Clinical and Genotypic aspects of Mitochondrial Disease

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Published work submitted for the degree of Doctor of Philosophy in the Faculty of Medical Sciences, Newcastle University, UK
Dóibh siúd a thug ar an tsaol mé,
Dóibh siúd a thug mé ar an saol,
Dóibh siúd a chruthaigh mé,
Dóibh siúd atá cruthaithe agam,

Mo bhuíochas.
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Proposal Summary

Mitochondrial myopathies are a clinically multifarious group of genetic disorders that affect the central nervous system and skeletal muscles and other organs heavily dependent on aerobic metabolism. They are typically characterised by multi-system involvement and have extensive phenotypic and disease burden variability. These diseases are often relentlessly progressive with high morbidity and mortality. The biochemical and molecular basis of many of the common mitochondrial myopathies has been elucidated over the last decade, yet the association between mitochondrial gene mutations and clinical symptoms, requires further elucidation. I propose to clearly define the clinico-pathological and molecular features of adults with mitochondrial disease and evaluate if there is a clear correlation between clinical phenotype and the underlying genetic defect. Identifying clear clinical features should help guide genetic diagnosis and enable tailored counselling regarding potential disease progression.

Unfortunately, to date, there are few effective treatments and no known cure for patients with mitochondrial myopathies. Exercise has been shown to hold significant positive effects upon skeletal muscle function and perceived health-related quality of life in patients with mitochondrial myopathies. The molecular basis of many of the common mitochondrial disorders has been elucidated over the last decade and although there is a vast spectrum of phenotypic expression throughout different genotypes, common symptoms are reported. Perceived fatigue is often a prominent symptom in patients with mitochondrial disease but to date, its prevalence, severity and aetiology is poorly understood. I wish to determine the prevalence and nature of perceived fatigue in a large, genetically heterogeneous group of patients with mitochondrial disease and systematically assess potential covariates of fatigue compared to healthy controls and patients with Myalgic Encephalopathy /Chronic Fatigue Syndrome.

Health-related quality of life is important for understanding the impact and progression of chronic disease and is increasingly recognised as a fundamental patient-based outcome measure in both clinical intervention and research. Generic outcome measures have been extensively validated to assess health-related quality of life across populations and different disease states. However, due to their inclusive construct, it is acknowledged that not all relevant aspects of a specific illness may be captured. Hence there is a need to develop a disease-specific health-related quality of life measures that centre on symptoms characteristic of a specific disease or condition and their impact. SF-36 and its abbreviated version SF-12 are currently the only tools used routinely for measuring patient-reported outcomes in our
patients with mitochondrial myopathies. I wish to explore the conceptualisation, development and preliminary psychometric evaluation (validity and reliability) of a mitochondrial disease-specific health-related quality of life measure, which may be used both in research and clinical settings. Indeed, in a condition where the natural history of the disease is poorly understood and therapeutic options are limited, long-term preservation of health-related quality of life in patients with mitochondrial disease poses a real challenge.
Author’s Declaration

This thesis is submitted to Newcastle University for the degree of Doctor of Philosophy. The research detailed within was conducted in the Wellcome Trust Centre for Mitochondrial Research, Institute of Neurosciences, under the supervision of Professor Douglass M Turnbull, Dr Robert McFarland and Professor Rita Horvath.

I certify that none of the material offered in this thesis has been previously submitted by me for a degree or other qualification at this or any other university.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>A</td>
<td>Adenine</td>
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<tr>
<td>AARS2</td>
<td>Alanyl-TRNA Synthetase 2, Mitochondrial</td>
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<tr>
<td>ABAT</td>
<td>4-Aminobutyrate Aminotransferase</td>
</tr>
<tr>
<td>AD</td>
<td>autosomal dominant</td>
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<td>ADP</td>
<td>Adenosine-5’-diphosphate</td>
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<td>AGK</td>
<td>Acylglycerol Kinase</td>
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<td>AIF1</td>
<td>Allograft Inflammatory Factor1</td>
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<td>Ala</td>
<td>Alanine</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>ANT1</td>
<td>Adenine nucleotide translocator 1</td>
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<td>APOPT1</td>
<td>Apoptogenic 1</td>
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<tr>
<td>AR</td>
<td>autosomal recessive</td>
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<tr>
<td>Arg</td>
<td>Arginine</td>
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<td>ARSAL</td>
<td>Autosomal recessive Spastic Ataxia with Leukoencephalopathy</td>
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<td>Asn</td>
<td>Asparagine</td>
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<td>ATP</td>
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<td>ATPAF2</td>
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<td>Adenosine triphosphatase</td>
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<td>Ubiquinol-Cytochrome C Reductase) Synthesis-Like</td>
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<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>C10orf2</td>
<td>Chromosome 10 Open Reading Frame 2</td>
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</table>
C12orf62  Chromosome 12 open reading frame 62
C12orf65  Chromosome 12 open reading frame 65
CABC1 (COQ8 or ADCK3)  Domain containing kinase 3
CARS2  Cysteinyl-TRNA Synthetase 2
CFS  Chronic Fatigue Syndrome
CHKB  Choline Kinase Beta
ClpB  Caseinolytic Peptidase B Homolog
CMT  Charcot Marie Tooth
CoQ10  Coenzyme Q10
COQ2  Coenzyme Q2 4-Hydroxybenzoate Polyprenyltransferase
COQ9  Coenzyme Q9
COX  Cytochrome c oxidase
COX10  Cytochrome C Oxidase Assembly Homolog 10
COX15  Cytochrome C Oxidase Assembly Homolog 15
CPEO  Chronic progressive external ophthalmoplegia
D-loop  Displacement loop
Da  Dalton
DARS2  Aspartyl-tRNA synthetase 2
DGUOK  Deoxyguanosine kinase
DNA  Deoxyribonucleic acid
dNTP  Deoxyribonucleotide
EARS  Glutamyl-TRNA Synthetase 2
EE  Ethylmalonic encephalopathy
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<td>EFTu</td>
<td>Elongation Factor Tu (Tu Translation Elongation Factor)</td>
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<td>ELAC2</td>
<td>E. Coli Homolog of 2</td>
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<td>EOEE</td>
<td>Early-onset epileptic encephalopathy</td>
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<td>ESS</td>
<td>Epworth sleepiness scale</td>
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<td>ETFDH</td>
<td>Electron-Transferring-Flavoprotein Dehydrogenase</td>
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<td>ETHE1</td>
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<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
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<td>FIS</td>
<td>Fatigue Impact Scale</td>
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<td>F-Box and Leucine-Rich Repeat Protein 4</td>
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<td>FMN</td>
<td>Flavin mononucleotide</td>
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<td>GARS</td>
<td>Glycyl-TRNA Synthetase</td>
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<td>GFER</td>
<td>Growth factor, augmenter of liver regeneration</td>
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<td>G elongation factor, mitochondrial 1</td>
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<td>G elongation factor, mitochondrial 2</td>
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<td>Guanosine triphosphate</td>
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<td>GTPBP3</td>
<td>GTP binding protein 3</td>
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<td>H-strand</td>
<td>Heavy strand of mtDNA</td>
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<td>H&amp;E</td>
<td>Haematoxylin and eosin</td>
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<td>HADS</td>
<td>Hospital Anxiety Depression scale</td>
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<td>HARS2</td>
<td>Histidyl-TRNA Synthetase 2</td>
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<td>HCM</td>
<td>Hypertrophic cardiomyopathy</td>
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<tr>
<td>His</td>
<td>Histidine</td>
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<tr>
<td>HRQOL</td>
<td>Health related quality of life</td>
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<td>HSD10</td>
<td>17β-hydroxysteroid dehydrogenase type 10</td>
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<td>HSMN</td>
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<td>HSP</td>
<td>Hereditary spastic paraplegia</td>
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<tr>
<td>HUPRA</td>
<td>hyperuricemia, pulmonary hypertension, renal failure, and alkalosis</td>
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<td>IARS</td>
<td>Isoleucyl-tRNA synthetase</td>
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<tr>
<td>Ile</td>
<td>Isoleucine</td>
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<td>ISCA2</td>
<td>Iron-Sulfur Cluster Assembly 2</td>
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<td>IUGR</td>
<td>Intrauterine growth retardation</td>
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<td>Lysyl-tRNA synthetase</td>
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<td>Kb</td>
<td>kilobase</td>
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<td>KDa</td>
<td>Kilo Dalton</td>
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<td>KSS</td>
<td>Kearns-Sayre syndrome</td>
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<td>LA</td>
<td>lactic acidosis</td>
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<td>LARS2</td>
<td>Leucyl-TRNA Synthetase 2</td>
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<td>LBSL</td>
<td>Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation</td>
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<td>LETM1</td>
<td>Leucine Zipper-EF-Hand Containing Transmembrane Protein 1</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>LHON</td>
<td>Leber hereditary optic neuropathy</td>
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<tr>
<td>LS</td>
<td>Leigh Syndrome</td>
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<td>LSFC</td>
<td>Leigh Syndrome French Canadian</td>
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<td>LSP</td>
<td>Light strand promoter</td>
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<td>LTBL</td>
<td>Leukoencephalopathy with thalamus and brainstem involvement and high lactate</td>
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<td>Methionyl-TRNA Synthetase 2</td>
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<td>MDS</td>
<td>Mitochondrial depletion syndrome</td>
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<tr>
<td>ME</td>
<td>Myalig Encephalopathy</td>
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<td>MELAS</td>
<td>Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes</td>
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<td>MERRF</td>
<td>Myoclonic epilepsy with ragged red fibres</td>
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<td>MFN1</td>
<td>Mitofusin 1</td>
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<td>MFN2</td>
<td>Mitofusin 2</td>
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<td>MGME</td>
<td>Mitochondrial Genome Maintenance Exonuclease 1</td>
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<td>MILS</td>
<td>Maternally inherited Leigh Syndrome</td>
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<td>MLPA</td>
<td>Multiplex ligation probe amplification assay</td>
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<td>MMA</td>
<td>methyl malonic aciduria</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<td>MNGIE</td>
<td>Mitochondrial neurogastrointestinal encephalomyopathy</td>
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<td>MPV17</td>
<td>MpV17 mitochondrial inner membrane protein</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<td><strong>MRPL12</strong></td>
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<td><strong>MRPS7</strong></td>
<td>Mitochondrial ribosomal protein S7</td>
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<td><strong>MRS</strong></td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td><strong>mt</strong></td>
<td>mitochondrial</td>
</tr>
<tr>
<td><strong>mt-RNA</strong></td>
<td>Mitochondrial transfer ribonucleic acid</td>
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<tr>
<td><strong>mtDNA</strong></td>
<td>Mitochondrial DNA</td>
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<tr>
<td><strong>mTERF</strong></td>
<td>Mitochondrial transcription termination factor</td>
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<tr>
<td><strong>MTFMT</strong></td>
<td>Mitochondrial Methionyl-TRNA Formyltransferase</td>
</tr>
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<td><strong>MTFMT1</strong></td>
<td>Methionyl-TRNA Formyltransferase</td>
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<td><strong>MTO1</strong></td>
<td>Mitochondrial TRNA Translation Optimization 1</td>
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<td><strong>mtSSB</strong></td>
<td>Mitochondrial single-stranded binding protein</td>
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<td>Mitochondrial transcription termination protein</td>
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<td><strong>NAD</strong></td>
<td>Nicotinamide adenine dinucleotide</td>
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<td><strong>NADH</strong></td>
<td>Nicotinamide adenine dinucleotide hydrogen</td>
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<td><strong>NARP</strong></td>
<td>Neurogenic weakness with ataxia and retinitis pigmentosa</td>
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<td><strong>NARS2</strong></td>
<td>Asparaginyl-TRNA Synthetase 2</td>
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<td><strong>nDNA</strong></td>
<td>Nuclear deoxyribonucleic acid</td>
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<tr>
<td>Term</td>
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<td>NMDAS</td>
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<td>NMQ</td>
<td>Newcastle Mitochondrial Quality of life measure</td>
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<tr>
<td>OA</td>
<td>Optic atrophy</td>
</tr>
<tr>
<td>OH</td>
<td>Heavy-strand origin of replication</td>
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<td>OL</td>
<td>Light-strand origin of replication</td>
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<td>Optic atrophy 1</td>
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<td>OXPHOS</td>
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<td>PAH</td>
<td>Pulmonary artery hypertension</td>
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<td>PARS2</td>
<td>Prolyl-TRNA Synthetase 2, Mitochondrial (Putative)</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
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<td>PEHO</td>
<td>Pontocerebellar hypoplasia, progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy</td>
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<td>PEO1</td>
<td>Progressive external ophthalmoplegia 1</td>
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<td>PET100, c19orf79</td>
<td>Protein PET100 Homolog, Chromosome 19 Open Reading Frame 79</td>
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<td>PGC</td>
<td>Primordial germ cell</td>
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<td>Pi</td>
<td>Inorganic phosphate</td>
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<td>Polymerase gamma</td>
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<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
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<td>Ribonucleotide incorporation throughout the lagging strand</td>
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<td>Required for meiotic nuclear division 1 homolog</td>
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<td>Abbreviation</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>RRF</td>
<td>Ragged red fibre</td>
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<td>Ribonucleotide Reductase M2 B (TP53 Inducible)</td>
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<td>Renal tubular acidosis</td>
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Chapter 1. Introduction to the mitochondrion
1.1 MITOCHONDRIAL MOLECULAR BIOLOGY

