet. We will then ask you to sign a consent form to show you have agreed to take part.

What will happen to me if I take part?
To gather information about your health and treatment we will look at your hospital notes. We will do this at all the hospitals you have been seen at. We will ask you to complete a questionnaire about your health.

We will ask you to complete questionnaires about your emotions and quality of life and to participate in an assessment of your IQ. This will last approximately 90 minutes. If you agree to take part you will be given a full explanation of the process, along with the results and what they mean for you. We will aim to do this when you are attending a routine clinic appointment so you do not have to make an extra journey. If it is not possible to do this in clinic we can arrange a time that is more convenient.

We would like to perform a blood test to see how many of your white cells are affected by being a carrier. We will also store part of this blood so that if we discover new tests in the future we could do this. We would also like you to provide us with a stool sample which we can store and in the future test it for common bugs found in the bowel to see if they are related to bowel problems.
What are the disadvantages to taking part?

We know it may be difficult to talk about your feelings and how they are affected by having a family member with CGD. If you think it would be helpful to see a trained psychologist we will arrange this.

What are the benefits to taking part?
We cannot promise the study will help you immediately, but the information from this study will help us understand about the health of carriers of CGD and may affect what treatments they, and you, are offered in the future. We will also be able to give more accurate information about what it means to be a carrier.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practices. All information about you will be handled in confidence. The details are included in Part 2.

This completes part 1. If the information has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2:
What will happen if I don't want to carry on with the study?
You can withdraw from the study at any time and it will not have an impact on your normal care or on the care of your relative. You do not have to give a reason for why you want to withdraw.

What happens if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure.

How will my taking part be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Information will be stored in an anonymous fashion with a code so nothing can be related back to the individual. With your consent, we will write to your GP to let them know you are involved in this study.

What will happen to the results of the study?
The results of this study will be published in medical journals and the findings will be communicated to the CGD Society so it can be passed on to patients and families. Anonymous data only will be used and no individual will be identifiable.

Who is funding this research?
This research is funded by The Bubble Foundation who fund research into primary immunodeficiency. No-one receives a payment for your inclusion in the study. All research in the NHS is looked at by independent group of people, (a Research Ethics Committee). This study has been reviewed by the NRES Committee North East – Newcastle and North Tyneside 1.

Contact details

Senior researcher
Dr Alex Battersby
4th Floor, Sir James Spence Institute
Royal Victoria Infirmary
Queen Victoria Road
Newcastle
NE1 4LP
0191 282 5234 email: a.battersby@newcastle.ac.uk

Project supervisor
Dr Andrew Gennery
Senior Lecturer/ Consultant in Paediatric Immunology
Old Children's Outpatients
Royal Victoria Infirmary
Newcastle
NE1 4LP
0191 282 5234 email: a.r.gennery@ncl.ac.uk

Patient age 12-15 information sheet – version 2 – 14.03.12

Great Ormond Street Hospital for Children NHS Trust

The Newcastle upon Tyne Hospitals NHS Foundation Trust

A collaborative study

4th Floor
Sir James Spence Institute
Royal Victoria Infirmary
Newcastle NE1 4LP

An Investigation into the General and Psychological Health of carriers of X-Linked Chronic Granulomatous Disease

Information Sheet for Patients
We are asking if you would take part in a research project to help us understand more about the health of carriers of CGD.

Before you decide if you want to join in it's important to understand why the research is being done and what it will involve for you. So please think about this leaflet and project carefully. Talk about it with your family, friends, doctor or nurse if you want to.

Part 1:
Why are we doing this study?
The point of this study is to look at the health of women and girls who carry the gene for Chronic Granulomatous Disease (CGD). We know that sometimes these people can have problems with their skin and rashes. We don't know how often this happens and if there are other symptoms they get more often than people without this gene.

Why have I been asked?
You have been asked as we know that you carry the gene for CGD. We would like to see if you have any of these medical problems.

Do I have to take part?
No. It is up to you. If you do, your doctor will ask you to sign a form giving your permission. Your parents or carer will also be asked to sign a form as it is important you all agree. You will be given a copy of this information sheet and your signed form to keep. You are free to stop taking part at any time during the study without giving a reason. If you decide to stop, this will not upset anyone or change the way we treat you or your family.

What will happen to me if I take part?
To gather information about your health and any treatment you may have had we will look at your hospital notes. We will ask you or your parents to provide a list of which hospitals you go to so we can look at all of your records. This information will be kept safe.

We will ask you with your parents to complete a survey about how you feel and the good and bad things in your life. We will ask you about any symptoms you may have had in a separate questionnaire about your health. We will ask you to come for a short interview where we will ask questions to measure your intelligence (IQ).

If you agree to the IQ measurement we will explain in detail how it works and what the answers mean for you. This will last no more than 90 minutes in total. We will aim to do this when you are at hospital for an appointment for you or your relative so you do not have to make an extra journey. If it is not possible we will arrange a separate time.

You will also be asked if it is ok for you to have a blood test to see how many of your white cells are affected by carrying this gene. We will also store a sample of this blood to look at in the future if we discover new information. This can be done when you come to the hospital for another reason and we can give you a cream or a spray so you do not feel it.

We will also ask you to provide us with a poo sample so that we can test it for any of the common bugs we usually find. We will store the sample to use at a later date.

Is there anything to be worried about if I take part?
We know that it may be difficult to talk about your feelings. If you, or your family, think it would be helpful to see someone to talk more, we can arrange this.

What are the benefits to taking part?
We cannot promise the study will help you immediately but the information we get from this study will tell us more about how people who carry the gene for CGD are affected and whether they should have different medicines and treatments.

Contact details
If you want to ask more questions please contact us. Our details are at the bottom of the information sheet.

Thank you for reading so far – if you are still interested, please go to Part 2*:*
Part 2:
What happens if there is a problem?
If you or your family is worried about any part of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you/ your family are still unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure.

Will anyone else know I am doing this?
We will keep your information private. This means we will only tell those who need to know. We will write to your GP to let them know you are involved in this study. Information will be stored with a code but no name or address so no-one else can tell who is in the study.

Who is funding this research?
This research is being funded by a grant from The Bubble Foundation, which is a charity which funds care and research into primary immunodeficiencies, like CGD. No-one gets paid for you taking part in this study.

Before any research goes ahead it has to be checked by a Research Ethics Committee. They make sure that the research is fair. Your project has been checked by the NRES Committee North East – Newcastle and North Tyneside 1.

Thank you for reading this – please ask any questions if you need to.

Contact details

Senior researcher
Dr Alex Battersby
4th Floor, Sir James Spence Institute
Royal Victoria Infirmary
Queen Victoria Road
Newcastle
NE1 4LP
0191 282 5234
email: a.battersby@newcastle.ac.uk

Project supervisor
Dr Andrew Gennery
Senior Lecturer/ Consultant in Paediatric Immunology
Paediatric Immunology Dept
Old Children's Outpatients
Royal Victoria Infirmary
Newcastle
NE1 4LP
0191 282 5234
email: a.r.gennery@ncl.ac.uk

Older Child 9 – 12 years

Patient age 9-12 years information sheet – version 2 – 16.08.13

An Investigation into the General and Psychological Health of carriers of X-Linked Chronic Granulomatous Disease

Great Ormond Street Hospital for Children NHS Trust

The Newcastle upon Tyne Hospitals NHS Foundation Trust

4th Floor
Sir James Spence Institute
Royal Victoria Infirmary
Newcastle NE1 4LP

A collaborative study

A Study of Carriers of CGD

We are asking if you would take part in a project to help us understand more about being a carrier of CGD.

Research is a way we try to find out the answers to questions. We want to find out what it is like to be a carrier of CGD and how it makes you feel. The researcher is also writing a project about this for university.

Why have I been asked to take part?
Your doctor has told us you are a carrier of CGD. We want to ask everyone like you to help us with this study.

Do I have to take part?
No. It is up to you and your family to decide if you want to be involved.

What will happen to me if I take part?
We will ask your family to answer some question about what your health is like and we will also meet you to ask you some questions. This will happen when you come to hospital for a normal check up. Your family can stay with you when we see you. This will all take about 45 minutes in total.

We will also look at the information your doctor keeps about how you are. We will write down and keep some of this information so we can find out how all CGD carriers are.

When you have your blood tests done by your doctor we will take an extra sample of blood.

Is there anything that might upset me?
We know that it may be difficult to talk about your feelings. We can arrange for you to see someone to help with this.

Will joining in help me?
We cannot promise the study will help you immediately but the information we get from this study will help improve the treatment of people who are also carriers of CGD.

What happens if I don't want to do this anymore?
If at any time you don't want to do the research anymore, just tell your parents, doctor or nurse. They will not be cross with you.

Contact details

Senior researcher

Dr Alex Battersby
4th Floor, Sir James Spence Institute
Royal Victoria Infirmary
Queen Victoria Road
Newcastle
NE1 4LP
0191 282 5234

email: a.battersby@newcastle.ac.uk

Project Supervisor

Dr Andrew Gennery
Senior Lecturer/ Consultant in
Paediatric Immunology
Old Children's Outpatients
Royal Victoria Infirmary
Newcastle
NE1 4LP
0191 282 5234

email: a.r.gennery@ncl.ac.uk

Younger Child 5- 8 years

Patient age 5-8 years information sheet – version 2 – 01.08.13

An Investigation into the General and Psychological Health of carriers of X-Linked Chronic Granulomatous Disease

Great Ormond Street Hospital for Children NHS Trust

The Newcastle upon Tyne Hospitals NHS NHS Foundation Trust

4th Floor
Sir James Spence Institute
Royal Victoria Infirmary
Newcastle NE1 4LP

A collaborative study

A Study of Carriers of CGD

We are asking if you to be in a project to help us learn about being a carrier of CGD.

Research is a way we try to find out the answers to questions. We want to find out what it is like to be a carrier of CGD and how it makes you feel. The person doing the study will write about the project for university.

Why have I been asked to take part?
Your doctor has told us you are a carrier of CGD. We want to ask everyone like you to help us with this study.

Do I have to take part?
No. It is up to you and your family to choose.

What will happen to me if I take part?
We will ask your family to answer some question about when you have been ill. We will also meet you to ask you some questions. This will happen when you come to hospital for a normal check up. Your family can stay with you when we see you.

We will also look at the information your doctor keeps about how you are. We will write down and keep some of this information so we can find out how all CGD carriers are.This will take about 45 minutes. You can have a break if you need to.

When you have your blood tests done by your doctor, we will ask them to take an extra bit for our project.

Is there anything that might upset me?
We know that it may be difficult to talk about your feelings. We can arrange for you to see someone to help with this.

Will joining in help me?
We cannot promise the study will help you immediately but the information we get from this study will help improve the treatment of people who are also carriers of CGD.

What happens if I don't want to do this anymore?
If at any time you don't want to do the research anymore, just tell your parents, doctor or nurse. They will not be cross with you.

Contact details

Senior researcher

Dr Alex Battersby
4th Floor, Sir James Spence Institute
Royal Victoria Infirmary
Queen Victoria Road
Newcastle
NE1 4LP
0191 282 5234

email: a.battersby@newcastle.ac.uk

Project Supervisor

Dr Andrew Gennery
Senior Lecturer/ Consultant in
Paediatric Immunology
Old Children's Outpatients
Royal Victoria Infirmary
Newcastle
NE1 4LP
0191 282 5234

email: a.r.gennery@ncl.ac.uk

Parent information sheet – version 1 – 14.03.12

Great Ormond Street Hospital for Children NHS Trust

The Newcastle upon Tyne Hospitals NHS Foundation Trust

A collaborative study

4th Floor
Sir James Spence Institute
Royal Victoria Infirmary
Newcastle NE1 4LP

An Investigation into the General and Psychological health of Carriers of X-Linked Chronic Granulomatous Disease

Information Sheet for Parents

We would like to invite your daughter to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask if anything is not clear or if you would like more information.

Part 1 tells you the purpose of this study and what will happen if your daughter takes part.
Part 2 gives more detailed information about the conduct of the study.

Part 1:

What is the purpose of this study?

The purpose of this study is to look at the health of carriers of the gene for Chronic Granulomatous Disease (CGD). We know that sometimes these women/girls can have problems with their skin, get rashes and sometimes other medical problems. We don't know how often this happens and if there are other symptoms that they suffer more frequently than people without this gene. We would like to know if these carriers suffer similar symptoms to the boys who have CGD.

Why has my daughter been invited?

Your daughter has been invited as we know she is a carrier of CGD as she has had this confirmed.

Do they have to take part?

It is up to you and your daughter to decide. We will give you time to read and consider this information sheet and discuss it with your daughter. As your daughter is under 16 years old we will then ask you to sign a consent form to show you have agreed for her to take part. We will also ask your daughter to sign a form to say she agrees to participate. She is free to withdraw at any time, without giving a reason. This would not affect the standard of care you, your daughter or your relative receives.

What will happen to my daughter if they take part?

To gather information about her health and treatment we will look at her hospital notes. We will do this at all of the hospitals she has been seen at. We will also ask you and her to complete a questionnaire about her health.

We will ask her to complete questionnaires about her emotions and quality of life. We will invite her to participate in a short interview to look at her IQ. This will last no more than 90 minutes. If she agrees to take part you will both be given a full explanation of the process, along with the results and what they mean for your daughter. We will aim to do this when you are attending a routine clinic appointment so you do not have to make an extra journey to hospital.

We would like to perform a blood test to see how many of your daughter's white cells are affected by being a carrier. We will also store part of this blood so that if we discover new tests in the future we could do this, We would also like your daughter to provide us with a stool sample which we can store and in the future test it for common bugs found in the bowel to see if they are related to problems with the bowel.

What are the disadvantages to taking part?

We know that it may be difficult to talk about feelings and how they are affected by having a family member with CGD. If you think it would be helpful for you or your daughter to see a trained psychologist we will arrange this.

What are the benefits to taking part?
We cannot promise the study will help your daughter immediately, but the information we get from this study will help us understand more about the health of carriers of CGD and may affect what treatments they, and your daughter, are offered in the future. It also means we will be able to give more accurate information about what it means to be a carrier to other carriers in the future.

Will my daughter taking part in the study be kept confidential?
Yes. The details are included in Part 2.

This completes part 1. If the information has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2:
What will happen if my daughter doesn't want to carry on with the study?
She can withdraw from the study at any time. It will not have an impact on your daughter's normal care or on the care of your relative. She does not have to give a reason for why she wants to withdraw.

What happens if there is a problem?
If you, or your daughter, have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you, or your daughter, remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure.

How will my daughter taking part be kept confidential?
All information collected about your daughter will be kept strictly confidential. Information will be stored in an anonymous fashion with a code so nothing can be related back to the individual. We will write to your daughter's GP to let them know she is involved in this study. We will follow ethical and legal practice.

What will happen to the results of the study?
The results of this study will be published in medical journals using anonymous data only. No individual will be identifiable. The findings will also be communicated to the CGD research trust so it can be passed on to patients and families. Again, no-one will be identifiable.

Who is funding this research?
This research is funded by The Bubble Foundation, who fund research into primary immunodeficiency. No-one receives a payment for your inclusion in the study. All research in the NHS is looked at by an independent group of people, (a Research Ethics Committee) to protect your safety, rights, wellbeing and dignity. This study has been reviewed by NRES Committee North East – Newcastle and North Tyneside 1.

Contact details

Senior researcher
Dr Alex Battersby
4th Floor, Sir James Spence Institute
Royal Victoria Infirmary
Queen Victoria Road
Newcastle
NE1 4LP
0191 282 5234
email: a.battersby@newcastle.ac.uk

Project supervisor
Dr Andrew Gennery
Senior Lecturer/ Consultant in Paediatric Immunology
Old Children's Outpatients
Royal Victoria Infirmary
Newcastle
NE1 4LP
0191 282 5234
email: a.r.gennery@ncl.ac.uk

Next of Kin Information sheet – version 1 – 14.03.12

Great Ormond Street Hospital for Children NHS Trust
The Newcastle upon Tyne Hospitals NHS Foundation Trust
A collaborative study

4th Floor
Sir James Spence Institute
Royal Victoria Infirmary
Newcastle NE1 4LP

An Investigation into the General and Psychological health of Carriers of X-Linked Chronic Granulomatous Disease

Information Sheet for Next of Kin

We would like to include your relative in a study about carriers of X-linked CGD. As your relative has died we would like to ask you, as their next of kin, for consent to include them in this study.

Before you decide you need to understand why the research is being done and what it would involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen if you agree to our relative being included. Part 2 gives more detailed information about the conduct of the study.
Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your relative to be included.

Part 1:
What is the purpose of this study?
The purpose of this study is to look at the health of carriers of the gene for Chronic Granulomatous Disease (CGD) in the United Kingdom. We know that sometimes these women/girls can have problems with their skin, get rashes and sometimes other medical problems. We don't know how often this happens and if there are other symptoms that they suffer more frequently than people without this gene. We would like to know if these carriers suffer similar symptoms to the boys who have CGD.

Why should my relative be included?
We know that your relative was a carrier of CGD as you or another family member have told us. Although your relative has died we would like to see if she suffered any symptoms related to CGD whilst she was alive.

Do I have to consent to them being included?
No. It is up to you and your family to decide. We will give you time to read and consider this information sheet and discuss it with your family. As your relative has died, we are asking you as the next of kin to consent to them being included. You are free to withdraw your consent at any time, without giving a reason. This would not affect the standard of care you or your relative receives.

What will happen if I agree to my relative being included?
To gather information about their health and treatment we will look at their hospital notes. We will ask you to provide a list of hospitals they were seen at so we can look at all of their records. We will also ask you to complete a questionnaire about their health. This information will be kept indefinitely in a secure fashion.

What are the disadvantages to taking part?
We know that it may be difficult to talk about relatives who have died and that you may be uncertain what your relative would want. We are happy for you to take your time about making this decision and to talk to other family members and your doctor.

What are the benefits to taking part?
We cannot promise the study will help your family immediately, but the information we get from this study

will help us understand about the health of carriers of CGD and may affect what treatments they are offered in the future. It also means we will be able to give more accurate information about what it means to be a carrier to other carriers in the future.

Will my relative's inclusion in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about your relative will be handled in confidence. Information will be kept anonymised so nothing can be related back to the individual. The details are included in Part 2.

This completes part 1. If the information has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2:
What will happen if I change my mind?
You can withdraw your relative from the study at any time and it will not have an impact on your normal care or on the care of your other relatives. You do not have to give a reason for why you want to withdraw.

What happens if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you, or your relatives, remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital where you are seen.

How will my relative's inclusion be kept confidential?
All information which is collected about your relative during the course of the research will be kept strictly confidential. Information will be stored in an anonymous fashion with a code so patients are not identifiable.

What will happen to the results of the study?
The results of this study will be published in medical journals using anonymous data only. No individual will be identifiable from these publications. The findings will also be communicated to the CGD Society so it can be passed on to patients and families. Again, no-one will be identifiable from this information.

Who is funding this research?
This research is being funded by a grant from The Bubble Foundation who fund research into primary immunodeficiency. No-one receives a payment for your inclusion in the study.
All research in the NHS is looked at by independent group of people (a Research Ethics Committee). This study has been reviewed by NRES Committee North East – Newcastle and North Tyneside 1.

Contact details

Senior researcher
Dr Alex Battersby
4th Floor, Sir James Spence Institute
Royal Victoria Infirmary
Queen Victoria Road
Newcastle
NE1 4LP
0191 282 5234
email: a.battersby@newcastle.ac.uk

Project supervisor
Dr Andrew Gennery
Senior Lecturer/ Consultant in Paediatric Immunology
Paediatric Immunology Dept
Old Children's Outpatients
Royal Victoria Infirmary
Newcastle
NE1 4LP
0191 282 5234
email: a.r.gennery@ncl.ac.uk

Appendix 2: Questionnaires

Introduction Form

General Information

Please complete this as best as you can. Leave blank anything you do not want to share with the researcher.

GP Name and Address:

Family Members (Please complete as much as you are able – add new lines if needed)

Family Member e.g. Son, Sister	Age	CGD Status e.g. diagnosed, carrier, unknown	Health Problems

Your Own Health:

Any Medical Conditions Known About:

Any Medications you take:

Do you see any doctors for these medical problems? If so name and which hospital?:

Are you a smoker?

How much do you exercise per week?

What is your weight?

What is your height?

ARA SLE Questionnaire

Modified ARA SLE Criteria Questionnaire

Dermatological Criteria

1. Have you ever had a prominent, red rash over your nose and cheeks? Yes / No

If Yes, please give details of diagnosis and treatment received:

2. Have you ever had a significant rash with raised patches? Have you ever seen a skin doctor (dermatologist)? Yes / No

If Yes, please give details:

3. Have you ever had a rash caused by exposure to sunlight? Yes / No

If yes, please give details:

Ulcers

1. Do you suffer from ulcers in your mouth or on your nose that may not hurt? Yes / No

If yes:
Do you get them often? Yes / No
Has your doctor ever seen them? Yes / No

Joints

Do you suffer from pain or swelling in any of your joints? Yes / No
If Yes:
Have you seen a doctor about this? Yes / No
Have they diagnosed you with arthritis? Yes / No
Have you ever had an X-Ray of the joint? Yes / No

Hearts and Lungs

1. Have you ever had a pleural effusion (fluid in the lungs)? Yes / No

If yes:
Did you get admitted to hospital? Yes / No
Did you have a chest X-Ray? Yes / No

Did you need treatment? Yes / No

Please give details:

2. Have you ever had pericarditis (fluid or inflammation around the heart)? Yes / No

Were you admitted to hospital? Yes / No

Please give details:

Neurological

1. Have you ever had a fit? Yes / No

If Yes:

Did the doctors think it was to do with medications, recreational drugs or alcohol? Yes / No

Was there a reason found for the fit? Yes / No

Please give details:

2. Have you ever been diagnosed as having a psychiatric event/problem, not related to drugs or alcohol? Yes / No

If yes, please give details:

Kidneys

Have you ever been told you had protein in your urine?