1.1.i Discovery of the mitochondrion

Swiss anatomist Rudolph Albert Von Kölliker (1817-1905) first identified ‘sarcosomes’, in 1857, as granular cytoplasmic compartments with their own membrane present in skeletal muscle. (Liesa, Palacin et al. 2009) The term ‘mitochondrion’ was first proposed by the German microbiologist Carl Benda (1857-1932) in 1898, derived from the Greek words ‘mitos’ meaning ‘thread’ and ‘chondron’ meaning ‘grain’ (Benda 1898), as he assumed their role was to help hold the shape of the cell and became widely accepted from the 1930s onwards to describe these granular organelles (Liesa et al., 2009).

With the evolution and rapid advances in biological electron microscopy (EM), several important discoveries were made. George Emil Palade (1912-2008), who with Albert Claude, and Christian de Duve, pioneered these techniques, determined mitochondria were ‘isolated’ organelles. These EM images showed the intimate structure of mitochondria and these techniques were sensitive enough to visualize what were later identified as the ATPase molecules (Palade, 1952). Formulation of the citric acid cycle and identification of other elements of the respiratory chain forged the way to the localization of cellular respiration to the mitochondria (Lehninger and Greville, 1953; Drahota et al., 1964) and its identification as the ‘powerhouse of cells’ (Philip (Siekevitz et al., 1958; Siekevitz, 1959; Liesa et al., 2009). These tiny cellular organelles would be shown, in time, to increase the amount of usable energy, in the form of ATP, from one molecule of glucose by 1700% over glycolysis alone.

Figure 1: Diagrammatic representation of a cell showing the nucleus and mitochondria in relation to other organelles.
Two decades later the ‘blueprint of a cell’s hereditary information, namely DNA, was discovered within mitochondria by EM (Nass and Nass, 1963a; Nass and Nass, 1963b) and its subsequent biochemical verification (Schatz, 1963; Schatz et al., 1964), spearheaded biochemical and molecular studies of human mitochondrial diseases.

1.1.ii Evolution of the mitochondrion

To date, there are two main theories on the evolution of mitochondria. These theories differ with regard to their conjectures on the attributes of the host organism, in addition to the physiological proficiency of the mitochondrial endosymbiont. Furthermore, the nature of the ecological interactions facilitating the resultant symbiosis is strikingly different.

1.1.iii Autogenous theory

This theory of the evolution of mitochondria purports that the recipient host was a prokaryote and ancestral mitochondrion, a facultative anaerobe, that is, able to survive with and without the presence of oxygen. It is speculated that production of hydrogen by the endosymbiont, acts as a source of energy and electrons for the host organism that was postulated to have been hydrogen dependent. This theory purports to account for the ubiquitous nature of mitochondria amongst all eukaryotic lineages and the various aerobic and anaerobic forms of mitochondria that are observed as independent functional entities (Taylor, 1976).

1.1.iv Endosymbiosis theory

An alternative and more widely accepted theory today suggests that the evolutionary origin of mitochondria, proposed by Lynn Margulis, suggests that a nucleated, eukaryote cell characterised metabolically by anaerobic respiration, ‘hosted’ the prokaryotic, aerobic, mitochondrion resulting in an obligate aerobic, mitochondrial endosymbiont with evolutionary advantages (Margulis and Fester, 1991).

1.1.v Anatomical structure of the mitochondrion

Mitochondria are tubular-shaped, double-membrane, ubiquitous, cellular organelles comprising an outer mitochondrial membrane, inner mitochondrial membrane, intermembrane space, cristae space and matrix (Ogata and Yamasaki, 1997).
1.1.vi **Outer mitochondrial membrane**

The outer mitochondrial membrane (OMM) is a relatively simple phospholipid bilayer and comprises a family of integral proteins known as ‘porins’ or voltage dependent anion channels. These form channels that traverse the OMM and facilitate the passive diffusion of molecules up to 10kDa in size. This allows the free permeability of substrates including nutrients, ions, ADP, and ATP.

1.1.vii **Inner mitochondrial membrane**

The inner mitochondrial membrane (IMM) unlike the OMM is relatively impermeable and only permits the passage of carbon dioxide, oxygen and water. Architecturally and functionally, it is extremely complex and contains highly specialized proteins including the complexes of the electron transport system, the ATP synthetase complex, and affiliated transport proteins.

1.1.viii **Intermembrane space**

The inner membrane space is the intervening space between the OMM and IMM and the seat of oxidative phosphorylation.

1.1.ix **Cristae space**

Intricate folding of the Inner mitochondrial membrane into lamellae or ‘cristae’ conspicuously increase the total surface area of the IMM.

1.1.x **Matrix**

The mitochondrial matrix acts as a site for important energy producing cellular processes that includes the citric acid cycle responsible for the oxidation of carbohydrates and fats and production of electrons for the electron transfer chain (ETC); in the IMM resulting in the production of ATP. Other functions include β -oxidation of fatty acids, and amino acid metabolism.

In addition, it also contains dissolved water, carbon dioxide, oxygen and the intermediate energy shuttles. IMM structure and relative proximity of the matrix to the cristae, facilitates the timely movement of matrix components to the inner membrane complexes and transport proteins.
1.1.xi Physiological function of the mitochondrion

Mitochondria play a central role in cellular metabolic energy production. These cytoplasmic organelles are critical integrators of intermediate metabolism in a variety of cellular metabolic pathways including oxidative phosphorylation, fatty acid oxidation, Kreb’s cycle (TCA cycle), urea cycle, gluconeogenesis and ketogenesis (Duchen, 2004). However, their function is not limited to cellular ATP production. Mitochondria play a cardinal role in several cellular processes including non-shivering thermogenesis, amino acids and lipid metabolism, biosynthesis of heme and iron-sulfur clusters, calcium homeostasis, and apoptosis (Lill and Kispal, 2000; Nedergaard et al., 2001; Newmeyer and Ferguson-Miller, 2003; Vieira and Kroemer, 2003; Green and Kroemer, 2004; Berdanier, 2005; Johnson et al., 2005; Lill and Mühlenhoff, 2005; Dolezal et al., 2006; Hopper et al., 2006; Ryan and Hoogenraad, 2007; Gvozdjáková, 2008; Hughes et al., 2009; Nunnari and Suomalainen, 2012).

1.1.xii Oxidative phosphorylation

Oxidative phosphorylation is the process in cell metabolism by which respiratory enzymes in the mitochondria generate and amplify ATP from ADP and inorganic phosphate during the oxidation of NADH. Whilst the metabolism of glucose generates two molecules of ATP during glycolysis, up to 38 molecules are generated by the mitochondrial respiratory chain (Berg et al.) (Figure 1).

1.1.xiii Complex I (NADH:ubiquinone oxidoreductase)

Complex I (NADH:ubiquinone oxidoreductase) is the largest (1MDa) of the five enzyme complexes constituting the OXPHOS system (mitochondrial respiratory chain), responsible for amplifying mitochondrial ATP production, by coupling electron transfer to the oxidative phosphorylation of ADP, generating an electrical and pH gradient across the inner mitochondrial membrane and stimulating mitochondrial ATP synthesis.

Mammalian complex I is a prodigious multiheteromeric enzyme that comprises 44 pre-assembled structural subcomplexes (Walker, 1992; Balsa et al., 2012a) that are assembled into an L-shape configuration with one-arm embedded in the inner mitochondrial membrane and a peripheral arm extending into the mitochondrial matrix (Clason et al., 2010). CI requires 14 evolutionary conserved core subunits for its catalytic function: seven mitochondrial DNA (mt-DNA)-encoded NADH-dehydrogenase (ND) core subunits (ND1-ND6, ND4L) and a further seven
subunits that are encoded by nDNA (NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS7 and NDUFS8) (Koopman et al., 2010; Hirst, 2011). The remaining accessory subunits, incorporating 16 additional subunits of the peripheral arm and 14 hydrophobic membrane arm components are thought to be intricately involved in regulating complex I assembly and stabilisation (Angerer et al., 2011). The assistance of more than 11 extrinsic assembly factors (AF) for CI assembly is also recognised yet their specific roles are yet to be fully elucidated (Andrews et al., 2013).

Mutations in the genes encoding subunits of this complex have been associated with Leigh syndrome; cardiomyopathy; epilepsy; encephalopathy; mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS); Leber hereditary optic neuropathy (LHON) and an overlap syndrome comprising clinical features of both LHON and MELAS (Kirby et al., 2004b; Valentino et al., 2004b; Blakely et al., 2005; Malfatti et al., 2007; Moslemi et al., 2008; Patsi et al., 2012; Delmiro et al., 2013)

1.1.xiv Complex II (succinate dehydrogenase; succinate ubiquinone oxidoreductase)

Complex II is a nuclear encoded, tetrameric enzyme that intricately links the tricarboxylic acid cycle (TCA) to the electron transport chain (ETC); uniquely constituting a membrane bound component of the TCA cycle and also forming the second component of the ETC. It has two major roles: firstly, it is responsible for succinate oxidation (to fumarate) in the matrix and secondly, ubiquinone reduction (to ubiquinol) in the inner mitochondrial membrane. This tetrameric structure is composed of two hydrophilic and two hydrophobic subunits. The hydrophilic components consist of a large subunit that houses a covalently bound flavin adenine dinucleotide (FAD) cofactor and the succinate-binding site (termed SdhA) and a smaller subunit (termed SdhB) that contains three iron-sulfur clusters (2Fe-2S, 3Fe-4S and 4Fe-4S) that transfer electrons from the flavin to ubiquinone. The remaining two hydrophobic subunits (termed SdhC and SdhD) comprise cytochrome b (six transmembrane α-helices, a haem b group and a ubiquinone binding site) (Sun et al., 2005).