If Yes,

Did this continue? Yes / No

Did you have a urine infection? Yes / No

Were you referred to a specialist? Yes / No

Did you have to see your GP again? Yes / No

Please give further details if possible:

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE
ORIGINAL ENGLISH VERSION

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

P.W. Jones, PhD FRCP
Professor of Respiratory Medicine,
St. George's University of London,
Jenner Wing,
Cranmer Terrace,
London SW17 ORE, UK.

Tel. +44 (0) 20 8725 5371
Fax +44 (0) 20 8725 5955

continued…

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question*:

		most days a week	several days a week	a few days a month	only with chest infections	not at all
1.	Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2.	Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3.	Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4.	Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one*:

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one:*

- No ☐
- Yes ☐

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please tick (✓) *one*:

The most important problem I have	☐
Causes me quite a lot of problems	☐
Causes me a few problems	☐
Causes no problem	☐

If you have ever had paid employment.

Please tick (✓) *one*:

My chest trouble made me stop work altogether	☐
My chest trouble interferes with my work or made me change my work	☐
My chest trouble does not affect my work	☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

Please tick (✓) in ***each box*** that applies to you ***these days***:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you <u>these days</u>.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

	Please tick (✓) in ***each box*** that applies to you ***these days***: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (✓) in ***each box*** that applies to you ***because of your breathing***:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest <u>usually</u> affects your daily life.

Please tick (✓) in ***each box*** that applies to you ***because of your chest trouble***:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

continued…

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog
Doing things at home or in the garden
Sexual intercourse
Going out to church, pub, club or place of entertainment
Going out in bad weather or into smoky rooms
Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

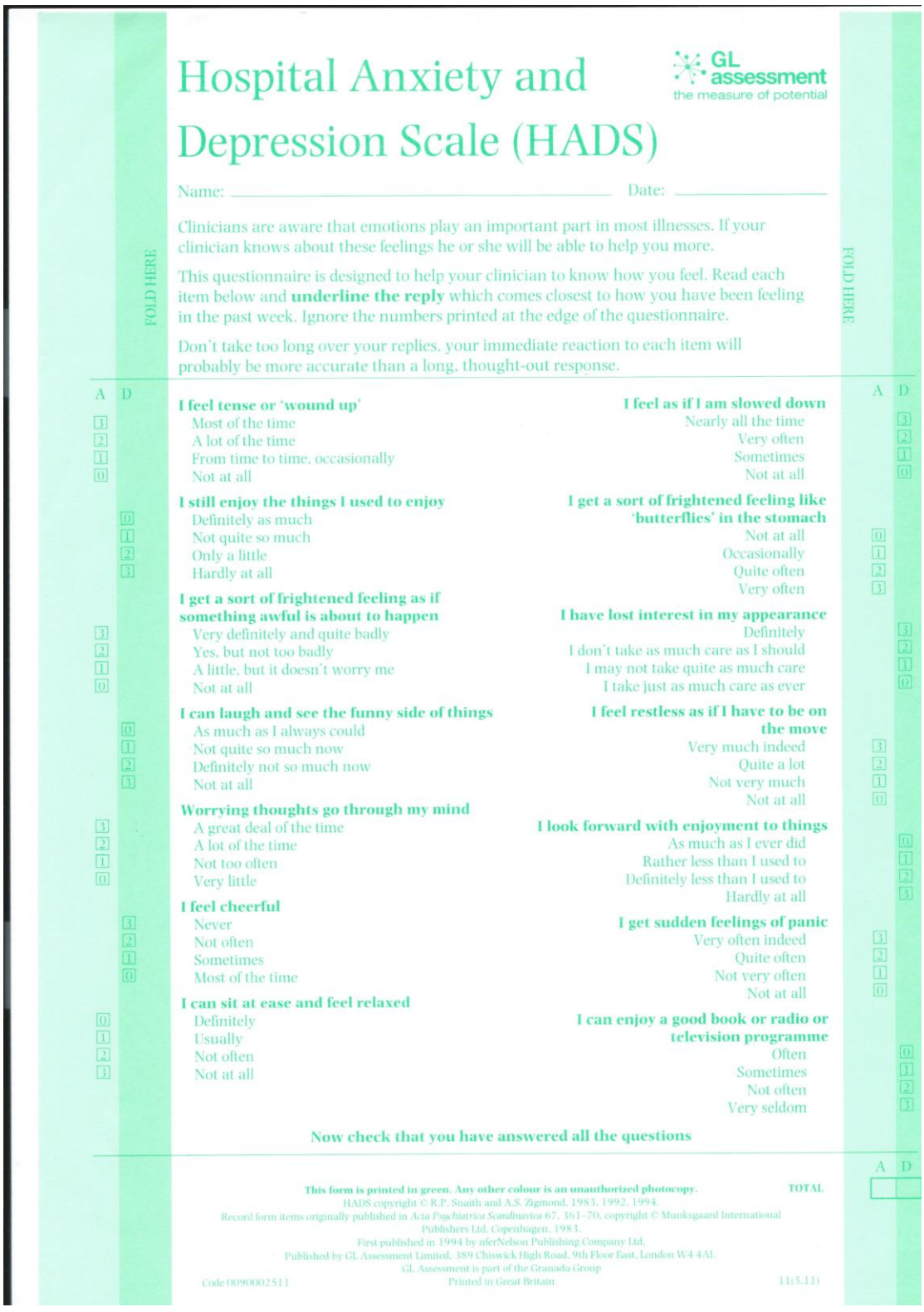
. . . . . .
. . . . . .
. . . . . .
. . . . . .

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐
It stops me doing one or two things I would like to do ☐
It stops me doing most of the things I would like to do ☐
It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

HADS



Hospital Anxiety and Depression Scale (HADS)

GL assessment
the measure of potential

Name: _____ Date: _____

Clinicians are aware that emotions play an important part in most illnesses. If your clinician knows about these feelings he or she will be able to help you more.

This questionnaire is designed to help your clinician to know how you feel. Read each item below and **underline the reply** which comes closest to how you have been feeling in the past week. Ignore the numbers printed at the edge of the questionnaire.

Don't take too long over your replies, your immediate reaction to each item will probably be more accurate than a long, thought-out response.

FOLD HERE

A	D		A	D
		I feel tense or 'wound up'		
3		Most of the time		
2		A lot of the time		
1		From time to time, occasionally		
0		Not at all		
		I still enjoy the things I used to enjoy		
	0	Definitely as much		
	1	Not quite so much		
	2	Only a little		
	3	Hardly at all		
		I get a sort of frightened feeling as if something awful is about to happen		
3		Very definitely and quite badly		
2		Yes, but not too badly		
1		A little, but it doesn't worry me		
0		Not at all		
		I can laugh and see the funny side of things		
	0	As much as I always could		
	1	Not quite so much now		
	2	Definitely not so much now		
	3	Not at all		
		Worrying thoughts go through my mind		
3		A great deal of the time		
2		A lot of the time		
1		Not too often		
0		Very little		
		I feel cheerful		
	3	Never		
	2	Not often		
	1	Sometimes		
	0	Most of the time		
		I can sit at ease and feel relaxed		
0		Definitely		
1		Usually		
2		Not often		
3		Not at all		
		I feel as if I am slowed down		
		Nearly all the time		3
		Very often		2
		Sometimes		1
		Not at all		0
		I get a sort of frightened feeling like 'butterflies' in the stomach		
		Not at all	0	
		Occasionally	1	
		Quite often	2	
		Very often	3	
		I have lost interest in my appearance		
		Definitely		3
		I don't take as much care as I should		2
		I may not take quite as much care		1
		I take just as much care as ever		0
		I feel restless as if I have to be on the move		
		Very much indeed	3	
		Quite a lot	2	
		Not very much	1	
		Not at all	0	
		I look forward with enjoyment to things		
		As much as I ever did		0
		Rather less than I used to		1
		Definitely less than I used to		2
		Hardly at all		3
		I get sudden feelings of panic		
		Very often indeed	3	
		Quite often	2	
		Not very often	1	
		Not at all	0	
		I can enjoy a good book or radio or television programme		
		Often		0
		Sometimes		1
		Not often		2
		Very seldom		3

Now check that you have answered all the questions

FOLD HERE

A	D

TOTAL

Rosenberg Self Esteem Questionnaire

NICHD SECCYD—Wisconsin

ROSENBERG SELF-ESTEEM SCALE

The next questions ask about your current feelings about yourself. For each of the following, please circle the number that corresponds with the answer that best describes how strongly you agree or disagree with the statement about yourself now.

	Strongly agree	Somewhat agree	Somewhat disagree	Strongly disagree
1. I feel that I am a person of worth, or at least on an equal plane with others.	1	2	3	4
2. I feel that I have a number of good qualities.	1	2	3	4
3. All in all, I'm inclined to feel that I am a failure.	1	2	3	4
4. I am able to do things as well as most other people.	1	2	3	4
5. I feel I do not have much to be proud of.	1	2	3	4
6. I take a positive attitude toward myself.	1	2	3	4
7. On the whole, I am satisfied with myself.	1	2	3	4
8. I certainly feel useless at times.	1	2	3	4
9. I wish I could have more respect for myself.	1	2	3	4
10. At times, I think I am no good at all.	1	2	3	4

Pediatric Inventory for Parents

PEDIATRIC INVENTORY FOR PARENTS

Below is a list of difficult events which parents of children who have (or have had) a serious illness sometimes face. Please read each event carefully, and circle HOW OFTEN the event has occurred for you in the past 7 days, using the 5 point scale below. Afterwards, please rate how DIFFICULT it was/or generally is for you, also using the 5 point scale. Please complete both columns for each item.

EVENT	HOW OFTEN? 1=Never, 2=Rarely, 3=Sometimes, 4=Often, 5=Very often	HOW DIFFICULT? 1=Not at all, 2=A little, 3=Somewhat, 4=Very much, 5=Extremely
1. Difficulty sleeping	1 2 3 4 5	1 2 3 4 5
2. Arguing with family member(s)	1 2 3 4 5	1 2 3 4 5
3. Bringing my child to the clinic or hospital	1 2 3 4 5	1 2 3 4 5
4. Learning upsetting news	1 2 3 4 5	1 2 3 4 5
5. Being unable to go to work/job	1 2 3 4 5	1 2 3 4 5
6. Seeing my child's mood change quickly	1 2 3 4 5	1 2 3 4 5
7. Speaking with doctor	1 2 3 4 5	1 2 3 4 5
8. Watching my child have trouble eating	1 2 3 4 5	1 2 3 4 5
9. Waiting for my child's test results	1 2 3 4 5	1 2 3 4 5
10. Having money/financial troubles	1 2 3 4 5	1 2 3 4 5
11. Trying not to think about my family's difficulties	1 2 3 4 5	1 2 3 4 5
12. Feeling confused about medical information	1 2 3 4 5	1 2 3 4 5
13. Being with my child during medical procedures	1 2 3 4 5	1 2 3 4 5
14. Knowing my child is hurting or in pain	1 2 3 4 5	1 2 3 4 5
15. Trying to attend to the needs of other family members	1 2 3 4 5	1 2 3 4 5
16. Seeing my child sad or scared	1 2 3 4 5	1 2 3 4 5
17. Talking with the nurse	1 2 3 4 5	1 2 3 4 5
18. Making decisions about medical care or medicines	1 2 3 4 5	1 2 3 4 5
19. Thinking about my child being isolated from others	1 2 3 4 5	1 2 3 4 5
20. Being far away from family and/or friends	1 2 3 4 5	1 2 3 4 5
21. Feeling numb inside	1 2 3 4 5	1 2 3 4 5
22. Disagreeing with a member of the health care team	1 2 3 4 5	1 2 3 4 5
23. Helping my child with his/her hygiene needs	1 2 3 4 5	1 2 3 4 5

EVENT	HOW OFTEN? 1=Never, 2=Rarely, 3=Sometimes, 4=Often, 5=Very often	HOW DIFFICULT? 1=Not at all, 2=A little, 3=Somewhat, 4=Very much, 5=Extremely
24. Worrying about the long term impact of the illness	1 2 3 4 5	1 2 3 4 5
25. Having little time to take care of my own needs	1 2 3 4 5	1 2 3 4 5
26. Feeling helpless over my child's condition	1 2 3 4 5	1 2 3 4 5
27. Feeling misunderstood by family/friends as to the severity of my child's illness	1 2 3 4 5	1 2 3 4 5
28. Handling changes in my child's daily medical routines	1 2 3 4 5	1 2 3 4 5
29. Feeling uncertain about the future	1 2 3 4 5	1 2 3 4 5
30. Being in the hospital over weekends/holidays	1 2 3 4 5	1 2 3 4 5
31. Thinking about other children who have been seriously ill	1 2 3 4 5	1 2 3 4 5
32. Speaking with my child about his/her illness	1 2 3 4 5	1 2 3 4 5
33. Helping my child with medical procedures (e.g. giving shots, swallowing medicine, changing dressing)	1 2 3 4 5	1 2 3 4 5
34. Having my heart beat fast, sweating, or feeling tingly	1 2 3 4 5	1 2 3 4 5
35. Feeling uncertain about disciplining my child	1 2 3 4 5	1 2 3 4 5
36. Feeling scared that my child could get very sick or die	1 2 3 4 5	1 2 3 4 5
37. Speaking with family members about my child's illness	1 2 3 4 5	1 2 3 4 5
38. Watching my child during medical visits/procedures	1 2 3 4 5	1 2 3 4 5
39. Missing important events in the lives of other family members	1 2 3 4 5	1 2 3 4 5
40. Worrying about how friends and relatives interact with my child	1 2 3 4 5	1 2 3 4 5
41. Noticing a change in my relationship with my partner	1 2 3 4 5	1 2 3 4 5
42. Spending a great deal of time in unfamiliar settings	1 2 3 4 5	1 2 3 4 5

MFSI-SF

Below is a list of statements that describe how people sometimes feel. Please read each item carefully, then circle the one number next to each item which best describes **how true each statement has been for you in the past 7 days.**

		Not at all	A little	Moderately	Quite a bit	Extremely
1.	I have trouble remembering things	0	1	2	3	4
2.	My muscles ache	0	1	2	3	4
3.	I feel upset	0	1	2	3	4
4.	My legs feel weak	0	1	2	3	4
5.	I feel cheerful	0	1	2	3	4
6.	My head feels heavy	0	1	2	3	4
7.	I feel lively	0	1	2	3	4
8.	I feel nervous	0	1	2	3	4
9.	I feel relaxed	0	1	2	3	4
10.	I feel pooped	0	1	2	3	4
11.	I am confused	0	1	2	3	4
12.	I am worn out	0	1	2	3	4
13.	I feel sad	0	1	2	3	4
14.	I feel fatigued	0	1	2	3	4
15.	I have trouble paying attention	0	1	2	3	4
16.	My arms feel weak	0	1	2	3	4
17.	I feel sluggish	0	1	2	3	4
18.	I feel run down	0	1	2	3	4
19.	I ache all over	0	1	2	3	4
20.	I am unable to concentrate	0	1	2	3	4
21.	I feel depressed	0	1	2	3	4
22.	I feel refreshed	0	1	2	3	4
23.	I feel tense	0	1	2	3	4
24.	I feel energetic	0	1	2	3	4
25.	I make more mistakes than usual	0	1	2	3	4
26.	My body feels heavy all over	0	1	2	3	4
27.	I am forgetful	0	1	2	3	4
28.	I feel tired	0	1	2	3	4
29.	I feel calm	0	1	2	3	4
30.	I am distressed	0	1	2	3	4

IBD Disability Score

PLEASE READ ALOUD THIS INSTRUCTIONS TO THE PATIENT
The first question is about the overall health of the patient, including both physical and mental health.

ANSWERS:1 = Very good; 2 = Good; 3 = Moderate; 4 = Bad; 5 = Very bad	1	2	3	4	5
Overall Health					
1. In general, how would you rate your health today?					

PLEASE READ ALOUD THESE INSTRUCTIONS TO THE PATIENT
Now I would like to review different functions of your body and activities of daily life. When answering these questions, I would like you to think about the last week, taking both good and bad days into account. When I ask about difficulty / problem, I would like you to consider how much difficulty / problem you have had, on an average, in the past week, while doing the activity in the way that you usually do it. By difficulty I mean that you require increased effort, that you have discomfort or pain, or that the activity is slower or that there are other changes in the way you do the activity. Please answer this question taking into account any assistance you have available.
(Read and show scale to respondent).

ANSWERS: 1 = None; 2 = Mild; 3 = Moderate; 4 = Severe; 5 = Extreme	1	2	3	4	5
Sleep and Energy					
2. Overall in the last week, how much of a problem did you have with sleeping, such as falling asleep, waking up frequently during the night or waking up too early in the morning? (b134)					
3. In the last week, how much of a problem did you have due to not feeling rested and refreshed during the day (e.g. feeling tired, not having energy)? (b130)					
Affect					
4. Overall in the last week, how much of a problem did you have with feeling sad, low or depressed? (b152)					
5. Overall in the last week, how much of a problem did you have with worry or anxiety? (b152)					
Body Image					
6. Overall in the last week, how much of a problem did you have with the way your body or body parts looked? (b1801)					
Pain					
7. Overall in the last week, how much of stomach or abdomen aches or pains did you have? (b28012)					

ANSWERS: 1 = None; 2 = Mild; 3 = Moderate; 4 = Severe; 5 = Extreme or cannot do	1	2	3	4	5
Regulating defecation					
8. Overall in the last week, how much difficulty did you have coordinating and managing defecation including choosing and getting to an appropriate place for defecation and cleaning oneself after defecation? (d5301)					
Looking after one's health					
9. Overall in the last week, how much difficulty did you have looking after your health, including maintaining a balanced diet? (d570)					
Interpersonal Activities					
10. Overall in the last week, how much difficulty did you have with personal relationships? (d7)					
11. Overall in the last week, how much difficulty did you have with participation in the community? (d920)					
Work and Education					
12. Overall in the last week, how much difficulty did you have with work or household activities? (d840-d859)					
13. Overall in the last week, how much difficulty did you have with school or studying activities? (d810-d899)					

(b525) Number of liquid or very soft stools in the last week _____
(b515) Body mass Index (BMI): _____ kg/m²
(b515) Do you feel that you have lost weight in the last week? ☐ Yes ☐ No
(s540) Blood in stool (weekly average): ☐ None ☐ Little ☐ A lot
(s770) Is arthritis or arthralgia present? ☐ Yes ☐ No

Please rate the extent to which the following aspects of the patient's environment positively or negatively influenced disease activity, body functions, and activities of daily life, which you have reviewed with the patient.

ANSWERS: NA = Not applicable; 1 = No positive effect; 2 = Mild positive effect; 3 = Moderate positive effect; 4 = Severe positive effect; 5 = Extreme positive effect	NA	1	2	3	4	5
+14. Overall in the last week, did the medication the patient take alleviate her/his problems and difficulties? (e1100)						
+15. Overall in the last week, did the food the patient take alleviate her/his problems and difficulties? (e1101)						
+16. Overall in the last week, did the patient's family alleviate her/his problems and difficulties? (e310)						
+17. Overall in the last week, did health professionals alleviate the patient's problems and difficulties? (e355)						

ANSWERS: NA = Not applicable; 1 = No negative effect; 2 = Mild negative effect; 3 = Moderate negative effect; 4 = Severe negative effect; 5 = Extreme negative effect	NA	1	2	3	4	5
-14. Overall in the last week, did the medication the patient take worsen her/his problems and difficulties? (e1100)						
-15. Overall in the last week, did the food the patient take worsen her/his problems and difficulties? (e1101)						
-16. Overall in the last week, did the patient's family worsen her/his problems and difficulties? (e310)						
-17. Overall in the last week, did health professionals worsen the patient's problems and difficulties? (e355)						

ANSWERS: 1 = No; 2 = yes	1	2
Social security and health services, systems and policies		
18. Does the patient benefit from the support he or she needs from the social security system? (e570)		
19. Does the patient receive the health care he or she needs? (e580)		

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.
Thank you for completing this survey!

For each of the following questions, please tick the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

		Yes, limited a lot	Yes, limited a little	No, not limited at all
a	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c	Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d	Climbing several flights of stairs	☐ 1	☐ 2	☐ 3
e	Climbing one flight of stairs	☐ 1	☐ 2	☐ 3
f	Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g	Walking more than a mile	☐ 1	☐ 2	☐ 3
h	Walking several hundred yards	☐ 1	☐ 2	☐ 3
i	Walking one hundred yards	☐ 1	☐ 2	☐ 3
j	Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

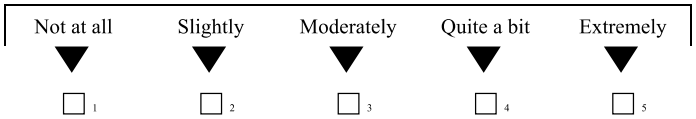
4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Had difficulty performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

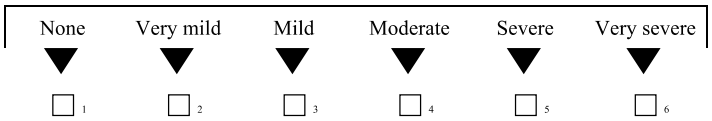
5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

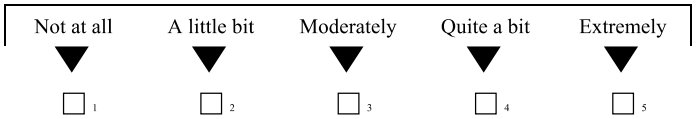
6. **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?**



7. **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?**



8. **During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?**



9. These questions are about how you feel and how things have been with you <u>during the past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the <u>past 4 weeks</u>…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f Have you felt downhearted and low?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a I seem to get ill more easily than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

Appendix 3: SOP for DHR and Autoantibodies by Immunofluorescence

SOP for DHR

Purpose of the Examination

This assay allows the investigation of altered neutrophil oxidative burst activity. Reduced or absent burst activity is observed in inbourne defects such as chronic granulomatous disease (CGD). CGD is characterised clinically by repeated and life-threatening infections caused by bacterial and fungal organisms. It may mimic inflammatory bowel disease and lead to malabsorption and obstruction of the bowel. Abscesses are another feature involving the liver, lungs or lymph nodes. Different forms of CGD are described (X-linked and autosomal recessive patterns).
Neutrophils from CGD patients fail to produce a significant oxidative burst following stimulation. NADPH oxidase is the enzyme system involved during intracellular killing as part of the phagocytic process. Superoxide anion is produced, which is quickly converted to hydrogen peroxide and hydroxyl radicals, which destroy bacteria in the phagosome. Abnormalities in the constitutive peptides of the NADPH oxidase enzyme system lead to the dysfunctions characteristic of CGD.
This assay is performed along side the nitro-blue tetrazolium (NBT) assay allowing a rapid and sensitive method for the diagnosis of CGD and for the detection of X-linked carriers.