Mutations in the genes encoding subunits of this complex cause highly variable phenotypic expression of mitochondrial disease and include Leigs disease (Riggs et al., 1984; Bourgeois et al., 1992; Parfait et al., 2000; Ghezzi et al., 2009), Kearns Sayer Syndrome (Rivner et al., 1989), dilated cardiomyopathy (Rustin et al., 1993; Reichmann and Angelini, 1994; Alston et al., 2012) and exercise intolerance and muscle weakness (Arpa et al., 1994).
1.1.xv  Complex III (cytochrome bc₁ or ubiqinol cytochrome c oxidoreductase)

Complex III is a homodimeric transmembrane protein. Each monomer comprises 10 nuclear encoded subunits and a single mitochondrial encoded subunit (cytochrome b) located within the inner mitochondrial membrane that facilitates two-electron quinone oxidation/reduction with one-electron cytochrome c reduction/oxidation. In addition, it is also now recognised that it likely plays as an important role in regulating electron flow, in response to bioenergetic fluxes within the cell. It comprises two respiratory, two core proteins and six low molecular weight protein subunits (Chen et al., 2003b; Acín-Pérez et al., 2004; Solmaz and Hunte, 2008).

Mutations in the genes encoding subunits of this complex often cause early onset, often fatal multisystem disorders including neonatal proximal tubulopathy, hepatic involvement, encephalopathy, lactic acidosis and Leigh-like syndromes (de Lonlay et al., 2001; De Meirleir et al., 2003; Fernandez-Vizarra et al., 2007; Blázquez et al., 2009; Ramos - Arroyo et al., 2009; Morón et al., 2010).

1.1.xvi  Complex IV (cytochrome c oxidase)

Cytochrome c oxidase (COX) is a large transmembrane protein responsible for the terminal enzymatic reaction of the ETC. It comprises 10 nuclear encoded subunits and three, hydrophobic, mitochondria-encoded subunits (cytochrome oxidase I, cytochrome oxidase II, and cytochrome oxidase III) (Saraste, 1983). Although the nuclear encoded subunits are thought to play a regulatory and structural role in COX, the mitochondria-encoded subunits are essential to its catalytic (MT-CO1, MT-CO2) and core structural (MT-CO3) roles. In addition, the mitochondrial subunits contain three copper atoms and two heme A molecules; that are integral to electron transfer and function as prosthetic groups in the holoenzyme complex (Balsa et al., 2012b).

Cytochrome c oxidase deficiency can be caused by mutations in both nuclear-encoded and mitochondrial-encoded genes. Mutations in the genes encoding subunits of this complex cause a heterogeneous group of clinical syndromes manifesting in early infancy or adulthood and ranging from isolated myopathy and exercise intolerance to severe multisystem disorders (Van Biervliet et al., 1977; Willems et al., 1977; DiMauro et al., 1987; Haller et al., 1989; Eshel et al., 1991; Chabrol et al., 1994; Bakker et al., 1996; Rubio-Gozalbo et al., 1999; Shoubridge, 2001b; Shoubridge, 2001a; Ghezzi et al., 2008; Lim et al., 2014).
1.1.xvii **Complex V (F₅Fₒ-ATP Synthase)**

Complex V is composed of 16 subunits, of which, only six are encoded by mtDNA. ATP synthase sits in the IMM and is a tripartite structure consisting of a membrane motor, a rotating transmission device and three catalytic sites. This machinery converts transmembrane electrochemical proton gradient energy (proton motive force) into subunit rotation and then transmits this to the catalytic sites where this mechanical energy is converted into the chemical bond energy of ADP and Pi, catalysed by F₅Fₒ-ATP Synthase (Senior *et al.*, 2002).

Mutations in the genes encoding subunits of this complex cause a myriad of syndromes including neonatal-onset hypotonia, lactic acidosis, hyperammonemia, hypertrophic cardiomyopathy, and 3-methylglutaconic aciduria, maternally inherited Leigh syndrome, bilateral striatal necrosis and neuropathy, ataxia and retinitis pigmentosa (NARP) (Mayr *et al.*, 2010).

**Figure 2.** The mitochondrial respiratory chain and oxidative phosphorylation. Reproduced from (Nijtmans *et al.*, 2004).
1.2 HUMAN MITOCONDRIAL GENETICS

1.2.i Mitochondrial biogenesis

This section will review the anatomical structure and biogenesis of the human mitochondrial genome.

1.2.ii Identification of mitochondrial DNA

The discovery of DNA, the ‘blueprint of a cell’s hereditary information’, within mitochondria by electron microscopy (Nass and Nass, 1963a; Nass and Nass, 1963b) and its subsequent biochemical verification (Schatz, 1963) paved the way for the biochemical and molecular studies of human mitochondrial (mt) diseases.

1.2.iii Structure of mitochondrial DNA

The human mtDNA genome is a circular, double stranded molecule composed of an inner light (L) strand and outer heavy (H) strand composed of only 16,569 base pairs. Although there are 37 genes found on mtDNA; only 13 essential respiratory chain polypeptides of the OXPHOS system: seven subunits (ND1-ND6 and ND4L) of complex I, cytochrome b of complex III, three catalytic subunits (COI-COIII) of complex IV and ATP6 and ATP8 of complex V are encoded by human mtDNA. Of the remaining genes, 22 are responsible for the formation of mt-transfer (t) RNA and two genes encode mt-ribosomal (r) RNAs (RNR1 (12S rRNA) and RNR2 (16S rRNA) for mitochondrial protein synthesis. The remaining major non-coding regions of the genome include the origin of L-strand replication and the 1.1 kb D-loop in which the origin of H-strand replication (OH) and regulatory elements and binding sequences for key factors involved in mtDNA transcription initiation and termination are located (Taylor and Turnbull, 2005a; Tuppen et al., 2010) (Figure 3).
Figure 3: Human mitochondrial (mt) genome. (A) Schematic diagram of the 16.6 kb circular, double-stranded human mitochondrial genome with an enhanced, linearised view of the D-loop and transcription termination regions. The outer circle represents the heavy (H) strand of the genome and the inner circle the light (L) strand. Human mtDNA encodes the two mt-rRNA genes (shown in red) RNR1 (12S rRNA) and RNR2 (16S rRNA), 22 mt-tRNAs (black bars) identified by their single letter abbreviation, and 13 essential respiratory chain polypeptides: seven subunits (ND1-ND6 and ND4L) of complex I (green), CYTB of complex III (purple), three catalytic subunits (COI-COIII) of complex IV (yellow) and ATP6 and ATP8 of complex V (blue). Major non-coding regions of the genome (grey) include the origin of L-strand replication (OL) and the 1.1 kb D-loop in which the origin of H-strand replication (OH) and regulatory elements and binding sequences for key factors involved in mtDNA transcription initiation and termination Reproduced from (Gorman and Taylor, 2011).

1.2.iv Functional organisation of mitochondrial DNA

The human mt genome which is 16.5kb in size is organised into compact protein–DNA complexes called mt-nucleoids (Satoh and Kuroiwa, 1991; Bereiter-Hahn and Vöth, 1998; Ashley et al., 2005). Each mitochondrion may contain between one and 10 mt-nucleoids; with ~2-10 mt genomes per nucleoid (Iborra et al., 2004; Legros et al., 2004; Malka et al., 2006). In addition, essential mtDNA proteins may also co-localise with these structures including TFAM (Ghivizzani et al., 1994), a non-specific DNA-binding protein and a key player in mtDNA transcription and nucleoid packaging (Fisher et al., 1992; Pohjoismäki et al., 2006; Kaufman et
al., 2007; Spelbrink, 2010), the mtDNA replication proteins including POLG (Di Re et al., 2009), Twinkle (Spelbrink et al., 2001) and mt-SSB (Bogenhagen et al., 2003; Garrido et al., 2003; Wang and Bogenhagen, 2006; Bogenhagen et al., 2008) and the tumour suppressor proteins BRCA1 and PRSS15; which are integral in protein degradation (Chen and Butow, 2005; Spelbrink, 2010).

The roles of several other proteins implicated in mt nucleoid organisation including ATAD3 (He et al., 2007; Holt et al., 2007) and human Dna2 (Duxin et al., 2009) have yet to be fully elucidated. One important function of these nucleoid proteins appears to be in the regulation of mt genome copy number.

1.2.v Control of mitochondrial DNA copy number

Human mitochondrial DNA replication and turn-over continues in post mitotic tissues; this is the antithesis of nuclear DNA replication. Although cellular mitochondrial genome copy number remains relatively stable during proliferation; copy number may vary widely depending on the energy demands of the tissue or organ (with estimated copy numbers up to 20,000 in the ovum) involved (Shoubridge, 2000; Moraes, 2001). The exact proteins and mechanisms regulating mtDNA copy number in human cells and the role of mt-nucleoids are not fully understood; and require further elucidation; beyond the remit of this thesis (Montier et al., 2009; Spelbrink, 2010).

1.2.vi Inheritance of mitochondrial DNA

Human mitochondrial DNA inheritance is strictly through the maternal lineage (Giles et al., 1980). A recent paper postulates the mechanism of strict maternal inheritance occurs during development when the head of the sperm (permatozoon) binds to the membrane of the oocyte and releases its contents into it, facilitating the fusion of the pronuclei contents of the sperm and egg, resulting in a diploid cell (zygote) with a complete complement of 46 chromosomes. Although the entire spermatozoon enters the oocyte during fertilisation, sequestration and elimination of sperm components including paternal mitochondria occurs within minutes of fertilisation by a process of autophagy instigated by the oocyte (Al Rawi et al., 2011) (Figure 4).
Figure 4: Maternal inheritance of mtDNA: A) Sperm binds to the membrane (zona pellucida) of the female egg and releases its contents into it. B) The mitochondria from the sperm rapidly undergo sequestration and elimination. C) The mitochondrial genetic material in the resulting embryo is inherited exclusively from the mother.

1.2.vii Replication of mitochondrial DNA

Currently there are several theories of mtDNA replication; but as yet, there is still no consensus on its exact mechanism. Two pivotal models of mitochondrial genome replication exist: the strand-displacement model (Clayton, 1982) and the strand coupling model (Holt et al., 2000). The core components of mtDNA replication machinery are related to their phage T7 analogues (Taanman, 1999). This includes the catalytic subunit of DNA polymerase γ (POLγ) (Carrodeguas et al., 2001; Bailey et al., 2009), the DNA helicase TWINKLE (Korhonen et al., 2003), and the mitochondrial RNA polymerase (POLRMT), which, during the initiation of mtDNA synthesis, constructs RNA primers (Saada, 2004; Falkenberg et al., 2007; Gowher, 2013). The asynchronous, strand-displacement model (Brown et al., 2005) of mitochondrial genome replication is initiated at the heavy strand origin of replication, within the D-loop, creating a leading H-strand, which proceeds in a clockwise manner until exposure of the light-strand (L-strand) origin of replication. This then initiates replication of the lagging L-strand, in a counterclockwise direction, resulting in the formation of two daughter mtDNA molecules at different time intervals. This process purports that mtDNA synthesis is continuous on both the
heavy and light strands of the mitochondrial genome with the hallmarks of this model being single-stranded DNA intermediates with the conspicuous absence of Okazaki fragments formation (McKinney and Oliveira, 2013). A modification of this model proposes that long stretches of RNA intermediates, termed RITOLS, (instead of DNA), are arranged on the mtDNA lagging-strand (Yasukawa et al., 2006). It is only during the maturation phase that these RNA segments are converted into DNA (Yang et al., 2002; Yasukawa et al., 2006). A second theory of replication, known as the strand coupling model, first proposed by Holt et al (Holt et al., 2000), proposes that mtDNA replication is initiated within a single replication zone and proceeds bi-directionally by means of coupled leading- and lagging-strand synthesis (Holt et al., 2000; Yasukawa et al., 2005).

**Figure 5**: Replication of mtDNA (a–c) *Asynchronous model of replication*. (a) mtDNA replication begins at Oₜ (in the D loop), displacing the L-strand from the H-strand. The L-strand is single stranded until synthesis of the incipient H-strand exposes Oₜ. (b,c) At this stage, replication of the L-strand begins in the opposite direction until both strands have been fully replicated. (d) *Synchronous or coupled model of replication*. Replication begins bidirectional from a zone of replication (OriZ) on the genome and proceeds synchronously via conventional coupled leading and lagging strand synthesis. (e–g) RITOLS model: the ribonucleotide incorporation throughout the lagging strand (RITOLS) model of replication initiates in the noncoding region close to or at Oₜ, displacing the light strand from the heavy strand. The RITOLS model is similar to the asynchronous mode of replication, but RNA intermediates are produced (dashed lines) on the L-strand before conversion to DNA. (Reproduced from Krishnan et al., 2008).
1.2.viii Transcription and translation of mitochondrial DNA

Three mitochondrial transcription sites within the D-loop are recognised: two on the H-strand at nucleotide positions 561 (ITH1) and 638 (ITH2) and one on the L-strand (ITL) at nucleotide position 407 (Ojala et al., 1980a; Ojala et al., 1981; Martin et al., 2005). The two H-strand initiation sites facilitate independent mRNA and rRNA synthesis; with ITH1 most frequently employed for the process. ITH1 produces two tRNAs (tRNA\textsubscript{Phe} and tRNA\textsubscript{Val}) and two rRNAs; whilst ITH2 transcription creates a large, polycistronic mRNA molecule (Ojala et al., 1980b; Montoya et al., 1983; Bonawitz et al., 2006). Key proteins essential to this process include TFAM-mediated recruitment of the mitochondrial RNA polymerase (POLRMT), to the promoter sites (Diaz and Moraes, 2008). Other essential regulatory mitochondrial transcription factors that have been identified to date include TFB1M, TFB2M; (Shoubridge, 2002; Ekstrand et al., 2004) factors that are required for efficient transcription and a combination of either MTIR1, MTIR2 (Wenz et al., 2009) or MTERF3 (Park et al., 2007; Rebelo et al., 2011); recognised as important terminators of the process.

Mitochondrial translation is less well understood. Only two subunits (12S, 16S) of mitochondrial ribosomes are of mitochondrial origin (O’Brien, 2003). The process is initiated by mitochondrial translational initiation factor 2 (IF2\textsubscript{mt}) which promotes the binding of tRNA\textsubscript{met} to the 12S subunit. Ribosomal initiation complex formation, on the other hand, is facilitated by mitochondrial translational initiation factor 3 (IF3\textsubscript{mt}) (Christian et al., 2009). Elongation of the resultant polypeptide chain is regulated by three leading mitochondrial elongation factors: EF-G1\textsubscript{mt}, EF-T\textsubscript{Smt}, and EF-T\textsubscript{Umt} while termination of the process is facilitated by RF1a\textsubscript{mt} (Christian and Spremulli, 2012).

1.3 MITOCHONDRIAL DNA MUTATIONS

1.3.i Location of mitochondrial DNA mutations

The mitochondrial genome exhibits a very high mutation rate, ranging from 10- to 17-fold higher than that observed in nuclear DNA (Tuppen et al., 2010). Since the discovery of the first pathogenic mtDNA mutations over two decades ago (Holt et al., 1988b; Wallace et al., 1988b) more than 250 novel, pathogenic mtDNA point mutations have been identified in association with human disease (Tuppen et al., 2010). Currently there are several mtDNA mutational hotspots that are recognised including point mutations in mitochondrial tRNA genes (Figure 6).
and genes encoding the structural subunits of complex I (Moraes et al., 1993; Chinnery et al., 2001; Kirby et al., 2004a; McFarland et al., 2004; Valentino et al., 2004a; McFarland et al., 2010).

**Figure 6**: Pathological mutations in tRNA\textsuperscript{LEU(UUR)} The significant variability in phenotypic expression is illustrated in the MTTL1 gene where different base substitutions (at nucleotide pair(np) 3243, 3252, 3271, and 3291) give rise to the same clinical syndrome (MELAS); whilst the m.3243 AA>G mutation can manifest as maternally inherited diabetes and deafness (MIDD) or a hypertrophic cardiomyopathy (HCM) phenotype. Other clinical syndromes associated with MTTL1 mutations include HCM and mitochondrial myopathy (MM) (np 3303 and 3260). (Reproduced from http://www.mitomap.org (Brandon et al., 2005)).

1.3.ii **Heteroplasmy and homoplasmy of mitochondrial DNA mutations**

There are unique genetic rules underpinning the clinical expression of mtDNA disease. Mitochondrial DNA is exclusively maternally-inherited and is present in multiple copies in all nucleated cells (Taylor and Turnbull, 2005a). The multicopy or polyploid nature (that is, multiple copies of mtDNA are present within each mitochondrion with several thousands of copies present in individual cells) means that mtDNA mutations can either be homoplasmic
(that is, all copies of mtDNA carry the mutation) or heteroplasmic (varying proportions of mutated and wild-type mtDNA). All offspring of a woman with homoplasmic mtDNA mutation will unequivocally carry the mtDNA mutation (Taylor and Turnbull, 2005a). However the situation with women carrying heteroplasmic mtDNA mutations is much more complicated. During the development of the oocyte there is a genetic bottleneck with a marked reduction in the copy number of mtDNA followed by a rapid expansion of mtDNA copy number during oocyte formation (Cree et al., 2008; Wai et al., 2008; Freyer et al., 2012). This means that some heteroplasmic mtDNA mutations (eg. single, large scale mtDNA deletions) are rarely transmitted due to purifying selection (Stewart et al., 2008). Other heteroplasmic mtDNA mutations (eg. the m.3243A>G MELAS mutation and the m.8344A>G MERRF mutations) are transmitted, but the developmental genetic bottleneck can lead to offspring being born with a range of different mtDNA mutation levels.

1.3.iii Replicative segregation and tissue variation in threshold

1.3.iv The mitochondrial bottleneck (purifying selection)

Mechanisms thought to be responsible for the mitochondrial bottleneck that determines the variability in heteroplasmy seen in off-spring of females harbouring mutant mtDNA, have raised much intrigue over the ensuing two decades and remain contentious (Jenuth et al., 1997; Cao et al., 2007; Cree et al., 2008; Stewart et al., 2008; Wai et al., 2008; Cao et al., 2009; Samuels et al., 2010). One theory suggests that during embryonic oogenesis, between generations variation in heteroplasmy results from the partition of mtDNA into different cells, both before and after implantation, followed by the segregation of replicating mtDNA between proliferating primordial germ cells (Cree et al., 2008). Another model suggests that this occurs during early post-natal folliculogenesis as a result of replication of a subpopulation of mtDNA molecules (Wai et al., 2008).

1.3.v Replicative segregation and the threshold effect

Replicative segregation is responsible for the percentage variation in generational heteroplasmic alleles during both mitotic and meiotic cell division. In the presence of such heteroplasmy, and as the percentage of mutant mtDNA increases, there is a threshold level of mutation that determines both clinical phenotype and biochemical defect expression (Poulton and Marchington, 2002; Taylor and Turnbull, 2005a; Wallace and Chalkia, 2013) (Figure 7).
Conversely, mutant mtDNA may disappear, as seen in fast-dividing tissues. This is perhaps best exemplified by the observation of an annual one percent reduction in m.3243A>G mutation levels in blood (Rahman et al., 2001); with selection against pathogenic mtDNA mutations occurring in a stem cell population (Rajasimha et al., 2008).

**Figure 7:** The distribution and degree of defective mitochondria in different tissues results in variation in clinical symptoms and severity.

1.3.vi Clinical syndromes of mitochondrial DNA

“Mitochondrial disease” conventionally defined by defective oxidative phosphorylation includes a wide, overlapping spectrum of diseases, with some groups of patients manifesting as distinct clinical syndromes. Mitochondrial disease arises not only from mutations in genes encoding oxidative phosphorylation subunits, but also to genes intimately involved in their translation and assembly, as well as determining their subcellular environment (Schon et al., 2012b) resulting in a huge variation of clinical phenotypes (Figure 8). On the one hand, the same genetic defect can cause different phenotypes in different members of the same family. On the other hand, a similar phenotypic spectrum can be seen with different genetic lesions in the nuclear or mitochondrial DNA. For mitochondrial DNA diseases, the concept of heteroplasmy further complicates the situation, with different percentage levels of mutation in
different cells within the same organ, and different levels between different organs in the same individual (Figure 7).

Hence mitochondrial disorders encompass a wide, overlapping spectrum of diseases, with only some groups of patients manifesting as distinct clinical syndromes including myoclonic epilepsy, lactic acidosis, stroke-like episodes (MELAS) (Pavlakis et al., 1984), myoclonic epilepsy, with ragged red fibres (MERRF) (Fukuhara et al., 1980; Chinnery et al., 1997), Kearns-Sayer Syndrome (KSS) (Kearns, 1965), Leber’s Hereditary Optic Neuropathy (LHON) (Leber, 1871), neurogenic weakness with ataxia and retinitis pigmentosa (NARP) (Holt et al., 1990) and Leigh syndrome (LS) (Ciafaloni et al., 1993)

Figure 8: The human mitochondrial genome demonstrating the common mtDNA mutation sites and associated clinical syndromes (Reproduced from (Tuppen et al., 2010)}
1.4 NUCLEAR BASIS OF MITOCHONDRIAL DISEASE

1.4.i Nuclear (n)DNA-mtDNA interactions

Over the past decade, there has been an exponential increase in the recognition of Mendelian inherited mitochondrial disorders of the nuclear genome cogent to the discovery of more than 1000 nuclear genes that encode mitochondrial proteins. More than half of all adult mitochondrial diseases result from genetic malfunction or disruption of the nuclear genome.

The pathological consequences of dysfunction of nuclear–mitochondrial interactions include defects of 1) mtDNA maintenance (secondary mtDNA mutations or mtDNA depletion); 2) mitochondrial protein synthesis; 3) coenzyme Q10 biosynthesis and 4) the mitochondrial respiratory chain complexes or their assembly (Chinnery, 2014). The mitochondrial genome is inextricably dependent on several nuclear encoded proteins, for replication and repair, including DNA polymerase gamma (Bolden et al., 1977), Twinkle helicase, ANT1 and thymidine phosphorylase (TYMP) (Kaukonen et al., 2000; Korhonen et al., 2004; Wanrooij et al., 2008).

Disruption in these nuclear encoded processes may manifest qualitatively in the generation of multiple point mutations or large scale mtDNA deletions that can appear over a patient’s lifetime (Ashley et al., 2007) or by the loss of the complete mitochondrial genome (mtDNA depletion) (Moraes et al., 1991) or both (Nishigaki et al., 2003).

Currently, nuclear maintenance genes appear to fall into distinct genetic categories. Firstly, there are those genes that affect protein function at the mitochondrial DNA replication fork such as POLG, POLG2, and PEO1 (also called C10orf2, encoding the Twinkle helicase) (Spelbrink et al., 2001; Van Goethem et al., 2001a; Longley et al., 2006); or secondly, those genes that encode proteins involved in nucleotide metabolism such as TK2, DGUOK, SUCLA2, SUCLG1, TYMP and RRM2B. (Nishino et al., 1999; Kaukonen et al., 2000; Mandel et al., 2001; Saada et al., 2001; Bourdon et al., 2007; Ostergaard et al., 2007; Dimmock et al., 2008b; Fratter et al., 2011).

Progressive external ophthalmoplegia (PEO) and ptosis is the most common presenting neurological feature seen in adults with mitochondrial DNA maintenance disorders. Of the known maintenance genes, the majority have been associated with PEO, although it is increasingly recognised that the clinical phenotype is not necessarily restricted to the extraocular muscles.

A skeletal muscle biopsy remains central to the diagnostic algorithm of adult-onset PEO with key diagnostic features of a mosaic pattern of cytochrome c oxidase (COX)-deficient fibres and
ragged-red fibres (indicative of mitochondrial sub-sarcolemmal accumulation) present in most (but not all) cases (Taylor et al., 2004).