2 Principle

Upon stimulation, neutrophils (and monocytes) produce reactive oxygen intermediates (ROI), such as superoxide anion, hydrogen peroxide and hypochlorous acid that destroy bacteria in the phagosome.
ROI's produced upon activation of DHR loaded normal neutrophils; react with the DHR and the resulting increase in fluorescence is detected by the flow cytometer FL-1 detector.
Chronic granulomatous disease (CGD) is characterised by the diminished or absent production of reactive oxygen intermediates (ROI). In the X-linked form, carriers can be detected by the presence of two different cell populations, one normal and one negative for ROI.

3 Safety considerations

Good laboratory practice is essential when performing all procedures.
Disposable gloves must be worn when handling samples, but this does not preclude washing hands regularly.

INDIRECT IMMUNOFLUORESCENCE FOR THE DETECTION OF ROUTINE AUTOANTIBODIES USING THE EUROIMMUNE BIOCHIPS AND THE TITERPLANE TECHNIQUE.

Contents

Appendix 4: Publications

J Clin Immunol. 2013 Nov;33(8):1276-84.

Clinical manifestations of disease in X-linked carriers of chronic granulomatous disease.

Battersby AC, Cale AM, Goldblatt D, Gennery AR.

J Clin Immunol
DOI 10.1007/s10875-013-9939-5

KEY REVIEW ARTICLE

Clinical Manifestations of Disease in X-Linked Carriers of Chronic Granulomatous Disease

A. C. Battersby &C. M. Cale &D. Goldblatt &A. R. Gennery

Received: 11 February 2013 / Accepted: 16 September 2013

Abstract Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiency due to a defect in one of the NADPH oxidase complex subunits; 70 % of cases are X-linked, due to a CYBB mutation, resulting in defective production of $gp91^{PHOX}$. Female carriers of X-linked CGD have previously been considered to be unaffected. It is increasingly recognized that they may suffer from similar problems to CGD patients. This review will examine the literature about clinical manifestations of disease in X-linked carriers of CGD.

Keywords Chronic granulomatous disease · X-linked carriers · primary immunodeficiency

Background

Chronic Granulomatous Disease (CGD) is a rare, inherited primary immunodeficiency in which a defect in one of the subunits of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase results in failure of phagocytes to generate reactive oxygen intermediates (ROI), and thus fail to kill bacteria and fungi. Patients present with recurrent infection, particularly with catalase positive organisms such as Staphylococcus aureus and Aspergillus, and inflammation.

A. C. Battersby · A. R. Gennery
Institute of Cellular Medicine, Newcastle University, London, UK

C. M. Cale
Clinical Immunology, Great Ormond Street Hospital, London, UK

D. Goldblatt
Institute of Child Health, University College London, London, UK

A. C. Battersby (*)
Department Paediatric Immunology & Infectious Diseases, Old Children's Outpatients, Royal Victoria Infirmary, Newcastle NE1 4LP, UK
e-mail: a.battersby@ncl.ac.uk

CGD is inherited in an X-linked manner in approximately 70 % of cases with the remainder being autosomal recessive [1]. X-linked disease is the result of a defective CYBB gene, encoding the $gp91^{phox}$ subunit.

X-linked CGD patients have no normally functioning phagocytes, although some may demonstrate reduced, rather than absent, NADPH activity [2]. Female carriers possess only one copy of the mutated gene on the X chromosome, and subsequent to lyonisation, have two populations of phagocytes: those which are $gp91^{phox}$ positive and those which are negative. Random X chromosome inactivation early in the fetal development of haematopoietic precursor cells may lead to two unequal populations.

The diagnosis of CGD is based upon the demonstration of an absent respiratory burst. Phagocytes in healthy controls upon stimulation produce superoxide which is the product of oxidase activation. Phagocytes from CGD patients, when stimulated, do not produce superoxide. X-linked carriers have a dual population of cells; those which are able to produce a respiratory burst and subsequently a superoxide and those which cannot.

Diagnosis may be made by either the Nitroblue Tetrazolium (NBT) reduction test or by using flow cytometry to detect altered fluorescence. Both methods will also detect X-linked carrier status, as it is possible to demonstrate the two populations of cells and to quantify the percentage of functioning neutrophils using either method.

The NBT reduction test is traditionally used to confirm the diagnosis of CGD. NBT is a yellow dye which is reduced to blue formazan by the superoxide produced as a result of the respiratory burst. The result is read manually; CGD phagocytes remain yellow as they do not reduce the NBT and normal phagocytes show the blue precipitate of formazan. X-linked carriers demonstrate both colours as the two populations of cells are present. Interpretation of results in this test is subjective and detection of X-linked carriers can be difficult as the two populations may not be distinct [3].

Published online: 29 September 2013

A flow cytometric method used in clinical practice to diagnosis CGD stimulates neutrophils with Phorbol-12-Myristate-13 Acetate (PMA) [4] to produce ROI such as hydrogen peroxide. The resulting ROI react with a fluorescent probe such as dihydrorhodamine (DHR) which increases fluorescence, detected by the flow cytometer. In healthy controls, all phagocytes produce an oxidative burst which is shown as a peak (Fig. 1). In patients with CGD the oxidative burst is absent in the majority of X-linked patients or substantially reduced, and no peak is produced upon stimulation. Carriers of X-linked disease demonstrate two populations of neutrophils (Fig. 2), as some phagocytes produce an oxidative burst when stimulated, whilst those in which the mutated X-chromosome is active i.e. those which are $gp91^{phox}$ negative, do not. Most carriers will exhibit between 20 % and 80 % normal burst activity.

Although DHR is the most commonly used fluorescent probe, others are available. However, DHR has been shown to provide the best distinction between normal and abnormal (CGD) phagocytes. When compared with two other fluorescent probes (2′7′-dichlorofluorescin and 5,6-carboxy-2′7′-dichlorofluorescein diacetate) DHR demonstrated a higher intensity fluorescence and the greatest separation of fluorescent signals between normal and abnormal phagocytes [5].

X-Linked Carriers

Female carriers of X-linked disorders are usually considered to be unaffected by the defective gene. However, it is increasingly recognised in clinical practice that female carriers of X-linked CGD may display similar clinical manifestations to patients which may significantly impact on their health.

This review will describe symptoms in X-linked carriers illustrated by vignettes and review the available literature about clinical manifestations of disease in this group.

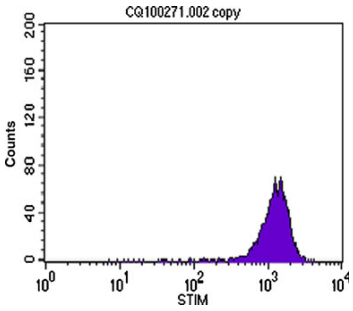


Fig. 1 Neutrophil oxidative burst in an unaffected individual

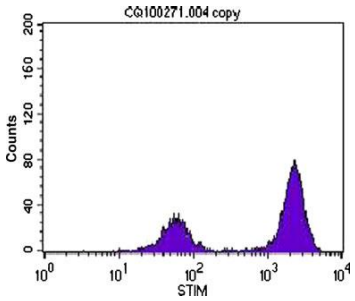


Fig. 2 Neutrophil oxidative burst in an X-linked carrier demonstrating the dual population upon stimulation

Skin Disease

The mother of a boy with known X-linked CGD suffered from a recurrent photosensitive rash in the malar distribution. The rash started in childhood but persisted and continues to be exacerbated by sunlight. After referral to dermatology at her local hospital a diagnosis of discoid lupus was made. Her neutrophil oxidative burst shows 73 % normally functioning neutrophils.

Skin rashes in X-linked carriers of CGD are well described and an association with discoid lupus erythematosis (DLE) long established [6–9]. The presence of a persistent facial rash resembling DLE in 2 X-linked carriers was first described in 1970 [10] and these findings have been corroborated in other carrier studies subsequently, most recently in 2007 when Cale et al. [9] found photosensitive skin rashes in 58 % of their carrier cohort.

Clinical and histopathological features are consistent in the literature, but severity and clinical course are not. Virtually all cases describe the presence of photosensitive eruptions on sun exposed surfaces with similar, if not identical, histological findings to DLE. The most common site described is in the malar distribution and on the hands. Age at onset of skin disease is not constant, with reports of early childhood appearances featuring alongside later presentations. Severity ranges from minor irritation, resolving in childhood, to treatment resistant disease persisting into late adulthood.

Photosensitivity alone is commonly described in X-linked carriers without the presence of classical DLE [7, 11]. Sillevis-Smitt et al. describe 10 of 16 X-linked carriers with relapsing skin eruptions, of whom 7 were provoked by sunlight, but not all had typical features of DLE [6]. Despite this link of DLE with X-linked carriers, DLE is unusual in patients with X-linked CGD, although has been occasionally reported [12].

Conversely, when X-linked CGD carrier status was sought in patients presenting with DLE, no carriers were found [13]. This was, however, a small scale study and the authors acknowledge that it remains important to look for X-linked

CGD in women presenting with DLE, particularly in the presence of related symptoms such as recurrent or suppurative infection or a family history of early childhood death. These findings were corroborated in a further study of 34 patients presenting with DLE were tested for carrier status with an NBT test. No carriers were found but the authors recommended screening for carrier stuatus particularly in the presence of aphthous stomatitis [14].

Alongside photosensitivity and DLE manifestations, aphthous stomatitis is reported with even greater prevalence. Brandrup reported as early as 1981 that 7 out of 9 X-linked carriers suffered recurrent aphthous-like stomatitis [7]. This was supported by a later survey in 1989 when 70 % of 16 carriers reported recurrent aphthous stomatitis [6] higher than the 63 % reporting recurrent skin eruptions. In both reports the symptoms have been severe. Case reports also corroborate these findings [15, 16].

A summary of the literature about skin disease in X-linked carriers of CGD is shown in Table I.

Gastrointestinal Manifestations

The sister of a patient with known X-linked CGD was seen in the paediatric department before her 8th birthday with recurrent aphthous ulcers. She had no other symptoms. She was re-referred to the paediatric department at the age of 15 years with abdominal pain, diarrhoea and urgency. She had poor appetite, weight loss and lethargy. Her height and weight were both on the 9th per centile. Endoscopy shortly after the clinical diagnosis was made showed severe active chronic colitis worse in the sigmoid colon. The overall appearances were consistent with chronic inflammatory bowel disease. Given the presence of small granulomata and the segmental distribution the diagnosis of active Crohn's disease was made.

She initially responded to corticosteroid treatment, but relapsed as treatment ceased and was commenced on azathioprine but continued to suffer abdominal pain and bloody stool. She was subsequently commenced on infliximab and

Table I Summary of literature describing skin manifestations in X-linked CGD carriers

Authors	Year	Number of carriers	Skin manifestations
Thompson et al. [10]	1970	8	2 persistent facial rash
Brandrup et al. [7]	1981	9	4 photosensitivity
			3 DLE (Skin and face)
Kragballe et al. [15]	1981	15	7 Photosensitivity
			5 DLE like skin lesions
			Irritant hand eczema
Barton et al. [38]	1986	1	Facial eruption with photosensitivity
			Folliculitis
Garioch et al. [11]	1989	5	Present in all
			All photosensitive
Sillevis Smitt at al [6]	1990	16	Present in 10
			7 photosensitive, 5 histological features of DLE
			3 recurrent skin infection
Yeaman et al. [26]	1992	1	Photosensitivity and DLE-like skin lesions
Hafner et al. [49]	1992	1	Photosensitivity
			Histologically typical DLE
Lovas et al. [50]	1995	1	Photosensitivity
			Discoid skin lesions (DLE on biopsy)
Martin-Villa et al. [28]	1999	13	DLE in 2 patients
Rupec et al. [51]	2000	2	Photosensitive rash
			Histopathological changes of LE Timidus
Rosen-Wolf et al. [16]	2001	1	Photosensitivity
Moltyaner et al. [20]	2003	1	Cutaneous LE
Foti et al. [8]	2004	1	Erythematous plaques (DLE-like rash)
Cale et al. [9]	2007	19	58 % Skin rash
Chollet-Martin et al. [36]	2007	2	Skin ulcers (Mycobacteria)
			Folliculitis
Cordoba-Guijarro et al. [27]	2008	1	Erythematous plaques
			Photosensitivity

is now maintained on weekly methotrexate, azithromycin and itraconazole. She remains well.

Her neutrophil oxidative burst confirmed carrier status with a dual population demonstrating 27 % normally functioning neutrophils most recently. Her autoantibody screen on initial presentation was negative but she has now developed positive anti-DS DNA antibodies and positive ENA-Ro antibodies.

Colitis is a common feature of CGD [17, 18] and has been particularly associated with X-linked disease [19]. There is considerable clinical and histopathological overlap between CGD colitis and inflammatory bowel disease (IBD) and in particular, Crohn's disease.

Gastrointestinal manifestations are described in X-linked carriers of CGD. Moltanyer et al. [20] describe a female carrier who presented at the age of 41 years with proven bronchocentric granulomatosis. Her background history revealed that at age 13 she had been diagnosed with ulcerative colitis and had a history of recurrent infection. On testing she had 10 % functioning neutrophils. Other gastrointestinal manifestations have also been seen in the carrier population including intermittent diarrhoea [9] and colonic polyposis [8].

These cases raise the question of whether there may be a higher prevalence than expected of X-linked CGD carriers in patients with IBD. A recent prospective study screened 120 paediatric patients with a diagnosis of IBD by performing a neutrophil oxidative burst. They failed to find any CGD patients or X-linked carriers within the IBD cohort [21]. However, this study is limited as it included children of all ages. Most CGD patients are diagnosed by the age of 5 years. The mean age in this study was 14.8 years, so it would be expected that any CGD patients would have already been diagnosed. The lack of X-linked carriers diagnosed may reflect the small scale of the study rather than a lack of association. No study to date has looked at X-linked carriers of CGD specifically for IBD or gastrointestinal symptoms. Anecdotal reports suggest that there may be more cases than currently reported in the literature.

Impaired neutrophil function, albeit with normal NBT reduction, has been found in Crohn's patients when compared to healthy controls [22]. No patients or X-linked carriers of CGD were found, but this finding highlights the close relationship between neutrophil dysfunction and inflammation, particularly within the gastrointestinal tract.

Treatment of X-linked carriers with IBD may require different considerations than those who have IBD alone. If the reduction in neutrophil function in X-linked carriers is sufficient to lead to inflammatory manifestations, they should be considered at increased risk of infection as well. More aggressive prophylaxis for infection should be considered when commencing immunosuppression in this group and aggressive treatment of existing infections prior to initiating therapy is perhaps merited.

Infliximab was used with success in the patient described in the vignette, and has been shown to improve outcome in Crohns disease and is known to be particularly effective when there are fistulae. The major side effect of infliximab is the increased risk of infection either by reactivation of latent infections such as tuberculosis or with new infections, although this has frequently been shown in the context of simultaneous corticosteroid use. CGD patients are at particular risk of this complication. In a case series of 5 CGD patients with inflammatory bowel disease treated with infliximab, all improved significantly, including resolution of fistulae, but all developed significant infection requiring the infliximab to be discontinued [23]. The success of infliximab in the vignette highlights the similarities between the gastrointestinal disease seen in CGD patients and X–linked carriers. It may also demonstrate that although X-linked carriers are at risk of inflammatory manifestations of CGD, the neutrophils which function normally are at sufficient levels to prevent the infective complications even when treated with immunosuppressive agents. Alternatively, it may be that simple antimicrobial and anti-fungal prophylaxis is sufficient to enable infliximab to be used without adverse effect in X-linked carriers.

The similarities in presentation of this X-linked carrier to boys with CGD, and the incomplete understanding of why some CGD patients suffer from severe colitis whilst others are spared, leads us to consider whether there may be some clinicopathological overlap between the gastrointestinial disease seen in the patients and in this, and potentially other, X-linked carriers. It is possible that inflammatory bowel disease is more common in X-linked carriers of CGD than hitherto suspected, but this has yet to be demonstrated.

A summary of the literature of X-linked carriers and gastrointestinal manifestations of CGD is shown in Table II.

Autoimmunity

A 39 year old lady, known to be an X-linked carrier of CGD as a result of a diagnosis in her son, was referred to hospital due to a persistent photosensitive rash in the malar distribution and over the chest associated with recurrent mouth ulcers, Raynaud's phenomenon and arthritis of both wrists. Her autoantibody screen was negative but a clinical diagnosis of 'Lupus-like Syndrome' was made and she was commenced

Table II Summary of literature about gastrointestinal manifestations in X-linked CGD

Author	Year	Number of carriers	Manifestation
Moltanyer [20]	2003	1	Ulcerative colitis
Foti [8]	2004	1	Polyposis of colon
Cale [9]	2007	19	1 intermittent diarrhoea

on hydroxychloroquine and nifedipine. Her most recent neutrophil oxidative burst demonstrated 48 % normally functioning neutrophils.

Autoimmune manifestations are recognised features of primary immunodeficiencies [24] and CGD is no exception. Autoimmunity other than IBD is described within CGD patients with Juvenile Idiopathic Arthritis, Cutaneous Lupus Erythematous (LE) and IgA nephropathy all reported [25]. However, the association with autoimmunity is better described in X-linked carriers of CGD rather than patients. Case reports of CGD patients frequently comment that their carrier female relatives suffer from features of autoimmune disease [6, 10, 25].

Reports about autoimmune manifestations in X-linked carriers have been published. Polyarthritis, recurrent aphthous ulcers and Raynaud's phenomenon are all reported [6, 7, 10, 20, 26]. The prevalence of these features appears to be more than expected in a healthy population.

These reports prompted Cale et al. [9] to perform a limited assessment of the physical health of 19 carriers of X-linked CGD to further investigate autoimmune manifestations. Aphthous stomatitis, photosensitivity and Raynauds phenomenon were frequently demonstrated [9]. Thirty-seven percent of X-linked carriers suffered from joint pain, with no other cause identified and nearly half of the cohort suffered from excessive fatigue [9]. This was the largest cohort of X-linked carriers studied to date and confirms what had previously been reported on a smaller scale, such as in Sillevis Smitt's study where 70 % of the X-linked carriers surveyed suffered recurrent apthous ulcers and 63 % had recurrent skin eruptions [6].

Autoantibody screening in X-linked carriers, even those with symptoms, has repeatedly been found to be negative, [9, 27] in contrast to those individuals with isolated autoimmune disease. Although, not universally screened, where autoantibody titres have been measured, they have been negative, with the exception of one study where it was found that autoantibodies were significantly more prevalent in the X-linked carriers than healthy, non-carrier family members [28]. Martin-Villa's study was one of the few studies which not only looked at X-linked carriers of CGD but also looked at the family members who were unaffected using them as a control group.

Many of the case reports and series describing X-linked carriers with skin disease also comment on associated features including arthralgia [29] and polyarthritis [15] and Raynaud's disease [6, 10] highlighting that X-linked carriers may be affected. This is further alluded to in case reports about patients with CGD, when references are also made to their relatives having higher incidences of autoimmune disease.

A summary of the literature about autoimmune features in X-linked carriers of CGD is shown in Table III.

Table III Summary of literature about autoimmune features in X-linked carriers of CGD

Authors	Year	Number of carriers	Autoimmune symptoms
Thompson [10]	1970	8	1 polyarthritis
Brandrup [7]	1981	9	7 recurrent aphthous stomatitis 1 acute glomerulonephritis
Kragballe [15]	1981	15	10 recurrent stomatitis 1 polyarthritis
Garioch [11]	1989	5	4 recurrent aphthous ulcers
Sillevis Smitt [6]	1990	16	11 recurrent aphthous ulcers 3 Raynaud's
Yeaman [26]	1992	1	Recurrent aphthous ulcers
Lovas [50]	1995	1	Recurrent ulcers with candidiasis
Martin-Villa [28]	1999		Autoantibodies
Rosen-Wolf [16]	2001	1	Aphthous stomatitis
Wolach [29]	2005	1	Arthralgia Aphthous stomatitis Pyoderma gangrenosum Vasculitis-like skin rash
Cale [9]	2007	19	8 recurrent aphthous ulcers 7 joint pain 8 excessive fatigue Negative autoantibodies
Cordoba-Guijarro [27]	2010	1	Suabcute lupus erythematous Autoantibodies negative

Chorioretinitis

Chorioretinitis as a manifestation of CGD has been reported since the 1970s [30]. It affects a significant number of patients and in some cases can lead to loss of vision [31]. Chorioretinitis in isolation, outside of CGD is rare, although can occur in other conditions such as toxoplasmosis. Although the lesions are usually non-progressive, cases of retinal detachment have been described [32] and it is therefore, an important finding.

In 1999, Goldblatt et al. [33] examined the eyes of a cohort of CGD patients and also their carrier female relatives. Of 38 patients screened almost 24 % had chorioretinal lesions demonstrated. Interestingly, 10 % of the carrier cohort had discrete typical lesions, compared to none of the non-carrier, non-patient control group. Lesions were rarely associated with visual disturbances. The lesions seen in both the X-linked carriers and patients were similar to previous descriptions of affected CGD patients; well circumscribe chorioretinal scars [33]. This

concurs with the findings of Brandrup et al. [7] who also described an historical case of Chorioretinitis in an X-linked carrier.

These findings add weight to the concept that X-linked carriers may be at significant risk of manifestations of CGD.