1.4.ii Qualitative defects of mitochondrial DNA

The mechanisms attributable to multiple mtDNA deletion formation remain a contentious issue (Krishnan et al., 2008) (Figure 9). The primary genetic defect in individuals with multiple mtDNA deletions involves nuclear genes encoding proteins involved in either mitochondrial nucleotide metabolism (TYMP and SLC25A4 or ANT1) or mtDNA (replication or repair) maintenance (C10orf2, POLG and POLG2). The majority of mtDNA deletions occur within the major arc of the mitochondrial genome flanked by short, homologous, direct repeats (Samuels et al., 2004; Bua et al., 2006; Krishnan et al., 2008) between the replication origins of O'H and O'L. Current theories of mtDNA deletion formation favour replication slippage errors as the causative mechanism (Krishnan et al., 2008). A slipped strand model of replication, cogent to the strand-displacement model of replication, (Clayton, 1982) proposes that during replication a single-stranded repeat of the L-strand misaligns with a newly exposed H-strand repeat. This process results in the generation of a downstream loop of L-strand, that is susceptible to breaks within the mtDNA molecule. Subsequent exonuclease degradation and ligation of the L-strand loop, with resumption of replication, results in the formation of a wild type and a deleted mtDNA molecule (Krishnan et al., 2008). However, discrepancies between this theory and recent findings in rapidly dividing colonic crypt cells have begun to emerge that fundamentally challenge this theory (Greaves et al., 2006).

An alternative model suggests that mtDNA deletion formation is not linked to replication; moreover to the process of repair of damaged DNA. This theory proposes that mtDNA deletions are secondary to double stranded breaks that are susceptible to exonuclease activity resulting in single strand formation. Subsequent repair mechanisms are inept, and result in the formation of a deleted mtDNA molecule (Krishnan et al., 2008).
Figure 9: Model of mtDNA replication. Slipped-strand model of replication (a) mtDNA molecule demonstrating two direct repeats (5' and 3'). (b) The process of mtDNA replication begins at O_h, (in the D loop) displacing the L-strand from the H-strand. (c) A single-stranded repeat of the L-strand misanneals with a newly exposed H-strand repeat. This process results in the generation of a downstream loop of L-strand, that is susceptible to breaks within the mtDNA molecule. (d) The damaged loop is degraded until it reaches the double-strand regions, and ligation of the free ends of the H-strand occurs. (e,f) Replication is resumed resulting in the production of a wild type and a deleted mtDNA molecule. (Reproduced from Krishnan et al., 2008).

1.4.iii Quantitative defects of mitochondrial DNA

1.4.iv Fission and fusion

Although originally thought of as static, isolated intracellular organelles, mitochondria constitute a group of organelles that form dynamic networks that constantly undergo fusion and fission (division), and programmed turnover; facilitating exchange of mitochondrial content in addition to replication and degradation of mtDNA. These processes of fission and fusion intimately regulate mitochondrial function via a number of processes, including recruitment, cellular exchange, morphology control, cytosolic communication, quality control and mtDNA integrity of mitochondria (Chen and Chan, 2005; Twig et al., 2008; Youle and Van Der Bliek, 2012). Hence these dynamic organelles can adapt rapidly to physiological or
environmental cues; however disruption of fission and/or fusion leads to dysfunction in cellular mechanisms and is thought to be the unifying mechanism underlying several mitochondrial diseases.

Mitochondrial fusion is regulated in humans by three proteins: Optic atrophy protein 1 (OPA1), (inner membrane fusion) Mfn1 and Mfn2 (outer membrane fusion) (Chen et al., 2003a; Cipolat et al., 2004; Chen and Chan, 2005; Lee et al., 2007). Mutations in OPA1 are responsible for dominant optic atrophy resulting in atrophy of the optic disc and visual impairment (Delettre et al., 2000). Mutations in Mfn2 cause Charcot Marie Tooth type 2A, a sensorimotor axonal neuropathy characterized by distal weakness and wasting (Züchner et al., 2004). Although initially perceived as phenotypically divergent, it is increasingly recognized, that disease manifestations may overlap in both disorders (Detmer and Chan, 2007).

Mitochondrial fission is regulated by another protein: dynamin-like protein (Drp1) (Smirnova et al., 2001; Yoon et al., 2003; Chang and Blackstone, 2010). This cytosolic dynamin-related GTPase, is recruited to the mitochondrion during fission and mediated by other outer membrane located proteins. The role of fission is postulated to ensure equal segregation of mitochondria, during cell division, into daughter cells in addition to augmenting the dissipation of mitochondria along cytoskeletal structures (Frank et al., 2001; Otera et al., 2013). Moreover, fission appears to be integral in the promotion of mitochondrial degradation via autophagy (‘mitophagy’), as it identifies defective sections of mitochondria (Poulton et al., 2010; Santos et al., 2010; Frank et al., 2012). If these integral systems fail, mitochondrial fission may espouse apoptosis. Disruption in mitochondrial dynamics has also been implicated in other common neurodegenerative disorders (Chen and Chan, 2009).

1.4.v Clonal expansion

Clonal expansion is a process whereby accumulation of progeny of one initial mutation expands within individual cells to attain suprathreshold levels resulting in a biochemical defect and cellular dysfunction (Elson et al., 2001). This is frequently characterized by a mosaic pattern of COX-deficient fibres in skeletal muscle of patients with nuclear gene mutations (Oldfors et al., 1992; Sciacco and Bonilla, 1996). Several causal theories governing clonal expansion include a combination of random genetic drift with or without selection to preserve wild-type (Coller et al., 2001a; Coller et al., 2002) or a replicative advantage for smaller mtDNA species (Fukui and Moraes, 2009; Nicholas et al., 2009). Neither theory accounts for other important factors that are likely to be influencing the rate of clonal expansion including
mitochondrial dynamics and mitophagy. Although conceptually central to our understanding of mitochondrial disorders, further validation of the processes driving clonal expansion are still warranted.

1.4.vi mtDNA mutation accumulation and Ageing

It is now increasingly recognized, that with normal ageing, there is an accumulation of both mtDNA point mutations (with a predilection for rapidly dividing tissues) and deletions (with a predilection for post mitotic tissues) at varying levels in different tissues including brain, muscle and heart (Corral-Debrinski et al., 1992; Simonetti et al., 1992; Pallotti et al., 1996; Krishnan et al., 2008; Meissner et al., 2008; Greaves and Turnbull, 2009).

Current evidence suggests that mtDNA mutations appear to arise spontaneously as the result of somatic mutation in post mitotic tissue and each individual mutant species may expand clonally (see section 1.4.V) in individual cells, resulting in mosaic respiratory chain deficiency (Larsson, 2010). This process is commonly seen in ageing muscle from normal subjects (Bodyak et al., 2001) and is identical to that seen in adult-onset Mendelian PEO (Vu et al., 2000; Schon et al., 2012b). The precise role of these somatic mutations in normal ageing is yet to be fully elucidated; but their presence in ageing tissue, has led to multiple studies focusing on these mtDNA defects to support the mitochondrial theory of ageing. It remains unknown whether such mutations are responsible for such age-related disorders as Parkinson’s disease, Alzheimer’s dementia and sarcopaenia.

1.4.vii Polg mouse models and Ageing

Increasing evidence has emerged to suggest that the majority of somatic mtDNA mutations arise from the indigenous error rate of mtDNA polymerase gamma (Larsson, 2010). Transgenic and knockout mouse models have been developed to interrogate the mitochondrial theory of ageing. Transgenic mouse models of mtDNA instability exhibiting a point mutation in a catalytic subunit of polymerase gamma (POLG) that is responsible for proofreading newly formed mtDNA have been developed by two independent groups of investigators (Trifunovic et al., 2004; Kujoth et al., 2005). Homozygous mutant mice (PolγD257A/D257A) exhibit a progressive accumulation of mtDNA point mutations and a marked reduction in lifespan compared to their wild type mice controls and a clinical phenotype consistent with accelerated ageing and characterised by muscle and weight loss, hearing loss, reduced bone density, infertility and anaemia (Kujoth et al., 2005). Interestingly at a molecular level, there was no difference detected in oxidative damage or mitochondrial ROS production between mutant mice and controls, suggesting the observed clinical phenotype was not directly mediated by
ROS production (Kujoth et al., 2005) or the loss of proof-reading had more complex and more far reaching effects on the mitochondrial genome (Jang and Van Remmen, 2009). However, Vermulst and colleagues (2007)(Vermulst et al., 2007), in a subsequent study demonstrated that heterozygous mice harbouring a single mutant allele (Polγ^{D257A}), exhibited almost a 500-fold increase in mtDNA point mutations, but a normal lifespan and phenotype. These findings were further supported by an ensuing study by the same authors evaluating the accumulation of mtDNA deletions in the same mouse model. They demonstrated that only the homogenous mutant Polγ^{D257A/D257A} mice had accelerated accumulation of such mtDNA defects and that perhaps more intriguingly that the rate at which the accumulation of mtDNA mutations reached a critical threshold of phenotypic expression varied widely between tissues. This led to Vermulst and colleagues (2008) concluding that the mtDNA deletions were driving the accelerated ageing phenotype in Polymerase gamma mutant mice (Vermulst et al., 2008). This was in contrast to a previous report of a transgenic mouse model with a mutated allele of mitochondrial helicase (TWINKLE) with marked elevation of mtDNA deletions but no associated accelerated ageing phenotype (Tyynismaa et al., 2005); however the discrepancies observed, maybe, in part, due to the experimental approaches employed (Jang and Van Remmen, 2009). These findings support the need for further work to provide insights into the role of mitochondrial dysfunction and somatic mtDNA mutations in the ageing process.

1.4.viii mtDNA mutations and Cancer

Genetic instability has been implicated in the development of both inherited and sporadic cancers. Although the precise molecular mechanisms remain unclear two models have been devised to explain models of cancer: 1) Mutator model; 2) Replication stress model. The ‘Mutator hypothesis’ of tumour formation suggests that genetic instability is present in precancerous lesions and drives tumorigenesis by increased spontaneous mutation rate (Loeb et al., 2008). This is supported by the identification of DNA repair gene mutations predominantly in inherited cancers. In the second model of cancer development, it is postulated that in sporadic cancer, oncogene-induced collapse of DNA replication and replisome stability at defective DNA replication forks results in genetic instability (Zeman and Cimprich, 2014).
Polyak and colleagues (1998) first suggested a role of mtDNA in carcinogenesis following the identification of numerous homoplasmic mtDNA mutations in cancer cell lines with normal mtDNA in adjacent healthy cells (Polyak et al., 1998); findings that have been prolifically reproduced several times since then (Yu, 2011). These findings led Polyak and colleagues to postulate that these mtDNA mutations bestowed the cancer cells with a physiological growth advantage (‘replicative advantage’) resulting in wild-type mtDNA being eventually replaced completely by the mutant mtDNA. However, this theory has subsequently been challenged with the high frequency of homoplasmic mtDNA mutations in cancer cells, postulated to have simply arisen due to other processes such as random genetic drift (Chinnery et al., 2000b; Coller et al., 2001b).

In cancer cells, mitochondrial function is altered in multifarious ways, allowing tumour cells to evade cell-death (Gogvadze et al., 2008). Firstly this may result from loss of p53 function via mutated or lost TP53 in tumour cells, resulting in either gain (Dittmer et al., 1993) or loss of function with depression of the respiratory chain (Wahl et al., 1996; Madan et al., 2011). Various cancers exhibit a high frequency of mutations in TP53, resulting in perturbations in p53 signalling pathways and (Muller and Vousden, 2014).

The regulation of cell death by apoptosis is intimately dependent on the balance between interacting pro-apoptotic factors and anti-apoptotic factors; with a shift towards the later in tumour cells (Schon et al., 2012a).