Infection

CGD patients suffer from recurrent infection and are particularly susceptible to infection with catalase positive organisms. Despite the reduced number of functioning neutrophils in X-linked carriers of CGD, recurrent infection is not well described. Johnston et al. [34] described a girl presenting with recurrent infections classical of CGD, including recurrent infective abscesses, pneumonia and multiple isolations of *S. aureus*, who was found to be a carrier of X-linked CGD. Lewis et al. [35] described a 16 year girl who presented with persisting pneumonia despite aggressive antibiotic therapy who was found to be an X-linked carrier. *Aspergillus fumigatus* was isolated from broncheoalveolar lavage and also sputum. She had been previously fit and well but did have a history of recurrent abscesses.

Recurrent skin abscesses requiring antibiotics and at times surgical drainage have also been described in X-linked carriers [15, 36–38] and have been shown to isolate CGD typical organisms including *S. aureus* [29]. Single episodes of infection have also been reported such as the case of persistent systemic salmonella infection in a 34 year old X-linked carrier [39]. As a single example of infection this perhaps does not carry significant weight to the hypothesis of X-linked carrier being at risk of similar infective organisms to the patients but in the context of the case reports there is a growing body of evidence that suggests X-linked carriers may be at greater risk of the infective manifestations of CGD as well as the inflammatory complications.

A summary of the literature about infection in X-linked carriers of CGD is shown in Table IV.

Table IV Summary of literature about infection in X-linked carriers of CGD

Author	Year	Number of carriers	Manifestation
Moelleri [39]	1970	1	Persistent salmonella infection
Kragballe [15]	1981	15	2 hidradenitis
Johnston [52]	1985	1	Frequent infection with *Staphylococcus aureus*, psuedomonas, E coli
			Multiple sites
Barton [38]	1986	3 Kindreds	Abscesses requiring drainage
			Hidradenitis suppurativia
			Folliculitis
Lun [37]	2002	1	Aspergillus fumigatus infection
			Cutaneous abscesses
			Recurrent pneumonia
			Severe salmonella sepsis
Moltanyer [20]	2003	1	Aspergillus colonisation
Wolach [29]	2005	1	Serratia marcescens sepsis
			Recurrent pneumonia
			S aureus abscess, acinetobacter skin abscess, escherichia coli
			Candida albicans urinary infection
			Providentia osteomyelitis
Chollet-Martin [36]	2007	2	Skin abscesses
			Mycobacteria skin ulcers
			Abdominal wall abscess
			Folliculitis
			Liver abscess
Lewis [35]	2008	1	Aspergillus fumigatus bronchopneumonia
Cordoba-Guijarro [27]	2010	1	Brucellosis
			Bacterial meningitis
			Pneumonia

Residual NADPH Oxidase

CGD is a heterogeneous condition with some patients presenting early in infancy with severe infection or inflammatory complications, whilst others do not present until late childhood or even early adulthood with apparently less severe features. Autosomal recessive disease was originally considered less severe [40] as evidenced by later presentation and less aggressive clinical course in many of the patients with AR disease.

X-linked patients have a higher disease-related mortality [41] and classically have a more severe form of disease including more severe gastrointestinal involvement.

However, more in depth scrutiny reveals that this is too simplistic an interpretation. There are reports of X-linked patients with single organ involvement [42] and of X-linked patients presenting in adulthood [43, 44] thereby complicating this simple distinction.

Consistent with the late presenting or less severe phenotypes was the finding that these individuals had preservation of residual NADPH oxidase function. This was described in the reports of the patients presenting in adulthood irrespective of whether they had AR or XL disease.

The hypothesis of residual function of NADPH oxidase led to Kuhn's et al. [2] examining a cohort of 287 CGD patients to determine a correlation between clinical symptoms and residual NADPH oxidase production. The degree of ROI production was quantified and based on this four subgroups or quartiles were defined independent of the mutation. Each quartile was then analysed for survival and markers of disease severity. Better survival rates were associated with higher superoxide production irrespective of the specific genetic mutation, supporting the hypothesis of the importance of residual NADPH oxidase function in disease severity.

Whilst residual ROI production was an independent predictor of severity, specific mutations correlated with residual ROI production. Mutations in $p47^{phox}$ and most missense mutations in $gp91^{phox}$ had a greater degree of residual ROI production than nonsense, frameshift, splice or deletion mutations in $gp91^{phox}$ [2].

Despite the importance of greater superoxide production in improved survival, this study demonstrated that gastrointestinal symptoms did not correlate with the degree of ROI production. Therefore it is clear that residual NADPH oxidase function alone does not protect from colitis. Other factors may contribute to disease manifestations and severity, including cytokine polymorphisms [45], and possibly gastrointestinal microbiota [46, 47].

If we consider X-linked carriers in the context of this study, it would seem logical that they are similar to CGD patients but have greater residual NADPH oxidase function preserved. The level of function at which symptoms and signs may appear is not clear. X-linked carriers are generally considered healthy. It has previously been suggested that as low as 5–10 % functioning neutrophils would require a massive exposure to an infectious agent to be considered at risk of infection [16] and only individuals with 3–5 % functioning neutrophils may be at risk of infection [37]. The cases in this review showed a range of functioning neutrophils from 26 % to 48 % when they were manifesting symptoms.

In the population of X-linked carriers of CGD, preservation of NADPH oxidase function is not the only factor in development of symptoms, given the reports of symptoms in carriers with a high percentage of functioning neutrophils. This is further strengthened by the lack of association between degree of ROI production and presence of gastrointestinal symptoms as it seems to suggest an independent mechanism for the development of these symptoms. It is possible that the inflammatory complications are also influenced by different factors. The study by Cale et al. [9], the largest cohort of X-linked carriers studied to date, concurred with this, showing no correlation with percentage functioning neutrophils and presence of symptoms. This was true whether specific symptoms or the presence of symptoms in general were considered.

Summary

The cases described, supported by the literature, suggest that X-linked carriers of CGD may be at greater risk of clinical disease manifestations than usually considered. Virtually all manifestations of CGD have now been described in X-linked carriers. Furthermore, there are reports that carriers of X-linked disease benefit from antibiotic and antifungal prophylaxis, alongside regular follow up in the same manner as patients [48] although this has only been shown in a very small subset.

However, the literature available is limited, consisting mainly of individual case reports and small case series. Much is inferred about the health of the X-linked carriers in reports of patients with CGD. Whilst these highlight the issues, they do not explore the extent or reason for them and do not describe prevalence. The largest published series focuses on skin disease rather than the full range of CGD-associated symptoms.

More information is needed about the extent to which X-linked carriers are affected and prevalence of symptoms amongst the cohort. Once this is established, attempts to predict which X-linked carriers are likely to be affected and to correlate this with percentage of functioning neutrophils would be useful for clinical practice.

As more is learnt about the genetics of CGD and the importance of residual NADPH oxidase function in the patients, it is becoming apparent that at least some X-linked carriers should be considered along the spectrum of disease, rather than as a separate entity.

References

1. Roos D, de Boer M, Kuribayashi F, Meischl C, Weening RS, Segal AW, et al. Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood. 1996;87(5):1663–81.
2. Kuhns D, Alvord WG, Heller TH, Feld JJ, Pike KM, Marciano BE, et al. Residual NADPH oxidase and survival in chronic granulomatous disease. N Engl J Med. 2010;363(27):2600–10.
3. Roesler J, Emmendorffer A. Diagnosis of chronic granulomatous disease. Blood. 1991;78:1387–9.
4. Jelsness-Jorgensen L-P et al. Chronic fatigue is more prevalent in patients with inflammatory bowel disease than in healthy controls. Inflamm Bowel Dis. 2011;17(7):1564–72.
5. Vowells S, Sekhsaria S, Malech HL, Shalit M, Fleisher TA. Flow cytometric analysis of the granulocyte respiratory burst: a comparison study of fluorescent probes. J Immunol Methods. 1995;178:89–97.
6. Sillevis Smitt J, Weening RS, Krieg SR, Bos JD. Discoid lupus erythematosus-like lesions in carriers of X-linked chronic granulomatous disease. Br J Dermatol. 1990;122(5):643–50.
7. Brandrup F, Koch C, Petri M, et al. Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease. Br J Dermatol. 1981;104:495–505.
8. Foti C, Cassano N, Martire B, Filotico R, Mastrandrea V, Vena G. Lupus erythematosus-like lesions in a carrier of X-linked chronic graulomatous disease: a case report and personal considerations. Int J Dermatol. 2004;43(11):840–2.
9. Cale CM, Morton L, Goldblatt D. Cutaneous and other lupus-like symptoms in carriers of X-linked chronic granulomatous disease: incidence and autoimmune serology. Clin Exp Immunol. 2007;148: 79–84.
10. Thompson EN. Chronic granulomatous disease - quantitative clinicopathological relationships. Arch Dis Child. 1970;45(239):24.
11. Garioch J, Sampson JR, Seywright T, Thomson J. Dermatoses in five related female carriers of X-linked chronic granulomatous disease. Br J Dermatol. 1989;121:391–6.
12. Ortiz-Romero P, Corell-Almuzara A, Lopez-Estebaranz JL, Arranz FR, Ruiz-Contreras J. Lupus like lesions in a patient with X-linked chronic granulomatous disease and recombinant X chromosome. Dermatology. 1997;195(3):280–3.
13. Humbert J, Fishman CB, Western WL, et al. Frequency of carrier state for X-linked chronic granulomatous disease among females with lupus erythematosus. Clin Genet. 1976;10(1):16–20.
14. Vanderpump MPJ et al. The incidence of thyroid-disorders in the community- a 20 year follow-up of the Whickham survey. Clin Endocrinol. 1995;43(1):55–68.
15. Kragballe K, Borregaard N, Brandrup F, Koch C, et al. Relation of monocyte and neutrophil oxidative metabolism to skin and oral lesions in carriers of chronic granulomatous disease. Clin Exp Immunol. 1981;43:390–8.
16. Rösen-Wolff A, Soldan W, Heyne K, Bickhardt J, Gahr M, Roesler J. Increased susceptibility of a carrier of X-linked chronic granulomatous disease (CGD) to Aspergillus fumigatus infection associated with age-related skewing of lyonization. Ann Hematol. 2001;80: 113–5.
17. Schäppi MG, Smith VV, Goldblatt D, Lindley KJ, Milla PJ. Colitis in chronic granulomatous disease. Arch Dis Child. 2001;84:147–51.
18. Sloan JM, Cameron CHS, Maxwell RJ, McCluskey DR, Collins JSA. Colitis complicating chronic granulomatous disease. A clinicopathological case report. Gut. 1996;38:619–22.
19. Marciano B, Rosenzweig SD, Holland SM, et al. Gastrointestinal involvement in chronic granulomatous disease. Pediatrics. 2004;114(2):462–8.
20. Moltanyer Y, Geerts WH, Chamberlain DW, Heyworth PG, Noack D, Rae J, et al. Underlying chronic granulomatous disease in a patient with bronchocentric granulomatosis. Thorax. 2003;58:1096–8.
21. Jaggi P, Scherzer R, Knieper R, Mousa H, Prasad V. Utility of screening for chronic granulomatous disease in patients with inflammatory. Bowel Dis J Clin Immunol. 2011;32(1):78–81.
22. Worsaae N, Staehr Johansen K, Christensen KC. Impaired in vitro function of neutrophils in Crohns disease. Scand J Gastroenterol. 1982;17(1):91–6.
23. Emmendörffer A et al. Evaluation of flow cytometric methods for diagnosis of chronic granulomatous disease variants under routine laboratory conditions. Cytometry. 1994;18(3):147–55.
24. Amos E. Immune deficiency and autoimmunity. Autoimmun Rev. 2003;2(6):364–9.
25. De Ravin S, Naumann N, Cowen EW, Friend J, Hilligoss D, Marquesen M, et al. Chronic granulomatous disease as a risk factor for autoimmune disease. J Allergy Clin Immunol. 2008;122(6): 1097–103.
26. Yeaman G, Froebel K, Galea G, Ormerod A, Urbaniak SJ. Discoid lupus erythematosus in an X-linked cytochrome-positive carrier of chronic granulomatous disease. Br J Dermatol. 1992;126:60–5.
27. Cordoba-Guijarro S, Feal C, Dauden E, Fraga J, Garcia-Diez A. Lupus erythematous-like lesions in a carrier of X-linked chronic granulomatous disease. J Eur Acad Dermatol Venereol. 2010;14(5):409–11.
28. Martin-Villa J, Corell A, Ramos-Amador J, Ruiz-Contreras J, Arnaiz-Villena A. Higher incidence of autoantibodies in X-linked chronic granulomatous disease carriers: random X-chromosome inactivation may be related to autoimmunity. Autoimmunity. 1999;31:261–4.
29. Wolach B et al. Unusual late presentation of X-linked chronic granulomatous disease in an adult female with a somatic mosaic for a novel mutation in CYBB. Blood. 2005;105(1):61–6.
30. Martyn L, Lischner HW, Pileggi AJ, Harley RD. Chorioretinal lesions in familial chronic granulomatous disease of childhood. Trans Am Ophthalmol Soc. 1971;69:84–112.
31. Seong-Joon K, Joong Gon K, Young Suk Y. Chorioretinal lesions in patients with chronic granulomatous disease. Retina. 2003;23(3): 360–5.
32. Valluri S, Chu FC, Smith ME. Ocular pathological findings of chronic granulomatous disease of childhood. Am J Ophthalmol. 1995;120(1):120–3.
33. Goldblatt D, Butcher J, Thrasher A, Russell-Eggitt I. Chorioretinal lesions in patients and carriers of chronic granulomatous disease. J Pediatr. 1999;134(6):780–3.
34. Boelaert K, Franklyn JA. Thyroid hormone in health and disease. J Endocrinol. 2005;187(1):1–15.
35. Lewis E, Singla M, Sergeant S, Koty PP, McPhail LC. X-Linked chronic granulomatous disease secondary to skewed X chromosome inactivation in a female with a novel CYBB mutation and late presentation. Clin Immunol. 2008;129:372–80.
36. Chollet-Martin S, Lopez A, Gaud C, Henry D, Stos B, El Benna J, et al. Severe X-linked chronic granulomatous disease in two unrelated females. Eur J Pediatr. 2007;166:153–9.
37. Lun A, Roesler J, Renz H. Unusual late onset of X-linked chronic granulomatous disease in an adult woman after unsuspicious childhood. Clin Chem. 2002;48:780–1.
38. Barton L, Johnson CR. Discoid lupus erythematosus and X-linked chronic granulomatous disease. Pediatr Dermatol. 1986;3(5):376–9.
39. Moelleri RC, Weinberg AN. Persistent salmonella infection in a female carrier for chronic granulomatous disease. Ann Intern Med. 1970;73(4):595.
40. Weening RS et al. Clinical differences in chronic granulomatous disease in patients with cytochrome-b-negative or cytochrome-b-positive neutrophils. J Pediatr. 1985;107(1):102–4.
41. Winkelstein JA et al. Chronic granulomatous disease: report on a national registry of 368 patients. Medicine (Baltimore). 2000;79: 155–69.
42. Gutierrez MJ et al. Residual NADPH oxidase activity and isolated lung involvement in x-linked chronic granulomatous disease. Case Rep Pediatr. 2012;974561(10):5.

43. Morte S et al. Production of interleukin-1 by peripheral blood mononuclear cells in patients with chronic fatigue syndrome. J Infect Dis. 1989;159(2):362.
44. Jendrossek V et al. An in-frame triplet deletion within the gp91-phox gene in an adult X-linked chronic granulomatous disease patient with residual NADPH-oxidase activity. Eur J Haematol. 1997;58(2):78–85.
45. Foster C, Lehrnbecher T, Mol F, et al. Host defense molecule polymorphisms influence the risk for immune-mediated complications in chronic granulomatous disease. J Clin Invest. 1998;102:2146–55.
46. Hinz A et al. Fatigue in patients with sarcoidosis, compared with the general population. Gen Hosp Psychiatry. 2011;33(5):462–8.
47. Martinez-Zamora MA, Cervera R, Balasch J. Recurrent miscarriage, antiphospholipid antibodies and the risk of thromboembolic disease. Clin Rev Allergy Immunol. 2012;43(3):265–74.
48. Roesler J. Carriers of X-linked chronic granulomatouse disease at risk. Clin Immunol. 2009;130:233.
49. Hafner J, Enderlin A, Seger RA, Wuthrich B, Bruckner-Tudermann, Panizzoni P, et al. Discoid lupus erythematosus-like lesions in carriers of X-linked chronic granulomatous disease. Br J Dermatol. 1992;127:446–7.
50. Lovas J, Issekutz A, Walsh N, Miller RAW. Lupus erythematosus-like oral mucosal and skin lesions in a carrier of chronic granulomatous disease. Oral Surg Oral Med Oral Pathol. 1995;80(1):78–82.
51. Rupec R, Petropoulou TH, Belohradsky BH, et al. Lupus erythematosus tumidus and chronic discoid lupus erythematosus in carriers of X-linked chronic granulomatous disease. Eur J Dermatol. 2000;10(3):184–9.
52. Johnston RB, Harbeck RJ. Recurrent severe infections in a girl with apparently variable expression of mosaicism for chronic granulomatous disease. J Pediatr. 1985;106(1):50–5.

Appendix 5: Oral Presentations

UK PIN, Liverpool, December 2013: The Health of X-linked Carriers of Chronic Granulomatous Disease in the United Kingdom

Appendix 6: Poster Presentations

European Society of Immunodeficiency (ESID), November 2014, Prague

ESID-0170 The Psychological Health of X-Linked Carriers of Chronic Granulomatous Disease (CGD) in the United Kingdom

THE PSYCHOLOGICAL HEALTH OF X-LINKED CARRIERS OF CHRONIC GRANULOMATOUS DISEASE IN THE UNITED KINGDOM

Alex Battersby[1*], Mark Pearce[2], Helen Braggins[3], Fiona McKendrick[4], Catherine Cale[5], David Goldblatt[6], Andrew Gennery[1]

[1] Institute of Cellular Medicine, Newcastle University, [2]Institute of Health & Society, Newcastle University, [3]CGD Society, Great Ormond Street Hospital, London [4]Newcastle upon Tyne Hospitals Foundation Trust [5]Clinical Immunology, Great Ormond Street Hospital, [6]Institute of Child Health, University College London,

Introduction

Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiency due to a defect in one of the NADPH oxidase complex subunits resulting in reduced or absent respiratory oxidative burst. UK incidence of CGD is approximately 1 in 125,000 and 70% of cases are X-linked (XL). XL-CGD carriers are frequently confirmed after the diagnosis of the disease in a close family member.

XL-CGD carriers have several potential risk factors for psychological health problems: a family member with chronic illness, their own medical problems and an association with Systemic Lupus Erythematosus (SLE). SLE is associated with anxiety and depression. No studies have evaluated psychological health in this group.

Aim

The aim of this study was to evaluate the psychological health of XL-CGD carriers in the UK

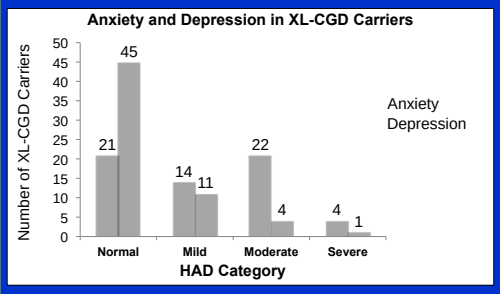
Methods

- XL-CGD families were identified from the UK CGD Registry and all families approached either in person or by post.
- Participants completed validated psychological health questionnaires; Hospital Anxiety and Depression Score (HADS) and Pediatric Inventory for Parents (PIP) .
- A HADS score above 7 (anxiety/depression) is abnormal [1].
- Symptom frequency was compared with parents of CF children [2]. HAD mean scores were compared with published data from SLE patients [3,4].
- The PIP scores distress due to parenting a chronically unwell child with sub-scores about frequency (PIP-F) and severity (PIP-S). PIP's validation in oncology patients provides comparison data [5]. Only mothers completed the PIP.
- Data were compared to published norms and published data from comparable group.

Recruitment and Demographics

80 Families identified
79 XL-CGD Carriers recruited (2 deceased)
Median Age: 43 years [3-77 years]
61 Returned completed HADS, 36 mothers completed PIP

Figure 1: Anxiety and Depression Scores in XL-CGD Carriers



Results

Table 1: Comparison of Anxiety Scores in XL-CGD Carriers with Other Populations

	CGD Carriers	UK 50th Centile	SLE (High Pain)[3]	SLE (Low Pain)[3]	SLE Patients [4]	CF Parents[2]
Number	61	1792	20	64	120	650
HAD-A (Mean)	9.54	6	9	4	9	7.52
p-value		<0.001	0.18	<0.01	0.18	0.0002

Table 2: PIP Scores in XL-CGD Carrier Mothers and Oncology Parents

	XL-CGD Carrier Mothers	Oncology Parents [5]	p-value
PIP-T	214.8 63.5	206	0.20
PIP-F	112.3 30.3	94.0 33.3	0.0005
PIP-S	103.0 35.3	112.4 35.1	0.06

Conclusions

This is the largest study of CGD carriers to date and the only one to examine psychological health.
XL-CGD carriers had significant rates of anxiety with scores significantly higher than the UK population and parents of CF patients. The scores are similar to SLE patients and particularly similar to SLE patients classified as having high pain.
The PIP scores are similar to those from parents of oncology patients suggesting similar degrees of distress irrespective of diagnosis.
Lack of correlation between HADS and PIP suggests the cause for anxiety was not solely related to their child's disease.
XL-CGD carriers have medical problems similar to SLE patients (poster ESID-0185) which may contribute to anxiety and account for the similar levels of anxiety in both conditions.
This study highlights that XL CGD carriers suffer unrecognised but potentially significant psychological health problems which may impact upon their own lives and their ability to cope with the medical needs of their affected children. Anxiety appears to be independent of being a carer.