Hypoxia- inducible factor 1(HIF1), a transcription factor mediating hypoxia mediated cell death has been implicated in tumorigenesis. This factor is stabilised by increased levels of TCA-derived succinate in tumours inhibiting its degradation and it is evasion of hypoxic-mediated cell death via this pathway that has been implicated in the development of such tumours such as phaeochromocytomas, paragangliomas and leiomyomas (Tomlinson et al., 2002; van Nederveen et al., 2009; Schon et al., 2012a).

Recently, it has been shown that tumours uniquely use the metabolite glycine that is produced by mitochondria from serine and tetrahydrofolate (as opposed to cytosolic glycine production), providing further evidence of the mediation of purine biosynthesis in the development of cancer by mitochondria (Jain et al., 2012).
1.4.ix mtDNA mutations and neurodegeneration

The potential role of mitochondrial dysfunction including somatic mtDNA mutations in age-related neurodegenerative disorders such as Parkinson’s disease, Alzheimer’s dementia, motor neuron disease, Huntington’s chorea and hereditary spastic paraplegia has gained significant momentum over recent years. Moreover, neurodegeneration would appear to be due primarily to inherent problems in mitochondrial dynamics (see section 1.4 iv) and/or quality control (such as autophagy) (Schon et al., 2012a), rather than to mutations in mtDNA (Bender et al., 2006; Kraytsberg et al., 2006; Khrapko and Vijg, 2009; Kukat and Trifunovic, 2009). For example, in Parkinson Disease associated with mutations in PINK1 and PARK2, altered mitochondrial quality control has been implicated in disease pathogenesis in both sporadic and familial cases (D’Aurelio et al., 2010; Vives-Bauza et al., 2010a; Vives-Bauza et al., 2010b; Vives-Bauza and Przedborski, 2011). In addition high levels of mtDNA deletions have been isolated from substantia nigra neurons from both healthy-aged controls and patients with Parkinson’s disease suggesting that these mtDNA defects may eventually have a deleterious effect on tissue function resulting in disease expression (Bender et al., 2006; Kraytsberg et al., 2006; Khrapko and Vijg, 2009; Kukat and Trifunovic, 2009). Further studies are currently warranted to help further elucidate these issues. And whilst altered mitochondrial trafficking has been implicated in the development of other neurodegenerative conditions including Amyotrophic Lateral Sclerosis (Shi et al., 2007; Bosco et al., 2010), relating pathology to specific defects in mitochondrial dynamics has proven difficult (Schon and Przedborski, 2011)(Schon 2011).

Hereditary spastic paraplegia (HSP) and Spinocerebellar atrophy (SCA), are another group of late-onset neurodegenerative disorders associated with mitochondrial dysfunction. The mechanism underlying the mitochondrial defects caused by mutations in specifically two causative genes, namely spastic paraplegin7 (SPG7) and AFG3L2 are discussed in relation to two original studies that will be presented later in this thesis (see Chapter 4, sections 4.4 and 4.5).

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogenous group of neurological disorders characterised by length-dependent distal axonal degeneration of the corticospinal tracts. HSP is defined as ‘uncomplicated’ in those individuals in whom their neurological deficits are limited to progressive spasticity and weakness and mild sensory dysfunction in the lower limbs that maybe associated with bladder dysfunction (Harding, 1983). In individuals, in whom, there are additional systemic clinical features such as dementia, ataxia, neuropathy, amyotrophy, extrapyramidal features and seizures; these cases are defined as ‘complex’ HSP (Fink, 1993). More recently HSP classification has been revised to refer mainly to their genetic classification (Durr, 2008). At the time of writing this thesis, 72 spastic
gait disease-loci and 55 HSP genes have been identified, including mutations in the SPG7 gene (Wedding et al., 2014).

SPG7, a relatively common form of autosomal recessive HSP, is similarly characterised by length dependent axonopathy of the corticospinal tracts, manifesting as lower limb spasticity and weakness. The molecular mechanisms underlying the phenotypic expression of HSP remains unknown. However, increasing evidence suggests dysfunction in the endoplasmic reticulum and intracellular membrane trafficking and distribution are the primary defects underlying HSP. At this stage, it would be important to revise the anatomy of the corticospinal tracts. Pyramidal neurons originating from layer V of the cerebral motor cortex, classically decussate in the caudal medulla and descend as the lateral corticospinal tracts. These corticospinal axons primarily synapse with spinal interneurons that then connect to lower motor neurons, with only a small number of corticospinal axons synapsing directly with lower motor neurons. The lower motor neurons then form specialised synapses at the neuromuscular junctions (Blackstone, 2012). An elaborate neuronal cytoskeleton scaffold facilitates complex intracellular machineries responsible for the distribution of essential proteins, lipids and mRNAs. A group of enzymes including kinesin, dynein and myosin proteins predominantly mediate anterograde and retrograde transport (Goldstein et al., 2008; Arnold, 2009).

Pathological and recent neurophysiological techniques have shown evidence of axonal degeneration in a retrograde degenerative manner involving the longest ascending sensory fibres and descending corticospinal tracts in HSP (Deluca et al., 2004). Two mitochondrial proteins mutated in HSP include paraplegin (ARS PG7) and HSP60 (ADSPG13).

SPG7 encodes paraplegin (a member of the AAA family), a mitochondrial protein composed of two peptide regions, a metallo-peptidase domain and an ATPase domain that mediates ribosomal assembly and protein quality control in the mitochondrial inner membrane (Casari et al., 1998). To date, almost all SPG7 variants are nonsense loss-of-function mutations or missense mutations in the metallo-peptidase domain (Casari et al., 1998); however, a number of mutations cause an amino acid substitution mapping in the AAA-domain. Paraplegin is implicated in several cellular processes including ribosomal assembly and the processing of mitochondrial proteins (Nolden et al., 2005; Karlberg et al., 2009).

SPG7 often manifests as uncomplicated or pure HSP; but cerebellar involvement is not uncommon. Additional clinical features have been extensively reported in complex cases of SPG7-related HSP including optic atrophy, deafness, pes cavus, neuropathy (motor and sensory), scoliosis and ophthalmoplegia (Casari and Marconi, 1993; Wedding et al., 2014).
Studies of skeletal muscle have shown evidence of COX negative fibres consistent with mitochondrial respiratory chain dysfunction (Casari et al., 1998; McDermott et al., 2001; Arnoldi et al., 2008; van Gassen et al., 2012); whilst fibroblast studies have shown variable evidence of complex I deficiency (Atorino et al., 2003). SPG7 null mice have been shown to exhibit evidence of impaired mitochondrial function and axonal transport associated with axonal swelling and accumulation of neurofilaments and mitochondria (Ferreirinha et al., 2004). Further supportive evidence of mitochondrial dysfunction has been observed in SPG13 HSP in which impaired HSP60 chaperone activity has been shown to result in impaired mitochondrial quality control (Bross et al., 2008). Dysfunction in the complex interplay between endoplasmic reticulum, plasma membranes, endosomes and mitochondrial have also been implicated in the development of HSP (Carrasco and Meyer, 2011; Toulmay and Prinz, 2011; Blackstone, 2012). Endoplasmic reticulum mitochondrial contacts have gained much interest recently in both mammalian and yeast models of HSP. A model of pulmonary arterial hypertension has been created, with reduced endoplasmic reticulum to mitochondrial phospholipid transfer and intra-mitochondrial calcium, exhibiting increased expression of Nozo-B, (an endoplasmic reticulum shaping protein of the reticular family), and resulting in disruption in the contacts between endoplasmic reticulum and mitochondria, providing further evidence of the role of endoplasmic reticulum shaping protein in mitochondrial dysfunction (Sutendra et al., 2011).

As outlined above, paraplegin co-assembling with AFG3L2 protein forming integral membrane proteins (Atorino et al., 2003) and forming a high molecular weight complex that appear absent in the fibroblasts of patients with HSP. The inactivation of parplegin-AFG3L2 complex causes a reduction in complex I activity that maybe reversed with the increased expression of wild type paraplegin, mediating mitochondrial ribosome assembly. Null or missense AFG3L2 mouse models have been shown to demonstrate conspicuous impairment of axonal development and transport resulting in severe early onset tetraparesis with complex I and III activity with abridged myelinated spinal cord fibres resulting in premature neonatal death (Maltecca et al., 2008) akin to a more severe phenotype than paraplegin-deficient mice.

I present a cohort of patients with complex PEO including spasticity and ataxia to varying degrees and associated with multiple mtDNA mutations in muscle. Fifteen patients were identified with either compound or single heterozygous mutations in SPG7 (Pfeffer et al., 2014) and a further two patients with novel heterozygous mutations in AFG3L2 (Gorman et al., 2015d). Simultaneously another group reported a further four adult patients with a combination of spastic paraplegia and PEO with muscle restricted mtDNA deletions due to mutations in the SPG7 gene (Wedding et al., 2014). These findings combined, suggest that
SPG7 and AFG3L2, are linked to mtDNA maintenance. Preliminary evidence is provided indicating that mutations in these genes may induce mitochondrial biogenesis. However significantly more work interrogating the molecular mechanisms underlying spasticity ataxia syndromes is required.

1.4.x Clinical syndromes of nDNA

Although qualitative and quantitative deficiencies of mtDNA are often associated with different mutations and clinical phenotypes, several clinical syndromes are historically recognised. These include Alpers-Huttenlocher syndrome (Davidzon et al., 2005), the most severe phenotype, characterised by hypotonia, intractable seizures, renal tubulopathy and liver failure, ataxia neuropathy syndromes and chronic progressive ophthalmoplegia (Cohen et al., 2012). More recently, a group of disorders with impaired mitochondrial dynamics, including mutations in OPA1 (optic atrophy, ataxia and deafness) and MFN2 (CMT type 2A), have emerged, that indirectly lead to qualitative deficiencies of mtDNA (Ishihara et al., 2006; Verhoeven et al., 2006).

1.4.xi Ataxia neuropathy syndromes

These groups of disorders encompass a myriad of clinical features and recognised syndromes frequently related to mutations in POLG. These include ataxia neuropathy spectrum (ANS) previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO) and myoclonic epilepsy myopathy sensory ataxia (MEMSA) previously known as spinocerebellar ataxia with epilepsy (SCAE) (Cohen et al., 2012). The cardinal clinical features of these syndromes include sensory axonal neuropathy with variable sensory and cerebellar ataxia. Other clinical manifestations include progressive external ophthalmoparesis, seizures, dysarthria, dementia, spasticity and myopathy.

1.4.xii Chronic progressive external ophthalmoplegia

Progressive external ophthalmoplegia (PEO) and ptosis, a common manifestation of adult mitochondrial disease, is a disorder of eye movements delineated by extra ocular muscle paresis that is biochemically defined by skeletal muscle restricted mitochondrial DNA
deletions. PEO is commonly associated with either primary mitochondrial DNA mutations or acquired mtDNA defects secondary to a nuclear genetic disorder of mtDNA maintenance.

To date, of the 12 known maintenance genes, three quarters have been identified in adult-onset mendelian PEO. These include recessive mutations in TYMP, encoding thymidine phosphorylase (Nishino et al., 1999), POLG1, encoding the catalytic alpha-subunit of DNA polymerase γ (Van Goethem et al., 2001a), C10ORF2 encoding Twinkle, the hexameric mtDNA helicase (Spelbrink et al., 2001), TK2 (Tyynismaa et al., 2012), RRM2B (Takata et al., 2011) and DGUOK (Ronchi et al., 2012) or dominant mutations in POLG1, POLG2, encoding the accessory beta-subunit of poly (Longley et al., 2006), SLC25A4, encoding adenine nucleotide translocator 1 (ANT-1) (Kaukonen et al., 2000), C10ORF2 (Spelbrink et al., 2001) or OPA1 (Hudson et al., 2008). Despite the recognition of these causative genes in patients with clinically confirmed PEO, the underlying nuclear gene defects remain unexplained in a significant number of patients. This is, in part, due to the poor characterization of genotype-phenotype correlates in PEO (Figure 10).