References

1.Zigmond, A.S. and R.P. Snaith, *The Hospital Anxiety and Depression Scale.* Acta Psychiatrica Scandinavica, 1983. 67(6): p. 361-370.
2.Besier, T., et al., *Anxiety, Depression, and Life Satisfaction in Parents Caring for Children With Cystic Fibrosis.* Pediatric Pulmonology, 2011. 46(7): p. 672-682.
3.Waldheim, E., et al., *Health-related quality of life, fatigue and mood in patients with SLE and high levels of pain compared to controls and patients with low levels of pain.* Lupus, 2013. 22(11): p. 1118-1127.
4. Tench, C.M., et al., *The prevalence and associations of fatigue in systemic lupus erythematosus.* Rheumatology, 2000. 39(11): p. 1249-1254.
5. Streisand, R., Braniecki, S, Tercyak, KP, Kazak, AE, *Childhood Illness-Related Parenting Stress: The Pediatric Inventory for Parents.* Journal of Pediatric Psychology, 2001. 26(3): p. 155-162.
Waldheim, E., et al., *Health-related quality of life, fatigue and mood in patients with SLE and high levels of pain compared to controls and patients with low levels of pain.* Lupus, 2013. 22(11): p. 1118-1127.

ESID-0182 Quality of Life (QOL) is Reduced in X-Linked Carriers of Chronic Granulomatous Disease (CGD)

QUALITY OF LIFE IS REDUCED IN X-LINKED CARRIERS OF CHRONIC GRANULOMATOUS DISEASE IN THE UNITED KINGDOM

Alex Battersby[1*], Mark Pearce[2], Helen Braggins[3], Catherine Cale[4], David Goldblatt[5], Andrew Gennery[1]
[1] Institute of Cellular Medicine, Newcastle University, [2]Institute of Health & Society, Newcastle University, [3]CGD Society, Great Ormond Street Hospital, London
[4]Clinical Immunology, Great Ormond Street Hospital, [5]Institute of Child Health, University College London, London.

Introduction

Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiecny (PID) in which there is a defect in one of the subunits of NADPH oxidase, resulting in a defective respiratory burst. Patients suffer recurrent incetion, inflammation and autoimmunity. 70% of cases are X-linked (XL). Female carriers are frequently confirmed after diagnosis of an affected individual.

Patients who do not undergo curative treatment have been demonstrated to heave poorer QoL than unaffected individuals [1]. XL-CGD carriers have several factors which may affect their QoL; unmet medical needs, caring for a child with chronic disease and excessive fatigue. No study to date has examined QoL in XL-CGD carriers.

Aims and Methods

- The aim of this study was to evaluate QoL in XL-CGD carriers in the UK
- XL-CGD carriers were identified through the UK CGD Registry and approached either in person or by post
- Participants completed the SF36v2 which is a validated QoL questionnaire [2]. The SF36v2 provides a numerical score for 8 domains, along with an overall score for mental health and physical health. Lower scores indicate poorer QoL
- Mean scores were compared with UK population data [3] and published data from other patient groups using a one sample t-test

Results

Recruitment and Demographics

80 Families identified
79 X-linked Carriers recruited to date (2 deceased)
Median Age: 43 years [3-77 years]
78 White British, 1 Chinese British
62 completed the SF36v2
55 (70%) were mothers of index case

Table 1: Mean Scores in XL-CGD carriers compared with UK population data

Domain	CGD Carriers	UK Norms (female age 35-54)[3]	P-value
Physical Function	79.28 (29.4)	89.4(18.3)	< 0.001
Role Physical	74.35(32.2)	84.0(32.0)	0.00235
Bodily Pain	66.24(30.5)	79.4(22.0)	0.002
General Health	56.31(28.5)	74.1(20.3)	< 0.001
Vitality	44.98(25.5)	58.2(19.9)	0.002
Social Function	69.68(29.5)	86.7 (20.5)	0.001
Role Emotional	71.88(31.3)	80.3 (33.6)	0.0341
Mental Health	63.16(16.8)	71.6(17.8)	0.005

Figure 1: XL-CGD Carriers compared to Adult CGD Patients [4]

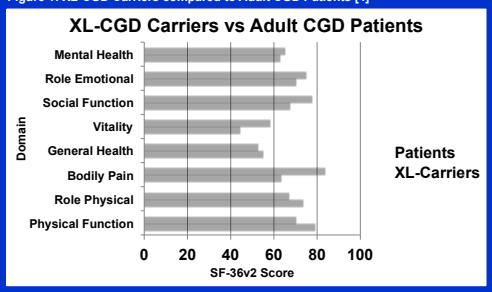


Figure 1 shows that XL-CGD carriers had similar QoL scores to adult CGD patients and in fact scored lower in 4 domains (Vitality, Emotional, Social fnction and mental health)
Tbale 1 shows the comparison of XL-CGD carriers and UK population data for females of similar age
No significant difference in scores between mothers and other relatives in any domain (data not shown)

Conclusions

- The lowest scores were seen in the vitality, general health and mental health domains
- QoL in XL-CGD carriers was significantly worse than UK population norms in all domains
- QoL in XL-CGD carriers was comparable with adult CGD patients and worse in four domains
- XL-CGD carriers have not previously been demonstrated to have significant problems. The importance of this study is that it demonstrates there is a significant impact upon their QoL
- The factors contributing to poor QoL are multiple and include poor medical health, caring for a realtive with chronic disease and psychological distress

References
1. Cole T et al, J Clin Immunol, 2013 Jan;33(1):8-13
2. Ware, JE et al, The MOS 36-item Shortf-Form Health Survey (SF-36), Medical Care, 1992;30(6):473-483
3. Jenkinson, C. et al *Short Form-36 (SF-36) Health Survey Questionnaire - Normative Data For Adults of Working Age.* British Medical Journal, 1993. 306(6890): p. 1437-1440.
4. Cole, T., et al., *Quality of life in adults with Chronic Granulomatous Disease over the last decade.* in *UK PIN.* 2013. Liverpool.

ESID-0185 The Health of Carriers of X-Linked Chronic Granulomatous Disease (CGD) in the United Kingdom

THE HEALTH OF X-LINKED CARRIERS OF CHRONIC GRANULOMATOUS DISEASE IN THE UNITED KINGDOM

Alex Battersby[1*], Mark Pearce[2], Helen Braggins[3], Dawn Barge[4], Catherine Cale[5], David Goldblatt[6], Andrew Gennery[1]
[1]Institute of Cellular Medicine, Newcastle University, [2]Institute of Health & Society, Newcastle University, [3]CGD Society, Great Ormond Street Hospital, London
[4]Newcastle upon Tyne Hospitals Foundation Trust [5]Clinical Immunology, Great Ormond Street Hospital, [6]Institute of Child Health, University College London

Introduction

Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiency due to a defect in one of the NADPH oxidase complex subunits resulting in reduced or absent respiratory oxidative burst. The incidence of CGD in the UK is approximately 1 in 125,000. 70% of cases are X-linked (XL), due to a *CYBB* mutation, resulting in defective production of gp91^{PHOX}. Patients suffer infective, inflammatory and autoimmune complications.

XL-CGD carriers have a dual population of wild-type and mutant phagocytes. The proportion of functioning cells varies between 20-80%. Current literature about XL-CGD carriers is limited to small case series and anecdotal reports. They are conventionally considered healthy although a high incidence of discoid lupus is well described.

Aims

- To evaluate the presence and severity of medical symptoms in XL-CGD carriers in the UK
- To compare the presence of medical symptoms with the degree of reduction in the neutrophil oxidative burst (NOB)

Methods

- XL-CGD families were identified from the UK CGD Registry and approached either in person or by post
- All enrolled participants complete detailed questionnaires about their medical history and presence of symptoms
- Hospital and GP records were reviewed
- A neutrophil oxidative burst and autoantibody panel were performed on enrolment.

Results

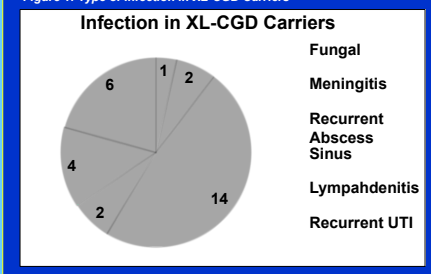
Recruitment and Demographics

80 Families identified
79 X-linked Carriers recruited (2 deceased). Complete information available for 77
Median Age: 43 years [3-77 years]; 78 White British

Infectious Complications

Infection was reported in 19 XL-CGD carriers. Type of infection is shown in figure 1

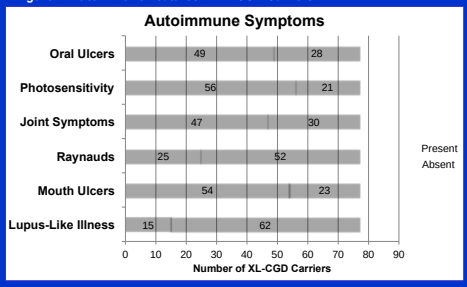
Figure 1: Type of Infection in XL-CGD Carriers



Neutrophil Oxidative Burst Results

Median 53%, Range 7-94%
Recurrent Skin Abscesses associated with lower NOB (p=0.02).
No other significant difference between affected and unaffected groups

Figure 2: Autoimmune Features in XL-CGD Carriers



Inflammatory Complications

- The most frequently occurring inflammatory manifestation were gastrointestinal symptoms (40/77 affected), which were similar in nature to that seen in CGD patients.
- Table 1 shows a comparison of prevalence of GI symptoms between patients and XL-CGD carriers
- Fatigue was reported in 50% (see poster ESID-0851)

Table 1: GI Symptoms in XL-CGD Carriers and CGD Patients

	CGD Carriers	CGD Patients [2]	p-value
Number Enrolled	77	140	
Abdominal Pain	34%	46 %	0.018
Diarrhoea	33%	33 %	0.51
Rectal Bleeding	23%	6 %	<0.0001
Constipation	11%	4 %	0.001

Conclusions

- This is the largest and most comprehensive study of XL-CGD carriers to date.
- NOB results are similar to previous with almost all carriers falling into the range of 20-80%.
- Autoimmune features are more common than previously reported and in particular joint problems occur more frequently than previously documented.
- Gastrointestinal symptoms have not previously been demonstrated and occurred in over 50% of this cohort.
- Skin manifestations concur in nature with previous studies, although are more frequent than previously described.
- Excessive fatigue was reported in half of patients which is a similar finding to other chronic inflammatory conditions.
- Symptoms do not appear to correlate with the degree of reduction in neutrophil oxidative burst.
- While carriers of XL-CGD may be asymptomatic, our survey shows an unexpected degree of clinical symptoms indicating carriers may require medical support
- Further work is required to identify which XL-CGD carriers are at risk and how they should be managed in the future.

ESID-0222 Identification of Autoantibodies in Carriers of X-Linked Chronic Granulomatous Disease by Autoantigen Microarray Analysis

AUTOANTIBODIES IN X-LINKED CARRIERS OF CHRONIC GRANULOMATOUS DISEASE

Katrina Abernethy[1,2], Alexandra Battersby[1], Mark Pearce[3], Dawn Barge[4], Jolan Walter[5], Luigi Notarangelo[2] , Catherine Cale[6], David Goldblatt[7] and Andrew Gennery[1]

1. Institute of Cellular Medicine, 2. Division of Immunology, Boston Children's Hospital, 3. Institute of Health and Society, Newcastle University, 4. Royal Victoria Infirmary, Newcastle Upon Tyne, 5. Division of Allergy/Immunology, Massachusetts General Hospital for Children, Clinical Immunology, Great Ormond Street Hospital, London, 6. Institute of Child Health, University College London

Background

- X-linked chronic granulomatous disease (XL-CGD) is a rare primary immunodeficiency due to mutations in *CYBB* causing reduction in, or absence of, phagocyte respiratory oxidative burst.
- Clinical manifestations of CGD include susceptibility to infection, granuloma formation and inflammatory/autoimmune disease.
- XL-CGD carriers have two populations of leukocytes, expressing either the wild type or the mutant *CYBB* gene. The percentage of wild-type population can be measured by the neutrophil oxidative burst (NOB).
- Discoid lupus has been observed in several XL-CGD carriers, and there is evidence that autoimmune phenomena are also more common. However, routine autoantibody screening in symptomatic carriers by indirect-immunofluorescence is usually negative.
- Novel microarray technology exhibits greater sensitivity for autoantibody detection than traditional techniques and permits rapid screening of a large set of autoantibody specificities with small volumes of sera.

Aims

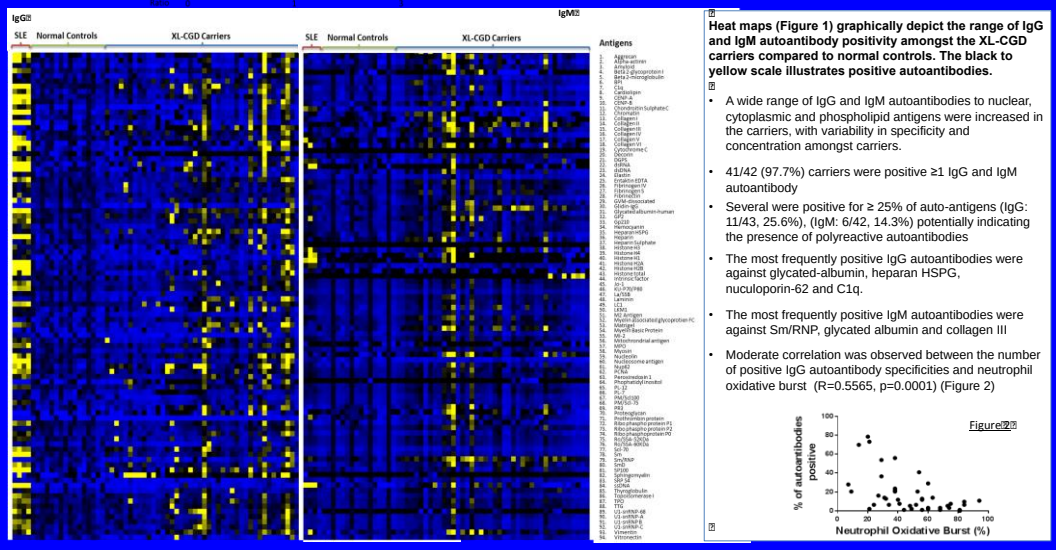
- To investigate the frequency and characteristics of autoantibodies in XL-CGD carriers using a high-throughput microarray method
- To assess the correlation between microarray data and ELISA experiments to validate the microarray data

Methods

- Serum samples from 42 XL-CGD carriers, identified via the UK CGD registry, normal controls and Systemic Lupus Erythematosus (SLE) positive control were probed for IgG and IgM antibodies against a panel of 94 auto-antigens at University of Texas South Western Microarray Core Facility. Normalised frequency intensity (NFI) data was provided.
- Data were converted into an optimised data set by calculating a ratio
- Positivity was defined as a ratio greater than 1
- Data were visualised using MultiExperiment Viewer (Open Source software available at http://mev-tm4.sourceforge.net)
- To validate the microarray findings, serum samples were tested for four IgG autoantibodies (against dsDNA, Nup62, PCNA and Scl70) using standard ELISA techniques.
- Arbitrary units were interpolated from the ELISA optical density at 1:40 dilution, using the SLE control as a standard curve. The correlation between microarray and ELISA data was assessed using Spearman's rank.
- NOB was measured by Dihydrorhodamine Flow Cytometric Assay at Newcastle Royal Victoria Infirmary.

Results

Figure 1



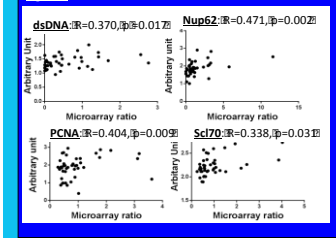
Heat maps (Figure 1) graphically depict the range of IgG and IgM autoantibody positivity amongst the XL-CGD carriers compared to normal controls. The black to yellow scale illustrates positive autoantibodies.

- A wide range of IgG and IgM autoantibodies to nuclear, cytoplasmic and phospholipid antigens were increased in the carriers, with variability in specificity and concentration amongst carriers.
- 41/42 (97.7%) carriers were positive ≥1 IgG and IgM autoantibody
- Several were positive for ≥ 25% of auto-antigens (IgG: 11/43, 25.6%), (IgM: 6/42, 14.3%) potentially indicating the presence of polyreactive autoantibodies
- The most frequently positive IgG autoantibodies were against glycated-albumin, heparan HSPG, nuculoporin-62 and C1q.
- The most frequently positive IgM autoantibodies were against Sm/RNP, glycated albumin and collagen III
- Moderate correlation was observed between the number of positive IgG autoantibody specificities and neutrophil oxidative burst (R=0.5565, p=0.0001) (Figure 2)

Figure 2

Results continued

Figure 3



Scatter plots (figure 3) displaying correlation between Microarray ratio and ELISA arbitrary units for XL-CGD carriers

- A significant weak positive correlation (R >0.3, p < 0.05) was observed between the microarray ratio and ELISA interpolated values.
- The differences may arise from distinct epitope presentation produced by the two methods of antigen fixation to solid surfaces

Conclusions

- XL-CGD carriers have increased autoantibodies detectable by antigen microarray compared to normal controls.
- This provides additional evidence that XL-CGD carriers are at increased risk of developing autoimmune symptoms
- There are differences between microarray and ELISA results . It is currently unclear which correlates better with clinical symptoms
- Further work is required to confirm the significance of these findings using ELISA techniques and further microarray studies as well as assessing the data in relation to clinical symptoms experienced by our carrier cohort.
- Microarray data may identify potential biomarkers to assist in identifying XL-CGD carriers at risk of autoimmune disease.

ESID-0851 High Fatigue Levels in XL-CGD Carriers Associated with Raised Serum IL-8

High serum levels of IL-8 are associated with excessive fatigue in female carriers of X-linked Chronic Granulomatous Disease

A Battersby[1], A.J. Martin[2], J.R. Tarn[1], W.F. Ng[1], C. Cale[3], D. Goldblatt[4], A.R. Gennery[1]

[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK [2]Newcastle University, [3] Clinical Immunology, Great Ormond Street Hospital, London. [4] Institute of Child Health, University College London, London.

Introduction

Chronic Granulomatous Disease (CGD) is a rare primary immunodeficiency in which one of the NADPH oxidase subunits is defective. Patients suffer recurrent, serious infection and inflammation.

70% of cases are X-linked (XL) due to the *CYBB* mutation, resulting in defective production of $gp91^{phox}$. XL-CGD carriers have a dual population of phagocytes, those which express $gp91^{phox}$ and function normally and those which do not.

There is increasing evidence that female carriers of XL-CGD experience a range of inflammatory symptoms [1] (Poster ESID-0185)

Aim

To investigate whether XL-CGD carriers have raised serum levels of inflammatory cytokines associated with fatigue.

Design

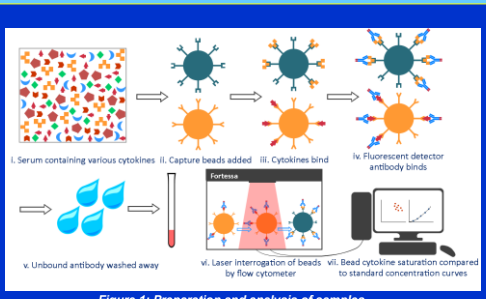


Figure 1: Preparation and analysis of samples

Design
Serum from 52 XL-CGD carriers was compared with inflammatory disease control groups of 10 high and 10 low fatigue Sjogren's disease patients and 15 healthy controls.

Data collection
Cytometric Bead Array (CBA) immunoassay assessed levels of IL-1α, IL-5, IL-8, IL-10, IL-17, IFNα and IFN-γ using BD Biosciences LSRFortessa™ cell analyser (Figure 1).

Data analysis
FACSDiva, BD Biosciences; FCAP Array, Soft Flow Inc.; SPSS, IBM (Mann-Whitney U Tests).

Results

25/52 (48%) of XL-CGD carriers reported fatigue on a validated questionnaire.[3]

IL-8 concentration (mean 1459u/ml) was significantly higher in XL-CGD carriers than in healthy controls (mean 72u/ml) (p=0.015) and Sjogren's disease controls (mean 203u/ml) (p=0.031) as a whole (high and low fatigue grouped).

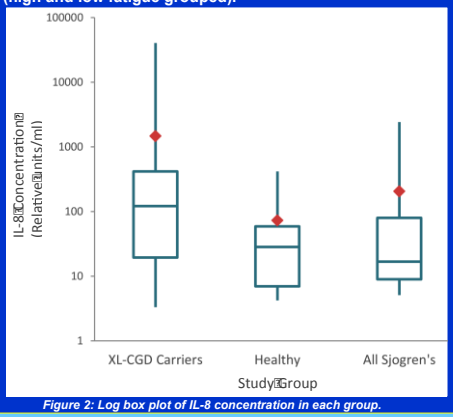


Figure 2: Log box plot of IL-8 concentration in each group.

IL-8 concentration was significantly higher in the subgroup of XL-CGD carriers who reported fatigue (mean 2405u/ml) than in those who did not (mean 400u/ml) (p=0.005).
Other investigated cytokines were not significantly raised.

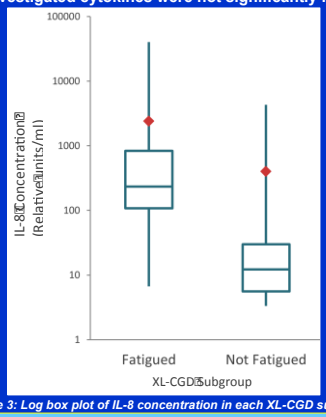


Figure 3: Log box plot of IL-8 concentration in each XL-CGD sub-group.

Conclusions

Serum IL-8 is significantly higher in XL-CGD carriers than in healthy and Sjogren's disease control groups.
Higher serum IL-8 levels are significantly correlated with higher levels of fatigue in XL-CGD carriers.
IL-8, which has been associated with fatigue,[4] may be a driver of fatigue in this group via an inflammatory process.
It is hoped that this initial finding will stimulate further research into how IL-8 influences fatigue in XL-CGD carriers and how its effects can be managed to improve the quality of life for these women.

References

[1] Battersby AC, Cale AM, Goldblatt D, Gennery AR. Clinical manifestations of disease in X-linked carriers of chronic granulomatous disease. Journal of Clinical Immunology 2013;33(8):1276-84
[2] Cale CM, Morton L, Goldblatt D. Cutaneous and other lupus-like symptoms in carriers of X-linked chronic granulomatous disease: incidence and autoimmune serology. Clinical & Experimental Immunology 2007;148(1):79-84
[3]Multidimensional Fatigue Symptom Inventory Short Form (MFSI-SF)
[4] Sorenson M, Jason L, Lerch A et al. The Production of Interleukin-8 Is Increased in Plasma and Peripheral Blood Mononuclear Cells of Patients with Fatigue. Neuroscience and Medicine 2012; 3, 47-53.
This research was supported by a scholarship grant from the Faculty of Medical Sciences, Newcastle University and The Bubble Foundation.

Appendix 7: Prizes

Best Poster Award, ESID 2014

ESID-0851 High Fatigue Levels in XL-CGD Carriers Associated with Raised Serum IL-8

A. Battersby [1], A. Martin[1], J. Tarn[1], C. Cale[2], D. Goldblatt[3], A. Gennery[1]

Chapter 12: References

1. Berendes, H., R. Bridges, and R. Good, *A fatal granulomatosus of childhood: the clinical study of a new syndrome.* Minnesota medicine, 1957. **40**(5): p. 309-12.
2. Bridges, R., H. Berendes, and R. Good, *A fatal granulomatous disease of childhood; the clinical, pathological, and laboratory features of a new syndrome.* A.M.A. journal of diseases of children, 1959. **97**(4): p. 387-408.
3. Baehner, R. and D. Nathan, *Leukocyte oxidase: defective activity in chronic granulomatous disease.* Science (New York, N.Y.), 1967. **155**(3764): p. 835-6.
4. Nathan, D., R. Baehner, and D. Weaver, *Failure of Nitro Blue Tetrazolium Reduction in the Phagocytic Vacuoles of Leukocytes in Chronic Granulomatous Disease.* The Journal of Clinical Investigation 1969. **48**: p. 1895-1904.
5. Windhorst, D., B. Holmes, and R. Good, *A Newly Defined X-Linked Trait in Man with Demonstration of the Lyon Effect in Carrier Females.* The Lancet, 1967. **289**(7493): p. 737-739.
6. Thompson, E.N., *Chronic Granulomatous Disease - Quantitative Clinicopathological Relationships.* Archives of Disease in Childhood, 1970. **45**(239): p. 24-&.
7. Chandra, R., W.A. Cope, and J.F. Soothill, *Chronic Granulomatous DIsease: Evidence for an Autosomal Mode of Inheritance.* Lancet, 1969. **2**(7611): p. 71-74.
8. Winkelstein, J., M. Marino, R. Johnston*, et al.*, *Chronic Granulomatous disease: Report on a national registry of 368 patients.* Medicine (Baltimore), 2000. **79**(155-169).
9. Fattahi, F., M. Badalzadeh, L. Sedighipour*, et al.*, *Inheritance Pattern and Clinical Aspects of 93 Iranian Patients with Chronic Granulomatous Disease.* Journal of Clinical Immunology, 2011. **31**(5): p. 792-801.
10. Van den Berg, J., E. van Koppen, A. Ahlin*, et al.*, *Chronic granulomatous disease: the European experience.* PLoS One, 2009. **4**: p. e5234.
11. Jones, L., P. McGrogan, T. Flood*, et al.*, *Chronic granulomatouse disease in the United Kingdom and Ireland: a comprehensive national aptient-based registry.* Clinical and Experimental Immunology, 2008. **152**: p. 211-218.
12. Hultqvist, M., L.M. Olsson, K.A. Gelderman*, et al.*, *The protective role of ROS in autoimmune disease.* Trends in Immunology, 2009. **30**(5): p. 201-208.
13. Gutierrez, M.J., G.D. McSherry, F.T. Ishmael*, et al.*, *Residual NADPH oxidase activity and isolated lung involvement in x-linked chronic granulomatous disease.* Case Rep Pediatr, 2012. **974561**(10): p. 5.

14. Kuhns, D., W. Alvord, T. Heller*, et al.*, *Residual NADPH Oxidase and Survival in Chronic Granulomatous Disease.* The New England Journal of Medicine, 2010. **363**(27): p. 2600-2610.

15. Schäppi, M.G., V.V. Smith, D. Goldblatt*, et al.*, *Colitis in chronic granulomatous disease.* Archives of Disease in Childhood, 2001. **84**: p. 147-151.

16. Meissner, F., R.A. Seger, D. Moshous*, et al.*, *Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease.* Blood, 2010. **116**(9): p. 1570-1573.

17. Glick, D., S. Barth, and K. Macleod, *Autophagy: cellular and molecular mechanisms.* The Journal of Pathology, 2010. **221**(1): p. 3-12.

18. Huang, J., V. Canadien, G.Y. Lam*, et al.*, *Activation of antibacterial autophagy by NADPH oxidases.* Proc Natl Acad Sci U S A, 2009. **106**(15): p. 6226-31.

19. Luca, A., S. Smeekens, A. Casagrande*, et al.*, *IL-1 receptor blockade restores autophagy and reduces inflammation in chronic granulomatous disease in mice and in humans.* Proc Natl Acad Sci USA, 2014. **111**(9): p. 3526-31.

20. Saitoh, T., N. Fujita, M.H. Jang*, et al.*, *Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production.* Nature, 2008. **456**(7219): p. 264-8.

21. Romani, L., F. Fallarino, A. De Luca*, et al.*, *Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease.* Nature, 2008. **451**(7175): p. 211-U12.

22. Rawat, A., S. Singh, D. Suri*, et al.*, *Chronic Granulomatous Disease: Two Decades of Experience From a Tertiary Care Centre in North West India.* J Clin Immunol, 2013.

23. Köker MY, Camcıoğlu Y, van Leeuwen K*, et al.*, *Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients.* Journal of Allergy and Clinical Immunology, 2013.

24. Roos, D., D.B. Kuhns, A. Maddalena*, et al.*, *Hematologically important mutations: X-linked chronic granulomatous disease (third update).* Blood Cells Molecules and Diseases, 2010. **45**(3): p. 246-265.

25. Ellson, C.D., K. Davidson, G.J. Ferguson*, et al.*, *Neutrophils from p40(phox-/-) mice exhibit severe defects in NADPH oxidase regulation and oxidant-dependent bacterial killing.* Journal of Experimental Medicine, 2006. **203**(8): p. 1927-1937.

26. Matute, J., A. Arias, N. Wright*, et al.*, *A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40(phox) and selective defects in neutrophil NADPH oxidase activity.* Blood, 2009. **114**(15): p. 3309-3315.

27. Roos, D., M. de Boer, F. Kuribayashi*, et al.*, *Mutations in the X-linked and Autosomal Recessive Forms of Chronic Granulomatous Disease.* Blood, 1996. **87**(5): p. 1663-1681.

28. Bylund, J., D. Goldblatt, and D. Speert, *Chronic Granulomatous Disease: From Genetic Defect to Clinical Presentation*, in *Hot Topics in Infection and Immunity in Children* A.J.P.a.A. Finn, Editor. 2005, Springer: New York.

29. Chang, Y.C., B.H. Segal, S.M. Holland*, et al.*, *Virulence of catalase-deficient Aspergillus nidulans in p47(phox-/-) mice - Implications for fungal pathogenicity and host defense in chronic granulomatous disease.* Journal of Clinical Investigation, 1998. **101**(9): p. 1843-1850.

30. Segal, B., E. DeCarlo, K. Kwon-Chung*, et al.*, *Aspergillus Niduluns infection in chronic granulomatous disease.* Medicine (Baltimore), 1998. **77**: p. 345-354.

31. Lublin, M., D. Bartlett, D. Danforth*, et al.*, *Hepatic Abscess in patients with chronic granulomatous disease.* Ann Surg, 2002. **235**: p. 383-91.

32. Bustamante, J., G. Aksu, G. Vogt*, et al.*, *BCG-osis and tuberculosis in a child with chronic granulomatous disease.* Journal of Allergy and Clinical Immunology, 2007. **120**(1): p. 32-38.

33. Lee, P.P.W., K.-W. Chan, L. Jiang*, et al.*, *Susceptibility to mycobacterial infections in children with X-linked chronic granulomatous disease - A review of 17 patients living in a region endemic for tuberculosis.* Pediatric Infectious Disease Journal, 2008. **27**(3): p. 224-230.

34. Goldblatt, D., J. Butcher, A. Thrasher*, et al.*, *Chorioretinal lesions in patients and carriers of chronic granulomatous disease.* The Journal of Pediatrics, 1999. **134**(6): p. 780-783.

35. Sloan, J.M., C.H.S. Cameron, R.J. Maxwell*, et al.*, *Colitis complicating chronic granulomatous disease. A clinicopathological case report.* Gut, 1996. **38**: p. 619-622.

36. Foster, C., T. Lehrnbecher, F. Mol*, et al.*, *Host defense molecule polymorphisms influence the risk for immune-mediated complications in chronic granulomatous disease.* J Clin Invest, 1998. **102**: p. 2146-2155.

37. Marciano, B., S. Rosenzweig, D. Kleiner*, et al.*, *Gastrointestinal Involvement in Chronic Granulomatous Disease.* Pediatrics, 2004. **114**(2): p. 462-468.

38. Marks, D.J.B., K. Miyagi, F.Z. Rahman*, et al.*, *Inflammatory Bowel Disease in CGD Reproduces the Clinicopathological Features of Crohn's Disease.* American Journal of Gastroenterology, 2009. **104**(1): p. 117-124.

39. Ramanuja, R., K. Wolf, M.A. Sadat*, et al.*, *Newly diagnosed chronic granulomatous disease in a 53 year old woman with Crohns Disease.* Annals of Allergy, Asthma and Immunology, 2005. **95**(2): p. 204-209.

40. Alimchandani, M., J.-P. Lai, P.P. Aung*, et al.*, *Gastrointestinal histopathology in chronic granulomatous disease: a study of 87 patients.* The American journal of surgical pathology, 2013. **37**(9): p. 1365-72.

41. Yu, J., S. De Ravin, G. Uzel*, et al.*, *High levels of Crohn's disease-associated anti-microbial antibodies are present and independent of colitis in chronic granulomatous disease.* Clinical Immunology, 2010. **138**: p. 14-22.

42. Seger, R.A., *Modern management of chronic granulomatous disease.* British Journal of Haematology, 2008. **140**(3): p. 255-266.

43. Aliabadi, H., R. Gonzalez, and P.G. Quie, *Urinary-Tract Disorders in Patients with Chronic Granulomatous-Disease.* New England Journal of Medicine, 1989. **321**(11): p. 706-708.

44. Martyn, L., H. Lischner, A. Pileggi*, et al.*, *Chorioretinal lesions in familial chronic granulomatous disease of childhood.* Transactions of the American Ophthalmological Society, 1971. **69**: p. 84-112.

45. Seong-Joon, K., J, K. oong Gon, and Y. Young Suk, *Chorioretinal Lesions in Patients with Chronic Granulomatous Disease.* Retina, 2003. **23**(3): p. 360-365.

46. De Ravin, S., N. Naumann, E. Cowen*, et al.*, *Chronic granulomatous disease as a risk factor for autoimmune disease.* Journal of Allergy and Clinical Immunology, 2008. **122**(6): p. 1097-1103.

47. Manzi, S., A.H. Urbach, A.B. McCune*, et al.*, *Systemic lupus erythematosus in a boy with chronic granulomatous disease - case report and review of the literature.* Arthritis and Rheumatism, 1991. **34**(1): p. 101-105.

48. Schmitt, C., K. Scharer, R. Waldherr*, et al.*, *Glomerulonephritis associated with chronic granulomatous disease and sytemic lupus erythematosus.* Nephrol Dial Transplant, 1995. **10**: p. 891-895.

49. Sanford, A., A. Suriano, D. Herche*, et al.*, *Abnormal apoptosis in chronic granulomatous disease and autoantibody production characteristic of lupus.* Rheumatology, 2006. **45**: p. 178-181.

50. Lugo Reyes, S.O., F. Suarez, R.-M. Herbigneaux*, et al.*, *Hodgkin lymphoma in 2 children with chronic granulomatous disease.* The Journal of allergy and clinical immunology, 2011. **127**(2): p. 543-544.e1-3.

51. Aguilera, D.G., T. Tomita, V. Rajaram*, et al.*, *Glioblastoma Multiforme in a Patient With Chronic Granulomatous Disease Treated With Subtotal Resection, Radiation, and Thalidomide Case Report of a Long-term Survivor.* Journal of Pediatric Hematology Oncology, 2009. **31**(12): p. 965-969.

52. Weel, E.A.M., W.K. Redekop, and R.S. Weening, *Increased risk of malignancy for patients with chronic granulomatous disease and its possible link to the pathogenesis of cancer.* European Journal of Cancer, 1996. **32A**(4): p. 734-735.

53. Emmendörffer, A., M. Nakamura, G. Rothe*, et al.*, *Evaluation of flow cytometric methods for diagnosis of chronic granulomatous disease variants under routine laboratory conditions.* Cytometry, 1994. **18**(3): p. 147-155.

54. Roos, D. and M. de Boer, *Molecular diagnosis of chronic granulomatous disease.* Clinical and experimental immunology, 2014. **175**(2): p. 139-49.

55. Roesler, J. and A. Emmendorffer, *Diagnosis of chronic granulomatous disease.* Blood, 1991. **78**: p. 1387-1389.

56. Klein, J.O., C. Buonomo, D.C. Shannon*, et al.*, *A 2-Year-old boy with recurrent infections, cervical lymphadenopathy and fever - Chronic Granulomatous-Disease - suppurative granulomatous lymphadenitis due to serratia-marcescens.* New England Journal of Medicine, 1993. **329**(10): p. 714-721.

57. *Reliability of acute illness dihydrorhodamine-123 testing for chronic granulomatous disease.* Clin Lab, 2013. **59**(1-2): p. 203-6.

58. Vowells, S., S. Sekhsaria, H. Malech*, et al.*, *Flow cytometric analysis of the granulocyte respiratory burst: a comparison study of fluorescent probes.* Journal of Immunological Methods, 1995. **178**: p. 89-97.

59. Köker, M., O. Sanal, M. de Boer*, et al.*, *Skewing of X-chromosome inactivation in three generations of carriers with X-linked chronic granulomatous disease within one family.* European Journal of Clinical Investigation 2006. **36**: p. 257-64.

60. Cole, T., M.S. Pearce, A.J. Cant*, et al.*, *Clinical outcome in children with chronic granulomatous disease managed conservatively or with hematopoietic stem cell transplantation.* Journal of Allergy and Clinical Immunology, 2013. **132**(5): p. 1150-1155.

61. Leiding, J.W., A.F. Freeman, B.E. Marciano*, et al.*, *Corticosteroid Therapy for Liver Abscess in Chronic Granulomatous Disease.* Clinical Infectious Diseases, 2012. **54**(5): p. 694-700.

62. Uzel, G., J.S. Orange, N. Poliak*, et al.*, *Complications of Tumor Necrosis Factor-alpha Blockade in Chronic Granulomatous Disease-Related Colitis.* Clinical Infectious Diseases, 2010. **51**(12): p. 1429-1434.

63. Martinez, C.A., S. Shah, W.T. Shearer*, et al.*, *Excellent survival after sibling or unrelated donor stem cell transplantation for chronic granulomatous disease.* Journal of Allergy and Clinical Immunology, 2012. **129**(1): p. 176-183.

64. Cole TS, Jones LKR, and M.P.e. al, *Emotional and behavioural difficulties in chronic granulomatous disease.* Arch Dis Child, 2011.

65. Grez, M., *Gammaretro and lentiviral vectors for the gene therapy of X-linked chronic Granulomatous disease.* Human Gene Therapy, 2013. **24**(12): p. A4-A4.

66. Kaufmann, K.B., M. Chiriaco, U. Siler*, et al.*, *Gene therapy for chronic granulomatous disease: current status and future perspectives.* Current gene therapy, 2014. **14**(6): p. 447-60.

67. Kawai, T., *Gene Therapy for a Patient with Chronic Granulomatous Disease.* Journal of Gene Medicine, 2014. **16**(7-8): p. 208-208.

68. Reichenbach, J., C. Brendel, G. Santilli*, et al.*, *Clinical gene therapy for X-linked chronic granulomatous disease.* Allergy, 2013. **68**: p. 91-91.

69. Cale, C.M., L. Morton, and D. Goldblatt, *Cutaneous and other lupus-like symptoms in carriers of X-linked chronic granulomatous disease: incidence and autoimmune serology.* Clinical and Experimental Immunology, 2007. **148**: p. 79-84.

70. Kragballe, K., N. Borregaard, F. Brandrup*, et al.*, *Relation of monocyte and neutrophil oxidative metabolism to skin and oral lesions in carriers of chronic granulomatous disease.* Clin Exp Immunol, 1981. **43**(390-398).

71. Rupec, R., T. Petropoulou, B. Belohradsky*, et al.*, *Lupus erythematosus tumidus and chronic discoid lupus erythematosus in carriers of X-linked chronic granulomatous disease.* European Journal of Dermatology, 2000. **10**(3): p. 184-9.

72. Sillevis Smitt, J., R. Weening, S. Krieg*, et al.*, *Discoid lupus erythematosus-like lesions in carriers of X-linked chronic granulomatous disease.* Br J Dermatol, 1990. **122**(5): p. 643-650.

73. Yeaman, G., Froebel, K, Galea, G, Ormerod, A, Urbaniak, SJ, *Discoid lupus erythematosus in an X-linked cytochrome-positive carrier of chronic granulomatous disease.* Br Journal of Dermatol, 1992. **126**: p. 60-65.

74. Lewis, E., M. Singla, S. Sergeant*, et al.*, *X-Linked chronic granulomatous disease secondary to skewed X chromosome inactivation in a female with a novel CYBB mutation and late presentation* Clinical Immunology, 2008. **129**: p. 372-380.

75. Rösen-Wolff, A., W. Soldan, K. Heyne*, et al.*, *Increased susceptibility of a carrier of X-linked chronic granulomatous disease (CGD) to Aspergillus fumigatus infection associated with age-related skewing of lyonization.* Ann Hematol, 2001. **80**: p. 113-115.

76. Lun, A., J. Roesler, and H. Renz, *Unusual Late Onset of X-Linked Chronic Granulomatous Disease in an Adult Woman after Unsuspicious Childhood.* Clin Chem, 2002. **48**: p. 780-1.

77. Chollet-Martin, S., A. Lopez, C. Gaud*, et al.*, *Severe X-linked chronic granulomatous disease in two unrelated females.* European Journal of Pediatrics, 2007. **166**: p. 153-159.

78. Clarke, A., *The Genetic Testing of Children.* Journal of Medical Genetics, 1994. **31**(10): p. 785-797.

79. James, C.A., N.A. Holtzman, and D.W. Hadley, *Perceptions of reproductive risk and carrier testing among adolescent sisters of males with chronic granulomatous disease.* American Journal of Medical Genetics Part C: Seminars in Medical Genetics, 2003. **119C**(1): p. 60-69.

80. Johnston, R.B. and R.J. Harbeck, *Recurrent Severe Infections in a girl with apparently variable expression of mosaicism for chronic granulomatous disease.* Journal of Pediatrics, 1985. **106**(1): p. 50-55.

81. Barton, L. and C. Johnson, *Discoid Lupus Erythematosus and X-linked Chronic Granulomatous Disease.* Pediatric Dermatology, 1986. **3**(5): p. 376-379.

82. Wolach, B., Y. Scharf, R. Gavrieli*, et al.*, *Unusual late presentation of X-linked chronic granulomatous disease in an adult female with a somatic mosaic for a novel mutation in CYBB.* Blood, 2005. **105**(1): p. 61-66.

83. Moelleri, R. and A.N. Weinberg, *Persistent Salmonella Infection in a female carrier for chronic granulomatous disease.* Annals of Internal Medicine, 1970. **73**(4): p. 595-&.

84. Brandrup, F., C. Koch, and M.e.a. Petri, *Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease.* Br Journal of Dermatol, 1981. **104**.

85. Foti, C., N. Cassano, B. Martire*, et al.*, *Lupus erythematosus-like lesions in a carrier of X-linked chronic graulomatous disease: A case report and personal considerations.* International Journal of Dermatology, 2004. **43**(11): p. 840-842.

86. Garioch, J., J. Sampson, T. Seywright*, et al.*, *Dermatoses in five related female carriers of X-linked chronic granulomatous disease.* Br Journal of Dermatol, 1989. **121**: p. 391-396.

87. Ortiz-Romero, P., A. Corell-Almuzara, J.L. Lopez-Estebaranz*, et al.*, *Lupus like lesions in a patient with X-linked chronic granulomatous disease and recombinant X chromosome.* Dermatology, 1997. **195**(3): p. 280-3.

88. Humbert, J., C. Fishman, W. Western*, et al.*, *Frequency of carrier state for X-linked chronic granulomatous disease among females with lupus erythematosus.* Clin Genet, 1976. **10**(1): p. 16-20.

89. Lindskov, R., J.M. Munkyad, and N.H. Valerius, *Discoid Lupus-Erythematosus and carrier status of X-Linked Chronic Granulomatous-Disease.* Dermatologica, 1983. **167**(5): p. 231-233.

90. Moltyaner Y., Geerts WH., Chamberlain DW.*, et al.*, *Underlying chronic granulomatous disease in a patient with bronchocentric granulomatosis.* Thorax, 2003. **58**: p. 1096-1098.

91. Jaggi, P., R. Scherzer, R. Knieper*, et al.*, *Utility of Screening for Chronic Granulomatous Disease in Patients with Inflammatory Bowel Disease* J Clin Immunol, 2011. **32**(1): p. 78-81.

92. Worsaae, N., Staehr Johansen K, and K. Christensen, *Impaired in vitro function of neutrophils in Crohns disease.* Scandinavian Journal of Gastroenterology, 1982. **17**(1): p. 91-96.