Figure 10: Mendelian disorders of mtDNA maintenance. A cartoon identifying the major genes (italicised in blue) which have been associated with disorders of mtDNA maintenance including adult-onset PEO and multiple mtDNA deletion syndromes and/or mtDNA depletion. In addition the sub-mitochondrial localisation of the proteins encoded by these genes are demonstrated. Reproduced from (Sommerville et al., 2014)
Mutations in the nuclear genome encoding essential mitochondrial processes may manifest as a spectrum of disorders as opposed to a discrete clinical syndrome; resembling that of mtDNA related mitochondrial disease. Next generation sequencing is currently revolutionising our approach to the diagnosis of mitochondrial disease, moving the goal posts from sequence detection, to the functional validation of new mutations. And perhaps this has been best evidenced in the rapid identification of nuclear gene mutations responsible for mitochondrial disorders. Pathogenic nuclear mutations are discussed in depth in chapter 4 and the current known and putative nuclear genes are listed (following) in table 1.
<table>
<thead>
<tr>
<th>Gene mutations</th>
<th>Clinical syndrome</th>
<th>RCD</th>
<th>Molecular role of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>AARS2</td>
<td>Hypertrophic cardiomyopathy (HCM), Cl and IV defect</td>
<td></td>
<td>aminoacylates alanyl-tRNA</td>
</tr>
<tr>
<td>ABAT</td>
<td>psychomotor retardation, intractable seizures, and hypotonia</td>
<td></td>
<td>catalyzes conversion of gamma-aminobutyrate and L-beta-aminoisobutyrate to succinate semialdehyde and methylmalonate semialdehyde</td>
</tr>
<tr>
<td>AGK</td>
<td>HCM, cataracts, lactic acidosis (LA), skeletal myopathy</td>
<td>+/-I</td>
<td>catalyzes formation of phosphatic and lysophosphatic acids</td>
</tr>
<tr>
<td>AIF1</td>
<td>Mitochondrial Encephalomyopathy</td>
<td>Combined</td>
<td>enhances activity of LCP1. Binds calcium; plays a role in phagocytosis</td>
</tr>
<tr>
<td>APOPT1</td>
<td>Leukoencephalopathy</td>
<td>IV</td>
<td>mediates mitochondria-induced cell death in vascular smooth muscle cells (release of cytochrome c; activation of the caspase cascade)</td>
</tr>
<tr>
<td>ATPAF2</td>
<td>Encephalopathy</td>
<td>V</td>
<td>encodes an assembly factor for the F(1) component of the mitochondrial ATP synthase</td>
</tr>
<tr>
<td>BCS1L</td>
<td>Encephalopathy, liver failure, renal tubulopathy</td>
<td>III</td>
<td>encodes a homolog of the S. cerevisiae bcs1 protein (involved in the assembly of complex III)</td>
</tr>
<tr>
<td>C12orf62</td>
<td>Brain hypertrophy, diffuse alteration of the white-matter myelination, numerous cavities in the parieto-occipital region, brainstem, and cerebellum, hepatomegaly, HCM, renal hypoplasia, adrenal-gland hyperplasia, fatal neonatal lactic acidosis</td>
<td>Combined; IV</td>
<td>plays a role in the assembly or stability of the cytochrome c oxidase complex (COX).</td>
</tr>
<tr>
<td>C12orf65</td>
<td>PEO, Leigh Syndrome (LS), optic atrophy, axonal neuropathy, intellectual disability, spastic paraparesis</td>
<td>Complex V</td>
<td>encodes a mitochondrial matrix protein; may help rescue stalled mitoribosomes during translation</td>
</tr>
<tr>
<td>CARS2</td>
<td>Epileptic encephalopathy, complex movement disorder; progressive myoclonic epilepsy</td>
<td>Combined</td>
<td>plays a critical role in protein biosynthesis by charging tRNAs with their affiliated amino acids</td>
</tr>
<tr>
<td>CHKB</td>
<td>Congenital muscular dystrophy</td>
<td>MDS</td>
<td>catalyzes 1st step in phosphatidylethanolamine biosynthesis</td>
</tr>
<tr>
<td>CLPB</td>
<td>Cataract, Neutropenia, Epilepsy, and Methylglutaconic Aciduria</td>
<td>I/III</td>
<td>may function as a regulatory ATPase and be related to secretion/protein trafficking process</td>
</tr>
<tr>
<td>COQ2, COQ9, CABC1, ETFDH</td>
<td>CoQ10 deficiency</td>
<td></td>
<td>involved in the ubiquinone biosynthetic pathway</td>
</tr>
<tr>
<td>Gene</td>
<td>Disorder</td>
<td>Pathway/Activity</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
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<tr>
<td><strong>COX10</strong></td>
<td>Leukodystrophy and renal tubulopathy</td>
<td>Converts protoheme IX and farnesyl diphosphate to heme O</td>
<td></td>
</tr>
<tr>
<td><strong>COX15, ELAC2</strong></td>
<td>HCM</td>
<td>I Putative role in the biosynthesis of heme A; Involved in tRNA maturation.</td>
<td></td>
</tr>
<tr>
<td><strong>DARS2</strong></td>
<td>LBSL: Leukoencephalopathy with Brain Stem and Spinal Cord Involvement and elevated Lactate progressive pyramidal and cerebellar dysfunction, dorsal column dysfunction and sometimes with axonal neuropathy</td>
<td>Aminoacylates aspartyl-tRNA; Protein homodimerization activity and aspartate-tRNA ligase activity</td>
<td></td>
</tr>
<tr>
<td><strong>DGUOK</strong></td>
<td>Encephalomyopathy and liver failure</td>
<td>Responsible for phosphorylation of purine deoxyribonucleosides and several nucleoside analogs</td>
<td></td>
</tr>
<tr>
<td><strong>EARS</strong></td>
<td>LTBL: Leukoencephalopathy with thalamus and brainstem involvement and high lactate</td>
<td>Catalyzes the attachment of glutamate to tRNA(Glu) in a two-step reaction</td>
<td></td>
</tr>
<tr>
<td><strong>EFTu</strong></td>
<td>Macrocystic leukodystrophy, polymicrogyria</td>
<td>Promotes binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis</td>
<td></td>
</tr>
<tr>
<td><strong>ELAC2</strong></td>
<td>HCM</td>
<td>I Involved in tRNA maturation, by removing a 3-trailer from precursor tRNA</td>
<td></td>
</tr>
<tr>
<td><strong>ETHE1</strong></td>
<td>Chronic diarrhea, EE, relapsing petechiae, acrocyanosis; glomerulonephritis</td>
<td>Metabolises hydrogen sulphide, preventing the accumulation of supraphysiological levels</td>
<td></td>
</tr>
<tr>
<td><strong>FARS2</strong></td>
<td>Alper syndrome</td>
<td>Responsible for the charging of tRNA(Phe) with phenylalanine in mitochondrial translation; catalyses direct attachment of m-Tyr (an oxidized version of Phe) to tRNA(Phe),</td>
<td></td>
</tr>
<tr>
<td><strong>FBXL4</strong></td>
<td>Neonatal LA, encephalomyopathy cerebral atrophy and variable involvement of the white matter, deep gray nuclei, and brainstem structures, dysmorphism, skeletal abnormalities, poor growth, gastrointestinal dysmotility, seizures, and episodic metabolic failure</td>
<td>MDS Encodes a member of the F-box protein family that function in phosphorylation-dependent ubiquitination</td>
<td></td>
</tr>
<tr>
<td><strong>FLAD1</strong></td>
<td>Myopathy</td>
<td>Combined Catalyzes the adenylation of flavin mononucleotide (FMN) to form flavin adenine dinucleotide (FAD) coenzyme</td>
<td></td>
</tr>
<tr>
<td><strong>GARS</strong></td>
<td>Myopathy, cardiomyopathy, MRC defect; Distal weakness (CMT2D/distal SMA type V-like), facial and respiratory muscle weakness/failure</td>
<td>Catalyzes the attachment of glycine to tRNA(Gly); produces diadenosine tetraphosphate (Ap4A), a universal pleiotropic signaling molecule needed for cell regulation pathways</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Function</td>
<td></td>
</tr>
<tr>
<td>------</td>
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<td></td>
</tr>
<tr>
<td>GFER</td>
<td>Myopathy with cataract</td>
<td>Isoform 1: regenerates the redox-active disulfide bonds in CHCHD4/MIA40, a chaperone essential for disulfide bond formation and protein folding in the mitochondrial intermembrane space; Isoform 2: may act as an autocrine hepatotrophic growth factor promoting liver regeneration</td>
<td></td>
</tr>
<tr>
<td>GFM1</td>
<td>IUGR, LS, mild microcephaly, LA, early fatal hepatomegaly;</td>
<td>Combined catalyzes the ribosomal translocation step during translation elongation</td>
<td></td>
</tr>
<tr>
<td>GFM2</td>
<td>microcephaly, simplified gyral pattern, and insulin-dependent diabetes</td>
<td>mediated the disassembly of ribosomes from messenger RNA at the termination of mt protein biosynthesis</td>
<td></td>
</tr>
<tr>
<td>GTPBP3</td>
<td>HCM, LA, encephalopathy</td>
<td>involved in the 5-carboxy-methylaminomethyl modification (mmn(S)(2s)(2s)(2s)(2s)U34) of the wobble uridine base in mt tRNAs</td>
<td></td>
</tr>
<tr>
<td>HARS2</td>
<td>Perrault syndrome: (AR) ovarian dysgenesis with SNHL</td>
<td>Involved in synthesis of histidyl-transfer RNA; regulation of protein biosynthesis</td>
<td></td>
</tr>
<tr>
<td>IARS</td>
<td>Fatal infantile cardiomyopathy</td>
<td>catalyze the aminocacylation of tRNA by its affiliated amino acid</td>
<td></td>
</tr>
<tr>
<td>ISCA2</td>
<td>Leukodystrophy, neuroregression</td>
<td>involved in the maturation of mitochondrial 4Fe-4S proteins functioning late in the iron-sulfur cluster assembly pathway</td>
<td></td>
</tr>
<tr>
<td>KARS</td>
<td>severe infantile visual loss, progressive microcephaly, developmental delay, seizures, and abnormal subcortical white matter</td>
<td>catalyzes two-step reaction: 1) amino acid (AA) is activated by ATP to form AA-AMP; 2) then transferred to the acceptor end of the tRNA</td>
<td></td>
</tr>
<tr>
<td>KARS, YARS, AARS</td>
<td>Charcot Marie Tooth (axonal)</td>
<td>catalyzes two-step reaction: 1) tyrosine is activated by ATP to form Tyr-AMP and 2) then transferred to the acceptor end of tRNA(Tyr). (or tRNA (Ala))</td>
<td></td>
</tr>
<tr>
<td>LARS2</td>
<td>Perrault syndrome</td>
<td>aminocacyl-tRNA editing activity and leucine-tRNA ligase activity</td>
<td></td>
</tr>
<tr>
<td>LETM1</td>
<td>Wolf-Hirschhorn syndrome (WHS)</td>
<td>maintains mitochondrial tubular shapes; required for normal mitochondrial morphology and cellular viability.</td>
<td></td>
</tr>
<tr>
<td>MARS2</td>
<td>(AR) Spastic Ataxia with Leukoencephalopathy (ARSAL)</td>
<td>functions as a monomer; predicted to localize to the mitochondrial matrix</td>
<td></td>
</tr>
<tr>
<td>Mfn2</td>
<td>CMT</td>
<td>mtDNA deletions</td>
<td>mediates mitochondrial fusion; clearance of damaged mitochondria via selective autophagy (mitophagy); acts as an</td>
</tr>
<tr>
<td>Protein</td>