93. Casanova, J.-L. and L. Abel, *Revisiting Crohn's disease as a primary immunodeficiency of macrophages.* The Journal of Experimental Medicine, 2009. **206**: p. 1839-1843.

94. Smith, A., F. Rahman, B. Hayee*, et al.*, *Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease.* The Journal of Experimental Medicine, 2009. **206**: p. 1883-1897.

95. Valluri, S., F.C. Chu, and M.E. Smith, *Ocular Pathological Findings of Chronic Granulomatous Disease of Childhood.* American Journal of Ophthalmology, 1995. **120**(1): p. 120-123.

96. Amos, E., *Immune deficiency and autoimmunity.* Autoimmunity Reviews, 2003. **2**(6): p. 364-369.

97. Suk See De Ravin, M., PhD,a Nora Naumann, MD,a Edward W. Cowen, MD, MHSc,c Julia Friend, MPH, PA-C,Dianne Hilligoss, RN, MSN, CRNP,a Martha Marquesen, NP,a James E. Balow, MD,b Karyl S. Barron, MD,a Maria L. Turner, and c.J.I.G. MD, MD,a and Harry L. Malech, MDa, *Chronic granulomatous disease as a risk factor for autoimmune disease.* Journal of Allergy and Clinical Immunology. **122**(6): p. 1097-1103.

98. Martin-Villa, J., A. Corell, J. Ramos-Amador*, et al.*, *Higher Incidence of Autoantibodies in X-linked Chronic Granulomatous Disease Carriers: Random X-Chromosome Inactivation may be Related to Autoimmunity.* Autoimmunity, 1999. **31**: p. 261-4.

99. Ammann, A., D. Wara, R. Pillarisetty*, et al.*, *The Prevalence of Autoantibodies in T-cell, B-cell, and Phagocytic immunodeficiency Disorders.* Clinical Immunology and Immunopathology, 1979. **14**: p. 456-466.

100. Cordoba-Guijarro, S., C. Feal, E. Dauden*, et al.*, *Lupus erythematous-like lesions in a carrier of X-linked chronic granulomatous disease.* Journal of European Academy of Dermatology and Venereology, 2010. **14**(5): p. 409-411.

101. Izuhara, K., Y. Arinobu, H. Sumimoto*, et al.*, *Association of the interleukin-4 receptor alpha chain with p47(phox), an activator of the phagocyte NADPH oxidase in B cells.* Molecular Immunology, 1999. **36**(1): p. 45-52.

102. Brunner, J., G. Dockter, A. Roesen-Wolff*, et al.*, *X-linked chronic granulomatous disease (CGD) caused by an intra-exonic splice mutation (CYBB exon 3, c.262G-> A) is mimicking juvenile sarcoidosis.* Clinical and Experimental Rheumatology, 2007. **25**(2): p. 336-338.

103. Takagi, N. and M. Sasaki, *Preferential Inactivation of Paternally Derived X-Chromosome in Extraembryonic Membrannes of Mouse.* Nature, 1975. **256**(5519): p. 640-642.

104. Amos-Langraf, J., A. Cottle, R. Plenge*, et al.*, *X Chromosome -Inactivation Patterns of 1005 Phenotypically Unaffected Females.* The American Journal of Human Genetics, 2006. **79**: p. 493-99.

105. El Kassar, N., G. Hetet, E. Briere*, et al.*, *X-chromosome inactivation in healthy females: incidence of excessive lyonization with age and comparison of assays involving DNA methylation and transcript polymorphisms.* Clinical Chemistry, 1998. **44**(1): p. 61-67.

106. Matthews, P.M., D. Benjamin, I. Vanbakel*, et al.*, *Muscle X-Inactivation Patterns and Dystrophin Expression in Duchenne Muscular-Dystrophy Carriers.* Neuromuscular Disorders, 1995. **5**(3): p. 209-220.

107. Gale, R.E., A.K. Fielding, C.N. Harrison*, et al.*, *Acquired skewing of X-chromosome inactivation patterns in myeloid cells of the elderly suggests stochastic clonal loss with age.* British Journal of Haematology, 1997. **98**(3): p. 512-519.

108. Allen, R.C., H.Y. Zoghbi, A.B. Moseley*, et al.*, *Methylation of HPAII and HHAI Sites Near The Polymorphic CAG Repeat In The Human Androgen-Receptor*

Gene Correlates With X-Chromosome Inactivation. American Journal of Human Genetics, 1992. **51**(6): p. 1229-1239.

109. Naumova, A.K., R.M. Plenge, L.M. Bird*, et al.*, *Heritability of X chromosome-inactivation phenotype in a large family.* American Journal of Human Genetics, 1996. **58**(6): p. 1111-1119.

110. Busque, L., R. Mio, J. Mattioli*, et al.*, *Nonrandom X-inactivation patterns in normal females: Lyonization ratios vary with age.* Blood, 1996. **88**(1): p. 59-65.

111. Hatakeyama, C., C.L. Anderson, C.L. Beever*, et al.*, *The dynamics of X-inactivation skewing as women age.* Clinical Genetics, 2004. **66**(4): p. 327-332.

112. Racchi, O., R. Mangerini, D. Rapezzi*, et al.*, *X chromosome inactivation patterns in normal females.* Blood Cells Molecules and Diseases, 1998. **24**(21): p. 439-447.

113. Brown, C.J. and W.P. Robinson, *The causes and consequences of random and non-random X chromosome inactivation in humans.* Clinical Genetics, 2000. **58**(5): p. 353-363.

114. Cattanach, B.M., E.P. Evans, and M. Burtenshaw, *X-Chromosomal location and variable inactivation of SXR (Sex Reversed) in the mouse.* Hereditas, 1983. **98**(1): p. 158-159.

115. Vulliamy, T.J., S.W. Knight, I. Dokal*, et al.*, *Skewed X-inactivation in carriers of X-linked dyskeratosis congenita.* Blood, 1997. **90**(6): p. 2213-2216.

116. Cazzola, M., A. May, G. Bergamaschi*, et al.*, *Familial-skewed X-chromosome inactivation as a predisposing factor for late-onset X-linked sideroblastic anemia in carrier females.* Blood, 2000. **96**(13): p. 4363-4365.

117. Gono, T., M. Yazaki, K. Agematsu*, et al.*, *Adult onset X-linked chronic granulomatous disease in a woman patient caused by a de novo mutation in paternal-origin CYBB gene and skewed inactivation of normal maternal X chromosome.* Intern Med, 2008. **47**(11): p. 1053-56.

118. Anderson-Coher, M., S. Holland, D. Kuhns*, et al.*, *Severe phenotype of chronic granulomatous disease presenting in a female with a de novo mutation in gp91-phox and a non familial, extremely skewed X chromosome inactivation* Clinical Immunology, 2003. **109**: p. 308-317.

119. Bushby, K., J. Goodship, L. Nicholson*, et al.*, *Variability in Clinical, Genetic and Protein Abnormalities in Manifesting Carriers of Duchenne and Becker Muscular Dystrophy.* Neuromusc Disord, 1993. **3**(1): p. 57-64.

120. Fishbein, M., R. Siegel, C. Thompson*, et al.*, *Sudden Death of a Carrier of X-Linked EMery-Dreifuss Muscular Dysrophy.* Annals of Internal Medicine, 1993. **119**(9): p. 900-905.

121. Ay, C., K. Thom, F. Abu-Hamdeh*, et al.*, *Determinants of factor VIII plasma levels in carriers of haemophilia A and in control women.* Haemophilia, 2010. **16**: p. 111-117.

122. Favier, R., J.M. Lavergne, J.M. Costa*, et al.*, *Unbalanced X-chromosome inactivation with a novel FVIII gene mutation resulting in severe hemophilia A in a female.* Blood, 2000. **96**(13): p. 4373-4375.

123. Okuya, M., H. Kurosawa, T. Kubota*, et al.*, *Hematopoietic stem cell transplantation for X-linked thrombocytopenia from a mild symptomatic carrier.* Bone Marrow Transplant, 2010. **45**(3): p. 607-609.

124. Sewry, C., A. Sansome, A. Clerk*, et al.*, *Manifesting Carriers of Xp21 Muscular Dystrophy; Lack of Correlation bewteen Dystrophin expression and clinical weakness.* Neuromusc Disord, 1993. **3**(2): p. 141-148.

125. Yoshioka, M., T. Yorifuji, and I. Mituyoshi, *Skewed X inactivation in manifesting carriers of Duchenne muscular dystrophy.* Clinical Genetics, 1998. **53**(2): p. 102-107.

126. Burry, J.N., *Lipstick and lupus erythematosus.* The New England journal of medicine, 1969. **281**(11): p. 620-1.

127. D'Cruz, D.P., *Systemic lupus erythematosus.* British Medical Journal, 2006. **332**(7546): p. 890-894B.

128. Hochberg, M.C., *Prevalence of Systemic Lupus-Erythematosus in England and Wales, 1981-2.* Annals of the Rheumatic Diseases, 1987. **46**(9): p. 664-666.

129. O'Neill, S. and R. Cervera, *Systemic lupus erythematosus.* Best Practice & Research in Clinical Rheumatology, 2010. **24**(6): p. 841-855.

130. Simard, J.F., K.H. Costenbader, M.H. Liang*, et al.*, *Exposure to maternal smoking and incident SLE in a prospective cohort study.* Lupus, 2009. **18**(5): p. 431-435.

131. Johnson, A.E., C. Gordon, R.G. Palmer*, et al.*, *The prevalence and incidence of Systemic Lupus-Erythematosus in Birmingham, England - Relationship to ethnicity and country of birth.* Arthritis and Rheumatism, 1995. **38**(4): p. 551-558.

132. Rullo, O.J. and B.P. Tsao, *Recent insights into the genetic basis of systemic lupus erythematosus.* Annals of the Rheumatic Diseases, 2013. **72**: p. 56-61.

133. Vaughn, S.E., L.C. Kottyan, M.E. Munroe*, et al.*, *Genetic susceptibility to lupus: the biological basis of genetic risk found in B cell signaling pathways.* Journal of Leukocyte Biology, 2012. **92**(3): p. 577-591.

134. Freemer, M.M., T.E. King, and L.A. Criswell, *Association of smoking with dsDNA autoantibody production in systemic lupus erythematosus.* Annals of the Rheumatic Diseases, 2006. **65**(5): p. 581-584.

135. Hardy, C.J., B.P. Palmer, K.R. Muir*, et al.*, *Smoking history, alcohol consumption, and systemic lupus erythematosus: A case-control study.* Annals of the Rheumatic Diseases, 1998. **57**(8): p. 451-455.

136. Tan, E., A. Cohen, J. Fries*, et al.*, *Special Article: The 1982 revised criteria for the classification of systemic lupus erythematosus.* Arthritis and Rheumatism, 1982. **25**: p. 1271-7.

137. Agmon-Levin, N., M. Mosca, M. Petri*, et al.*, *Systemic lupus erythematosus one disease or many?* Autoimmunity Reviews, 2012. **11**(8): p. 593-595.

138. *The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes.* Arthritis and Rheumatism, 1999. **42**(4): p. 599-608.

139. Moraes-Fontes, M.F., I. Lucio, C. Santos*, et al.*, *Neuropsychiatric features of a cohort of patients with systemic lupus erythematosus.* ISRN rheumatology, 2012. **2012**: p. 989218-989218.

140. Tench, C.M., I. McCurdie, P.D. White*, et al.*, *The prevalence and associations of fatigue in systemic lupus erythematosus.* Rheumatology, 2000. **39**(11): p. 1249-1254.

141. Sterling, K.L., K. Gallop, P. Swinburn*, et al.*, *Patient-reported fatigue and its impact on patients with systemic lupus erythematosus.* Lupus, 2014. **23**(2): p. 124-132.

142. Hoffman, B.I. and W.A. Katz, *The Gastrointestinal Manifestations of Systemic Lupus-Erythematosus - A Review of the Literature.* Seminars in Arthritis and Rheumatism, 1980. **9**(4): p. 237-247.

143. Garcia Carrasco, M., C. Mendoza Pinto, A. Lopez Colombo*, et al.*, *Irritable bowel syndrome-type symptoms in female patients with mild systemic lupus erythematosus: frequency, related factors and quality of life.* Neurogastroenterology and Motility, 2013. **25**(12): p. 958-966.

144. Dubois, E.L. and D.L. Tuffanelli, *Clinical Manifestations of Systemic Lupus Erythematosus. Computer Analysis of 520 Cases.* JAMA : the journal of the American Medical Association, 1964. **190**: p. 104-11.

145. Miyakis, S., M.D. Lockshin, T. Atsumi*, et al.*, *International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS).* Journal of Thrombosis and Haemostasis, 2006. **4**(2): p. 295-306.

146. Martinez-Zamora, M.A., R. Cervera, and J. Balasch, *Recurrent miscarriage, antiphospholipid antibodies and the risk of thromboembolic disease.* Clin Rev Allergy Immunol, 2012. **43**(3): p. 265-74.

147. Verstegen, G., M.C. Duyck, P. Meeus*, et al.*, *Detection and Identification of Antinuclear Antibodies (ANA) in a Large Community Hospital.* Acta Clinica Belgica, 2009. **64**(4): p. 317-323.

148. Marin, G.G., M.H. Cardiel, H. Cornejo*, et al.*, *Prevalence of Antinuclear Antibodies in 3 Groups of Healthy Individuals Blood Donors, Hospital Personnel, and Relatives of Patients With Autoimmune Diseases.* Jcr-Journal of Clinical Rheumatology, 2009. **15**(7): p. 325-329.

149. Abeles, A.M. and M. Abeles, *The Clinical Utility of a Positive Antinuclear Antibody Test Result.* American Journal of Medicine, 2013. **126**(4): p. 342-348.

150. Rekvig, O.P., C. Putterman, C. Casu*, et al.*, *Autoantibodies in lupus: Culprits or passive bystanders?* Autoimmunity Reviews, 2012. **11**(8): p. 596-603.

151. Katzav, A., I. Solodeev, O. Brodsky*, et al.*, *Induction of autoimmune depression in mice by anti-ribosomal P antibodies via the limbic system.* Arthritis and Rheumatism, 2007. **56**(3): p. 938-948.

152. Kowal, C., L.A. DeGiorgio, T. Nakaoka*, et al.*, *Cognition and immunity: Antibody impairs memory.* Immunity, 2004. **21**(2): p. 179-188.

153. Watanabe, A., M. Kodera, K. Sugiura*, et al.*, *Anti-DFS70 antibodies in 597 healthy hospital workers.* Arthritis and Rheumatism, 2004. **50**(3): p. 892-900.

154. Dellavance, A., V.S.T. Viana, E.R. Leon*, et al.*, *The clinical spectrum of antinuclear antibodies associated with the nuclear dense fine speckled immunofluorescence pattern.* Journal of Rheumatology, 2005. **32**(11): p. 2144-2149.

155. Mariz, H.A., E.I. Sato, S.H. Barbosa*, et al.*, *Pattern on the Antinuclear Antibody-HEp-2 Test Is a Critical Parameter for Discriminating Antinuclear Antibody-Positive Healthy Individuals and Patients With Autoimmune Rheumatic Diseases.* Arthritis and Rheumatism, 2011. **63**(1): p. 191-200.

156. Agmon-Levin, N., J. Damoiseaux, C. Kallenberg*, et al.*, *International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies.* Annals of the Rheumatic Diseases, 2014. **73**(1): p. 17-23.

157. Hellier, M.D. and J.G. Williams, *The burden of gastrointestinal disease: implications for the provision of care in the UK.* Gut, 2007. **56**(2): p. 165-166.

158. Rothfuss, K.S., E.F. Stange, and K.R. Herrlinger, *Extraintestinal manifestations and complications in inflammatory bowel diseases.* World J Gastroenterol, 2006. **12**(30): p. 4819-31.

159. Vrijmoet-Wiersma, C.M.J., J.E.H.M. Hoekstra-Weebers, W.M.G.M. de Peinder*, et al.*, *Psychometric qualities of the Dutch version of the Pediatric Inventory for Parents (PIP): a multi-center study.* Psycho-Oncology, 2010. **19**(4): p. 368-375.

160. Xavier, R.J. and D.K. Podolsky, *Unravelling the pathogenesis of inflammatory bowel disease.* Nature, 2007. **448**(7152): p. 427-434.

161. Abraham, C. and J.H. Cho, *Mechanisms Of Disease Inflammatory Bowel Disease.* New England Journal of Medicine, 2009. **361**(21): p. 2066-2078.

162. Cleynen, I., J.R. Gonzalez, C. Figueroa*, et al.*, *Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project.* Gut, 2013. **62**(11): p. 1556-1565.

163. Ward, M., *Pathogenesis of Crohns-Disease.* Lancet, 1977. **2**(8044): p. 903-905.

164. Sanderson, I.R., W.M. Bisset, P.J. Milla*, et al.*, *Chronic Inflammatory Bowel-Disease in Glycogen-Storage-Disease TYPE-1B.* Journal of Inherited Metabolic Disease, 1991. **14**(5): p. 771-776.

165. Couper, R., J. Kapelushnik, and A.M. Griffiths, *Neutrophil-dysfunction in glycogen storage disease 1B associated with Crohns like colitis.* Gastroenterology, 1991. **100**(2): p. 549-554.

166. Verspaget, H.W., M.A.C. Mieremetooms, I.T. Weterman*, et al.*, *Partial Defect of Neutrophil Oxidative-Metabolism in Crohns-Disease.* Gut, 1984. **25**(8): p. 849-853.

167. Muise, A.M., W. Xu, C.H. Guo*, et al.*, *NADPH oxidase complex and IBD candidate gene studies: identification of a rare variant in NCF2 that results in reduced binding to RAC2.* Gut, 2012. **61**(7): p. 1028-35.

168. Papa, E., M. Docktor, C. Smillie*, et al.*, *Non-Invasive Mapping of the Gastrointestinal Microbiota Identifies Children with Inflammatory Bowel Disease.* Plos One, 2012. **7**(6).

169. van Langenberg, D.R. and P.R. Gibson, *Systematic review: fatigue in inflammatory bowel disease.* Alimentary Pharmacology & Therapeutics, 2010. **32**(2): p. 131-143.

170. Mitchell, A., G. Guyatt, J. Singer*, et al.*, *Quality of Life in Patients with Inflammatory Bowel Disease.* Journal of Clinical Gastroenterology, 1988. **10**(3): p. 306-310.

171. Rupp, I., H.C. Boshuizen, C.E. Jacobi*, et al.*, *Impact of fatigue on health-related quality of life in rheumatoid arthritis.* Arthritis & Rheumatism-Arthritis Care & Research, 2004. **51**(4): p. 578-585.

172. Basu, N., G.T. Jones, N. Fluck*, et al.*, *Fatigue: a principal contributor to impaired quality of life in ANCA-associated vasculitis.* Rheumatology, 2010. **49**(7): p. 1383-1390.

173. Jelsness-Jorgensen, L.P., T. Bernklev, M. Henriksen*, et al.*, *Chronic fatigue is associated with impaired health-related quality of life in inflammatory bowel disease.* Alimentary Pharmacology & Therapeutics, 2011. **33**(1): p. 106-114.

174. Glaus, A., R. Crow, and S. Hammond, *A qualitative study to explore the concept of fatigue/tiredness in cancer patients and in healthy individuals.* European journal of cancer care, 1996. **5**(2 Suppl): p. 8-23.

175. Romberg-Camps, M.J.L., Y. Bol, P.C. Dagnelie*, et al.*, *Fatigue and Health-related Quality of Life in Inflammatory Bowel Disease: Results from a Population-Based Study in the Netherlands: The IBD-South Limburg Cohort.* Inflammatory Bowel Diseases, 2010. **16**(12): p. 2137-2147.

176. Mattsson, M., B. Moller, I. Lundberg*, et al.*, *Reliability and validity of the Fatigue Severity Scale in Swedish for patients with systemic lupus erythematosus.* Scand J Rheumatol, 2008. **37**(4): p. 269-77.

177. Rosa, K., M. Fu, L. Gilles*, et al.*, *Validation of the Fatigue Severity Scale in chronic hepatitis C.* Health Qual Life Outcomes, 2014. **12**: p. 90.

178. van Nes, S.I., E.K. Vanhoutte, C.G. Faber*, et al.*, *Improving fatigue assessment in immune-mediated neuropathies: the modified Rasch-built fatigue severity scale.* J Peripher Nerv Syst, 2009. **14**(4): p. 268-78.

179. Hjollund, N.H., J.H. Andersen, and P. Bech, *Assessment of fatigue in chronic disease: a bibliographic study of fatigue measurement scales.* Health and Quality of Life Outcomes, 2007. **5**.

180. Lewis, G. and S. Wessely, *The Epidemiology of Fatigue - More Questions than Answers.* Journal of Epidemiology and Community Health, 1992. **46**(2): p. 92-97.

181. Pawlikowska, T., T. Chalder, S.R. Hirsch*, et al.*, *Population-based Study of Fatigue and Psychological Distress.* British Medical Journal, 1994. **308**(6931): p. 763-766.

182. Hinz, A., M. Fleischer, E. Braehler*, et al.*, *Fatigue in patients with sarcoidosis, compared with the general population.* General Hospital Psychiatry, 2011. **33**(5): p. 462-468.

183. Bager, P., R. Befrits, O. Wikman*, et al.*, *Fatigue in out-patients with inflammatory bowel disease is common and multifactorial.* Alimentary Pharmacology & Therapeutics, 2012. **35**(1): p. 133-141.

184. Jelsness-Jorgensen, L.-P., T. Bernklev, M. Henriksen*, et al.*, *Chronic Fatigue Is More Prevalent in Patients with Inflammatory Bowel Disease than in Healthy Controls.* Inflammatory Bowel Diseases, 2011. **17**(7): p. 1564-1572.

185. Rosse, R.B., *Fatigue In Multiple-Sclerosis.* Archives of Neurology, 1989. **46**(8): p. 841-842.

186. Huet, P.M., J. Deslauriers, A. Tran*, et al.*, *Impact of fatigue on the quality of life of patients with primary biliary cirrhosis.* American Journal of Gastroenterology, 2000. **95**(3): p. 760-767.

187. Michielsen, H.J., M. Drent, T. Peros-Golubicic*, et al.*, *Fatigue is associated with quality of life in sarcoidosis patients.* Chest, 2006. **130**(4): p. 989-994.

188. Tack, B.B., *Self-reported fatigue in rheumatoid arthritis. A pilot study.* Arthritis care and research : the official journal of the Arthritis Health Professions Association, 1990. **3**(3): p. 154-7.

189. Wolfe, F., D.J. Hawley, and K. Wilson, *The prevalence and meaning of fatigue in rheumatic disease.* Journal of Rheumatology, 1996. **23**(8): p. 1407-1417.

190. Yount, S., M.V. Sorensen, D. Cella*, et al.*, *Adalimumab plus methotrexate or standard therapy is more effective than methotrexate or standard therapies alone in the treatment of fatigue in patients with active, inadequately treated rheumatoid arthritis.* Clinical and Experimental Rheumatology, 2007. **25**(6): p. 838-846.

191. de Raaf, P.J., S. Sleijfer, C.H.J. Lamers*, et al.*, *Inflammation and fatigue dimensions in advanced cancer patients and cancer survivors.* Cancer, 2012. **118**(23): p. 6005-6011.

192. Rohleder, N., M. Aringer, and M. Boentert, *Role of interleukin-6 in stress, sleep, and fatigue.* Annals of the New York Academy of Sciences, 2012. **1261**: p. 88-96.

193. Ohsugi, Y. and T. Kishimoto, *The recombinant humanized anti-IL-6 receptor antibody tocilizumab, an innovative drug for the treatment of rheumatoid arthritis.* Expert Opinion on Biological Therapy, 2008. **8**(5): p. 669-681.

194. Strombeck, B., C. Ekdahl, R. Manthorpe*, et al.*, *Physical capacity in women with primary Sjogren's syndrome: A controlled study.* Arthritis & Rheumatism-Arthritis Care & Research, 2003. **49**(5): p. 681-688.

195. Fox, R.I., H.I. Kang, D. Ando*, et al.*, *Cytokine Messenger-RNA Expression in Salivary-Gland Biopsies of Sjogrens-Syndrome.* Journal of Immunology, 1994. **152**(11): p. 5532-5539.

196. d'Elia, H.F., C. Bjurman, E. Rehnberg*, et al.*, *Interleukin 6 and its soluble receptor in a central role at the neuroimmunoendocrine interface in Sjogren syndrome: an explanatory interventional study.* Annals of the Rheumatic Diseases, 2009. **68**(2): p. 285-286.

197. Bower, J.E., *Cancer-related fatigue: Links with inflammation in cancer patients and survivors.* Brain Behavior and Immunity, 2007. **21**(7): p. 863-871.

198. Bower, J.E., P.A. Ganz, M.L. Tao*, et al.*, *Inflammatory Biomarkers and Fatigue during Radiation Therapy for Breast and Prostate Cancer.* Clinical Cancer Research, 2009. **15**(17): p. 5534-5540.

199. PM, M., in *Fundamental Immunology*, P. WE, Editor. 2008.

200. Sorenson M, Jason L, Lerch A*, et al.*, *The Production of Interleukin-8 is Increased in Plasma and Peripheral Blood Mononuclear Cells of Patients with Fatigue.* Neuroscience and Medicine, 2012. **3**: p. 47-53.

201. Piazza-Waggoner, C., C.D. Adams, L. Cottrell*, et al.*, *Child and caregiver psychosocial functioning in pediatric immunodeficiency disorders.* Annals of Allergy Asthma & Immunology, 2006. **96**(2): p. 298-303.

202. Barlow, J.H. and D.R. Ellard, *The psychosocial well-being of children with chronic disease, their parents and siblings: an overview of the research evidence base.* Child Care Health and Development, 2006. **32**(1): p. 19-31.

203. Patterson, J.M., H.I. McCubbin, and W.J. Warwick, *The Impact of Family Functioning on Health Changes in Children with Cystic-Fibrosis.* Social Science & Medicine, 1990. **31**(2): p. 159-164.

204. McDowell, E., P. Titman, and S. Davidson, *Parents' Experiences One Year On from Their Child's Hematopoietic Stem Cell Transplant for Primary Immunodeficiency.* Journal of Health Psychology, 2010. **15**(6): p. 897-904.

205. Vrijmoet-Wiersma, C., R. Egeler, H. Koopman*, et al.*, *Parental stress and perceived vulnerability at 5 and 10 years after pediatric SCT.* Bone Marrow Transplantation, 2010. **45**: p. 1102-1108.

206. Manne, S., K. DuHamel, J. Ostroff*, et al.*, *Anxiety, depressive, and posttraumatic stress disorders among mothers of pediatric survivors of hematopoietic stem cell transplantation.* Pediatrics, 2004. **113**(6): p. 1700-1708.

207. Kenneson, A. and J.K. Bobo, *The effect of caregiving on women in families with Duchenne/Becker muscular dystrophy.* Health & Social Care in the Community, 2010. **18**(5): p. 520-528.

208. Gray, W.N., D.M. Graef, S.S. Schuman*, et al.*, *Parenting Stress in Pediatric IBD: Relations with Child Psychopathology, Family Functioning, and Disease Severity.* Journal of Developmental and Behavioral Pediatrics, 2013. **34**(4): p. 237-244.

209. Moskowitz, J.T., E. Butensky, P. Harmatz*, et al.*, *Caregiving time in sickle cell disease: Psychological effects in maternal caregivers.* Pediatric Blood & Cancer, 2007. **48**(1): p. 64-71.

210. Nereo, N.E., R.J. Fee, and V.J. Hinton, *Parental stress in mothers of boys with Duchenne muscular dystrophy.* Journal of Pediatric Psychology, 2003. **28**(7): p. 473-484.

211. Finocchiaro, C.Y., A. Petruzzi, E. Lamperti*, et al.*, *The burden of brain tumor: a single-institution study on psychological patterns in caregivers.* Journal of Neuro-Oncology, 2012. **107**(1): p. 175-181.

212. Hoge, E.A., A. Ivkovic, and G.L. Fricchione, *Generalized anxiety disorder: diagnosis and treatment.* British Medical Journal, 2012. **345**.

213. Bol, Y., A.A. Duits, C.E.R. Vertommen-Mertens*, et al.*, *The contribution of disease severity, depression and negative affectivity to fatigue in multiple sclerosis: A comparison with ulcerative colitis.* Journal of Psychosomatic Research, 2010. **69**(1): p. 43-49.

214. Fonseca, R., M. Bernardes, G. Terroso*, et al.*, *Silent Burdens in Disease: Fatigue and Depression in SLE.* Autoimmune diseases, 2014. **2014**: p. 790724-790724.

215. Gao, H.-X., S.R. Campbell, M.-H. Cui*, et al.*, *Depression is an early disease manifestation in lupus-prone MRL/lpr mice.* Journal of Neuroimmunology, 2009. **207**(1-2): p. 45-56.

216. Van Gool, A.R., W.H.J. Kruit, F.K. Engels*, et al.*, *Neuropsychiatric side effects of interferon-alfa therapy.* Pharmacy World & Science, 2003. **25**(1): p. 11-20.

217. Guloksuz, S., M. Wichers, G. Kenis*, et al.*, *Depressive Symptoms in Crohn's Disease: Relationship with Immune Activation and Tryptophan Availability.* Plos One, 2013. **8**(3).

218. Graff, L.A., J.R. Walker, and C.N. Bernstein, *Depression and Anxiety in Inflammatory Bowel Disease: A Review of Comorbidity and Management.* Inflammatory Bowel Diseases, 2009. **15**(7): p. 1105-1118.

219. Kishida KT, Hoeffer CA, Hu D*, et al.*, *Synaptic Plasticity Defecits and Mild Memory Impairments in Mouse Models of Chronic Granulomatous Disease.* Molecular and Cellular Biology, 2006. **26**(15): p. 5908-5920.

220. James, C., D. Hadley, N. Holtzman*, et al.*, *How does the mode of inheritance of a genetic condition influence families? A study of guilt, blame, stigma, and understanding of inheritance and reproductive risks in families with X-*

linked and autosomal recessive diseases. Genetics In Medicine, 2006. **8**(4): p. 234-242.

221. Fayers, P. and D. Machin, *Quality of Life: Assessment, Analysis and Interpretation* 2000.

222. Ware, J.E. and C.D. Sherbourne, *The MOS 36-Item Short-Form Health Survey (SF-36) .1. Conceptual-Framework and Item Selection.* Medical Care, 1992. **30**(6): p. 473-483.

223. Stewart, S.T., D.M. Cutler, and A.B. Rosen, *Comparison of Trends in US Health-Related Quality of Life Over the 2000s Using the SF-6D, HALex, EQ-5D, and EQ-5D Visual Analog Scale Versus a Broader Set of Symptoms and Impairments.* Medical care, 2014. **52**(12): p. 1010-6.

224. Zigmond, A.S. and R.P. Snaith, *The Hospital Anxiety and Depression Scale.* Acta Psychiatrica Scandinavica, 1983. **67**(6): p. 361-370.

225. Soresina, A., R. Nacinovich, M. Bomba*, et al.*, *The Quality of Life of Children and Adolescents with X-Linked Agammaglobulinemia.* Journal of Clinical Immunology, 2009. **29**(4): p. 501-507.

226. Driscoll, K.A., K. Montag-Leifling, J.D. Acton*, et al.*, *Relations Between Depressive and Anxious Symptoms and Quality of Life in Caregivers of Children With Cystic Fibrosis.* Pediatric Pulmonology, 2009. **44**(8): p. 784-792.

227. Havermans, T., L. Wuytack, J. Deboel*, et al.*, *Siblings of children with cystic fibrosis: quality of life and the impact of illness.* Child Care Health and Development, 2011. **37**(2): p. 252-260.

228. Huang, I.C., M. Anderson, P. Gandhi*, et al.*, *The Relationships Between Fatigue, Quality of Life, and Family Impact Among Children With Special Health Care Needs.* Journal of Pediatric Psychology, 2013. **38**(7): p. 722-731.

229. Waldheim, E., A.C. Elkan, S. Pettersson*, et al.*, *Health-related quality of life, fatigue and mood in patients with SLE and high levels of pain compared to controls and patients with low levels of pain.* Lupus, 2013. **22**(11): p. 1118-1127.

230. Pao M, Wiggs EA, Anastacio MM*, et al.*, *Cognitive Function in Patients with Chronic Granulomatous Disease: A Preliminary Report.* Psychosomatics, 2004. **45**: p. 230-4.

231. Thompson, R.J., K.E. Gustafson, S. Meghdadpour*, et al.*, *The role of biomedical and psychosocial processes in the intellectual and academic functioning of children and adolescents with cystic fibrosis.* Journal of Clinical Psychology, 1992. **48**(1): p. 3-10.

232. Cole, T.S., F. McKendrick, A.J. Cant*, et al.*, *Cognitive Ability in Children with Chronic Granulomatous Disease: A Comparison of Those Managed Conservatively with Those Who Have Undergone Hematopoietic Stem Cell Transplant.* Neuropediatrics, 2013. **44**(4): p. 230-232.

233. Bliss, T.V.P. and G.L. Collingridge, *A Synaptic Model of Memory - Long-Term Potentiation in the Hippocampus.* Nature, 1993. **361**(6407): p. 31-39.

234. Thiels, E., N.N. Urban, G.R. Gonzalez-Burgos*, et al.*, *Impairment of long-term potentiation and associative memory in mice that overexpress extracellular superoxide dismutase.* Journal of Neuroscience, 2000. **20**(20): p. 7631-7639.

235. Bindokas, V.P., J. Jordan, C.C. Lee*, et al.*, *Superoxide production in rat hippocampal neurons: Selective imaging with hydroethidine.* Journal of Neuroscience, 1996. **16**(4): p. 1324-1336.

236. Knapp, L.T. and E. Klann, *Role of reactive oxygen species in hippocampal long-term potentiation: Contributory or inhibitory?* Journal of Neuroscience Research, 2002. **70**(1): p. 1-7.

237. Manzur, A.Y., M. Kinali, and F. Muntoni, *Update on the management of Duchenne muscular dystrophy.* Archives of Disease in Childhood, 2008. **93**(11): p. 986-990.

238. Bae YJ, Y. Kim, Park CS*, et al.*, *Reliability and validity of the St George's Respiratory Questionnaire for asthma.* International Journal of Tuberculosis and Lung Disease, 2011. **15**(7): p. 966-71.

239. Yorke, J., P. Jones, and S. J, *Development and validity testing of an IPF-specific version of the St George's Respiratory Questionnaire.* Thorax, 2010. **65**: p. 921-926.

240. Santiveri, C., M. Espinalt, F. Diaz Carrasco*, et al.*, *Evalutation of male COPD patients' health status by proxies.* Respiratory Medicine, 2007. **101**(3): p. 439-45.

241. Wilson, C., P. Jones, C. O'Leary*, et al.*, *Validation of the St. George's Respiratory Questionnaire in bronchiectasis. .* American Journal of Respiratory and Critical Care Medicine, 1997. **156**(2 Pt 1): p. 536-41.

242. Jones PW, Quirk FH, and B. CM, *The St George's Respiratory Questionnaire.* Respir Med, 1991. **85** ((Suppl B)): p. 25-31.

243. NICE. *Managing overweight and obesity in adults* 2014 [cited 2014 05.08.14]; Available from: http://www.nice.org.uk/Guidance/PH53/chapter/glossary#body-mass-index.

244. Peyrin-Biroulet, L., A. Cieza, W. Sandborn*, et al.*, *Development of the first disability index for inflammatory bowel disease based on the international classification of functioning, disability and health.* Gut, 2011.

245. Stein, K.D., S.C. Martin, D.M. Hann*, et al.*, *A multidimensional measure of fatigue for use with cancer patients.* Cancer Practice, 1998. **6**(3): p. 143-152.

246. Stein, K.D., P.B. Jacobsen, C.M. Blanchard*, et al.*, *Further validation of the multidimensional fatigue symptom inventory-short form.* Journal of Pain and Symptom Management, 2004. **27**(1): p. 14-23.

247. Pien, L.-C., H. Chu, W.-C. Chen*, et al.*, *Reliability and validity of a Chinese version of the Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF-C).* Journal of Clinical Nursing, 2011. **20**(15-16): p. 2224-2232.

248. Crawford, J.R., J.D. Henry, C. Crombie*, et al.*, *Normative data for the HADS from a large non-clinical sample.* British Journal of Clinical Psychology, 2001. **40**: p. 429-434.

249. Herrmann, C., *International experiences with the hospital anxiety and depression scale - A review of validation data and clinical results.* Journal of Psychosomatic Research, 1997. **42**(1): p. 17-41.

250. Bjelland, I., A.A. Dahl, T.T. Haug*, et al.*, *The validity of the Hospital Anxiety and Depression Scale - An updated literature review.* Journal of Psychosomatic Research, 2002. **52**(2): p. 69-77.

251. Besier, T., A. Born, G. Henrich*, et al.*, *Anxiety, Depression, and Life Satisfaction in Parents Caring for Children With Cystic Fibrosis.* Pediatric Pulmonology, 2011. **46**(7): p. 672-682.

252. Rosenberg, M., *The association between self-esteem and anxiety.* Journal of psychiatric research, 1962. **1**.

253. Kaplan, H.B. and A.D. Pokorny, *Self-derogation and psychosocial adjustment.* The Journal of nervous and mental disease, 1969. **149**(5).

254. Klass, W.H. and S.E. Hodge, *Self-Esteem in Open and Traditional Classrooms.* Journal of Educational Psychology, 1978. **70**(5): p. 701-705.

255. Streisand, R., S. Braniceki, K. Tercyak*, et al.*, *Childhood Illness-Related Parenting Stress: The Pediatric Inventory for Parents.* Journal of Pediatric Psychology, 2001. **26**(3): p. 155-162.

256. Phipps, S., M. Dunavant, S. Lensing*, et al.*, *Patterns of distress in parents of children undergoing stem cell transplantation.* Pediatric Blood & Cancer, 2004. **43**(3): p. 267-274.

257. Jenkinson, C., A. Coulter, and L. Wright, *Short Form-36 (SF-36) Health Survey Questionnaire - Normative Data For Adults of Working Age.* British Medical Journal, 1993. **306**(6890): p. 1437-1440.

258. Cole, T., T. Flood, A. Cant*, et al. Quality of life in adults with Chronic Granulomatous Disease over the last decade*. in *UK PIN*. 2013. Liverpool.

259. England, P.H., *Adult Weight Factsheet*, P.H. England, Editor. 2012.

260. Roesler, J., M. Hecht, J. Freihorst*, et al.*, *Diagnosis of chronic granulomatous disease and of its mode of inheritance by dihydrorhodamine 123 and flow microcytofluorometry.* European Journal of Pediatrics, 1991. **150**(3): p. 161-165.

261. Meyer, J., S.A. Harris, I. Satti*, et al.*, *Comparing the safety and immunogenicity of a candidate TB vaccine MVA85A administered by intramuscular and intradermal delivery.* Vaccine, 2013. **31**(7): p. 1026-33.

262. Dhillon, S.S., R. Fattouh, A. Elkadri*, et al.*, *Variants in Nicotinamide Adenine Dinucleotide Phosphate Oxidase Complex Components Determine Susceptibility to Very Early Onset Inflammatory Bowel Disease.* Gastroenterology, 2014. **147**(3): p. 680-+.

263. Sharp, A., D. Robinson, and P. Jacobs, *Age- and tissue-specific variation of X chromosome inactivation ratios in normal women.* Human Genetics, 2000. **107**(4): p. 343-349.

264. Lodes, M.J., Y.Z. Cong, C.O. Elson*, et al.*, *Bacterial flagellin is a dominant antigen in Crohn disease.* Journal of Clinical Investigation, 2004. **113**(9): p. 1296-1306.

265. Ho, R., *Prevalence of depressive and anxiety symptoms in patients with inflammatory bowel diseases: A systemic review, meta-analysis and meta-regression.* Journal of Psychosomatic Research, 2014. **76**(6): p. 504-504.

266. Matsuoka, K. and T. Kanai, *The gut microbiota and inflammatory bowel disease.* Semin Immunopathol, 2014.

267. Stewart, C.J., E.C. Marrs, S. Magorrian*, et al.*, *The preterm gut microbiota: changes associated with necrotizing enterocolitis and infection.* Acta Paediatr, 2012. **101**(11): p. 1121-7.

268. Townshend, J., S. Hails, and M. McKean, *Diagnosis of asthma in children.* British Medical Journal, 2007. **335**(7612): p. 198-202.

269. Sangha, K.K., M.D. Stephenson, C.J. Brown*, et al.*, *Extremely skewed X-chromosome inactivation is increased in women with recurrent spontaneous abortion.* American Journal of Human Genetics, 1999. **65**(3): p. 913-917.

270. Haidar, Z.A., A. Malshe, and D. McKenna, *Chronic granulomatous disease carrier with recurrent poor obstetric outcome.* Obstetrics & Gynecology, 2014. **123**(2 Pt 2 Suppl 2): p. 484-6.

271. Goundry, B., L. Bell, M. Langtree*, et al.*, *Diagnosis and management of Raynaud's phenomenon.* British Medical Journal, 2012. **344**.

272. Harries, M.J., J. Sun, R. Paus*, et al.*, *Management of alopecia areata.* Bmj-British Medical Journal, 2010. **341**.

273. Szczawinska-Poplonyk, A., D.D.S. Gerreth, K. Breborowicz*, et al.*, *Oral manifestations of primary immune deficiencies in children.* Oral surgery, oral medicine, oral pathology, oral radiology and endodontics 2009. **108**(3): p. e9-20.

274. Perez de Diego, R., A. Lopez Lera, and A. Ferreira Cerdan, *High frequency in the delay in primary tooth loss in X-linked chronic granulomatous disease.* Clinical Immunology, 2012. **145**(1): p. 59-60.

275. Wang, Y., B.E. Marciano, D. Shen*, et al.*, *Molecular identification of bacterial DNA in the chorioretinal scars of chronic granulomatous disease.* Journal of Clinical Immunology, 2013. **33**(5): p. 917-24.

276. Mason, J.C. and P. Libby, *Cardiovascular disease in patients with chronic inflammation: mechanisms underlying premature cardiovascular events in rheumatologic conditions.* Eur Heart J, 2014.

277. Schmeding, A. and M. Schneider, *Fatigue, health-related quality of life and other patient-reported outcomes in systemic lupus erythematosus.* Best Practice & Research in Clinical Rheumatology, 2013. **27**(3): p. 363-375.

278. Cole, T., F. McKendrick, P. Titman*, et al.*, *Health Related Quality of Life and Emotional Health in Children with Chronic Granulomatous Disease: A Comparison of Those Managed Conservatively with Those That Have Undergone Haematopoietic Stem Cell Transplant.* Journal of Clinical Immunology, 2013. **33**(1): p. 8-13.

279. Guilfoyle, S.M., W.N. Gray, M. Herzer-Maddux*, et al.*, *Parenting stress predicts depressive symptoms in adolescents with inflammatory bowel disease.* Eur J Gastroenterol Hepatol, 2014. **26**(9): p. 964-71.

280. Cook, C.J. and J.M. Fletcher, *Interactive effects of in utero nutrition and genetic inheritance on cognition: New evidence using sibling comparisons.* Economics & Human Biology, 2014. **13**: p. 144-154.

281. Kan, K.-J., J.M. Wicherts, C.V. Dolan*, et al.*, *On the Nature and Nurture of Intelligence and Specific Cognitive Abilities: The More Heritable, the More Culture Dependent.* Psychological Science, 2013. **24**(12): p. 2420-2428.

282. Donzis, E.J. and N.C. Tronson, *Modulation of learning and memory by cytokines: Signaling mechanisms and long term consequences.* Neurobiol Learn Mem, 2014. **115C**: p. 68-77.