<td>Description</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MGME (Ddk1, C20orf72)</strong></td>
<td>Upstream regulator of EIF2AK3 and suppresses EIF2AK3 activation under basal conditions. PEO, emaciation, respiratory failure</td>
<td>Single-stranded DNA (ssDNA) exonuclease involved in mitochondrial genome maintenance</td>
<td></td>
</tr>
<tr>
<td><strong>MPV17, POLG, C10orf2, DGUOK</strong></td>
<td>Hepatoencephalopathy; LA; hypoglaemia</td>
<td>MPV17: involved in mitochondria homeostasis; may be involved in the metabolism of ROS and control of oxidative phosphorylation and (mtDNA) maintenance</td>
<td></td>
</tr>
<tr>
<td><strong>MRPL12</strong></td>
<td>Growth retardation</td>
<td>Encodes a 39S subunit protein which forms homodimers; RNA binding and structural constituent of ribosome.</td>
<td></td>
</tr>
<tr>
<td><strong>MRPL3</strong></td>
<td>HCM, psychomotor retardation</td>
<td>Encodes a 39S subunit protein that belongs to the L3P ribosomal protein family.</td>
<td></td>
</tr>
<tr>
<td><strong>MRPL44</strong></td>
<td>HCM, pigmentary retinopathy, hemiplegic migraine, Leigh-like lesions, renal insufficiency, hepatopathy</td>
<td>Assembly/stability of nascent mitochondrial polypeptides exiting the ribosome</td>
<td></td>
</tr>
<tr>
<td><strong>MRPP1</strong></td>
<td>Progressive neurological abnormalities and cardiomyopathy (17β-Hydroxysteroid dehydrogenase type 10; HSD10 disease)</td>
<td>Functions in mitochondrial tRNA maturation; component of mitochondrial ribonuclease P, an enzyme composed of MRPP1/RG9MTD1, MRPP2/HSD17B10 and MRPP3/KIAA0391, which cleaves tRNA molecules in their 5'-ends</td>
<td></td>
</tr>
<tr>
<td><strong>MRPS5</strong></td>
<td>Agenesis of corpus callosum, dysmorphism, fatal neonatal lactic acidosis</td>
<td>I and IV multispecific organic anion transporter, with oxidized glutatione, cysteinyl leukotrienes</td>
<td></td>
</tr>
<tr>
<td><strong>MRPS7</strong></td>
<td>SNHL, hepatic and renal failure, LA</td>
<td>Encodes a 28S subunit protein involved in protein synthesis</td>
<td></td>
</tr>
<tr>
<td><strong>MRPS16</strong></td>
<td>Agenesis of corpus callosum, dysmorphism, fatal neonatal LA</td>
<td>I and IV encodes a 28S subunit protein that belongs to the ribosomal protein S16P family</td>
<td></td>
</tr>
<tr>
<td><strong>MRPS22</strong></td>
<td>Severe muscle hypotonia, marked lactic acidaemia and hyperammonaemia, HCM, tubulopathy, or Cornelia de Lange-like dysmorphic features, brain abnormalities and HCM</td>
<td>Combined encodes a 28S subunit protein</td>
<td></td>
</tr>
<tr>
<td><strong>MTFMT</strong></td>
<td>LS</td>
<td>I or Combined formylates methionyl-tRNA</td>
<td></td>
</tr>
<tr>
<td><strong>MTO1</strong></td>
<td>HCM, LA; isolated SNHL</td>
<td>Combined involved in the 5-carboxymethylaminomethyl modification (mnm(5)s(2)U34) of the wobble uridine base in mt tRNAs</td>
<td></td>
</tr>
<tr>
<td><strong>NARS2</strong></td>
<td>Alpers syndrome</td>
<td>Putative member of the class II family of aminoacyl-tRNA synthetases; plays a critical role in protein biosynthesis by charging tRNAs with their affiliated amino acids</td>
<td></td>
</tr>
<tr>
<td><strong>NDUFAB1</strong></td>
<td></td>
<td>Carrier of the growing fatty acid chain in fatty acid biosynthesis; accessory and non-catalytic subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase</td>
<td></td>
</tr>
<tr>
<td><strong>NDUF11</strong></td>
<td>Microphthalmia with linear skin defects syndrome</td>
<td>Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I)</td>
<td></td>
</tr>
<tr>
<td><strong>NDUFS1, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFSV</strong></td>
<td>LS</td>
<td>Core subunits of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I)</td>
<td></td>
</tr>
<tr>
<td><strong>NDUF2</strong></td>
<td>Cardiomyopathy and encephalopathy</td>
<td>Core subunits of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I)</td>
<td></td>
</tr>
<tr>
<td><strong>PARS2</strong></td>
<td>Alpers syndrome</td>
<td>Catalyses the ligation of proline to tRNA molecules</td>
<td></td>
</tr>
<tr>
<td><strong>PET100</strong></td>
<td>LS</td>
<td>Plays a role in the biogenesis of complex IV</td>
<td></td>
</tr>
<tr>
<td><strong>PNPLA8</strong></td>
<td>Mitochondrial myopathy, hypotonia, LA</td>
<td>Catalyses the hydrolysis of the sn-2 position of glycerophospholipids, PtdSer and to a lower extent PtdCho.</td>
<td></td>
</tr>
<tr>
<td><strong>PNPT1</strong></td>
<td>Chorioretinal defect, microcephaly, seizures, SNHL</td>
<td>Processing and polyadenylation of mt mRNAs</td>
<td></td>
</tr>
<tr>
<td><strong>POLG</strong></td>
<td>Alpers syndrome</td>
<td>Catalytic subunit of mt DNA polymerase. The encoded protein contains a polyglutamine tract near its N-terminus that may be polymorphic; Involved in mt DNA replication</td>
<td></td>
</tr>
<tr>
<td><strong>POLG, C10orf2, OPA1, SPG7, AFG3L2</strong></td>
<td>Ataxia neuropathy syndromes</td>
<td>OPA1: required for mitochondrial fusion and regulation of apoptosis; may form a diffusion barrier for proteins stored in mitochondrial cristae; putative role in mitochondrial genome maintenance; SPG7: involved in diverse cellular processes including membrane trafficking, intracellular motility, organelle biogenesis, protein folding, and proteolysis;</td>
<td></td>
</tr>
<tr>
<td><strong>Gene</strong></td>
<td><strong>MIM</strong></td>
<td><strong>Description</strong></td>
<td><strong>Function</strong></td>
</tr>
<tr>
<td>----------</td>
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<td>-----------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>AFG3L2</strong></td>
<td></td>
<td>ATP-dependent protease which is essential for axonal development</td>
<td></td>
</tr>
<tr>
<td><strong>POLG, PARS2, NARS2</strong></td>
<td></td>
<td>Alpers syndrome</td>
<td>NARS2: thought to catalyse the ligation of asparagine to tRNA molecules; PARS2: catalyse the ligation of proline to tRNA</td>
</tr>
<tr>
<td><strong>POLG, POLG2, C10orf2, SLC25A4</strong></td>
<td></td>
<td>Autosomal PEO</td>
<td>C10orf2: encodes a hexameric DNA helicase which unwinds short stretches of double-stranded DNA in the 5’ to 3’ direction and, along with mitochondrial single-stranded DNA binding protein and mtDNA polymerase gamma; key role in mtDNA replication; SLC25A4: catalyzes exchange of cytoplasmic ADP with mitochondrial ATP across MIM</td>
</tr>
<tr>
<td><strong>PTCD1</strong></td>
<td></td>
<td>Cardiomyopathy</td>
<td>Combined implicated in negative regulation of leucine tRNA levels, and negative regulation of mitochondria-encoded proteins and COX activity</td>
</tr>
<tr>
<td><strong>PUS1, YARS2</strong></td>
<td></td>
<td>Myopathy and sideroblastic anemia</td>
<td>Converts specific uridines to PSI in a number of tRNA substrates.</td>
</tr>
<tr>
<td><strong>QARS</strong></td>
<td></td>
<td>EOEE; progressive microcephaly with diffuse cerebral atrophy (+/- cerebellar), severely deficient myelination, intractable seizures, and developmental arrest</td>
<td>Catalyze the aminocacylation of tRNA by its affiliated amino acid</td>
</tr>
<tr>
<td><strong>RARS2</strong></td>
<td></td>
<td>Pontocerebellar hypoplasia/PEHO-like syndrome</td>
<td>Arginyl-tRNA synthetase; arginine-tRNA ligase activity</td>
</tr>
<tr>
<td><strong>RMND1</strong></td>
<td></td>
<td>Severe encephalopathy, LA, and intractable seizures; neonatal LA, infantile onset renal failure, deafness, severe myopathy, dysautonomia</td>
<td>Mitochondrial translation,? by coordinating assembly or maintenance of the mt ribosome</td>
</tr>
<tr>
<td><strong>RRM2B</strong></td>
<td></td>
<td>Hypotonia, LA, renal tubulopathy</td>
<td>Repairs damaged DNA in a p53/TP53-dependent manner; supplies deoxyribonucleotides for DNA repair in cells arrested at G1 or G2; forms an active ribonucleotide reductase (RNR) complex with RRM1 which is expressed both in resting and proliferating cells in response to DNA damage.</td>
</tr>
<tr>
<td><strong>SARS2</strong></td>
<td></td>
<td>Hyperuricemia, PAH, renal failure in infancy and alkalosis, HUPRA syndrome</td>
<td>Catalyses attachment of serine to tRNA(Ser); aminoacylate tRNA(Sec) with serine, to form misacylated tRNA L-seryl-tRNA(Sec)</td>
</tr>
<tr>
<td><strong>SCO1</strong></td>
<td></td>
<td>Hepatopathy and ketoacidosis</td>
<td>Plays a role in cellular copper homeostasis, mitochondrial redox</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Function/Action</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>SCO2</strong></td>
<td>Cardiomyopathy and encephalopathy</td>
<td>Signaling or insertion of copper into the active site of COX.</td>
<td></td>
</tr>
<tr>
<td><strong>SDHA</strong></td>
<td>LS or OA and ataxia</td>
<td>Involved in complex II of the mitochondrial electron transport chain; responsible for transferring electrons from succinate to ubiquinone; may act as a tumor suppressor.</td>
<td></td>
</tr>
<tr>
<td><strong>SDHAF1</strong></td>
<td>Leukodystrophy</td>
<td>Assembly of SDH; promotes maturation of the iron-sulfur protein subunit SDHB of the SDH catalytic dimer; may act together with SDHAF3.</td>
<td></td>
</tr>
<tr>
<td><strong>SERAC1</strong></td>
<td>LS, transient neonatal LA and hyperammonemia, SNHL, OA, dystonia, tubular aggregates</td>
<td>May catalyze the remodeling of phosphatidylglycerol and be involved in the transacylation-acylation reaction to produce phosphatidylglycerol-36:1; may be involved in bis-(monoacylglycerol)phosphate biosynthetic pathway.</td>
<td></td>
</tr>
<tr>
<td><strong>SLC25A3</strong></td>
<td>Cardiomyopathy, lactic acidosis (mt-phosphate carrier defect)</td>
<td>Transport of phosphate groups from the cytosol to the mitochondrial matrix; may play a role regulation of the mitochondrial permeability transition pore.</td>
<td></td>
</tr>
<tr>
<td><strong>SUCLA2</strong></td>
<td>LS with MMA and/or hypotonia, movement disorder</td>
<td>Provides instructions for making a beta subunit of succinate-CoA ligase; catalyzes the ATP-dependent ligation of succinate and CoA to form succinyl-CoA.</td>
<td></td>
</tr>
<tr>
<td><strong>SURF1, LRPPRC</strong></td>
<td>LS; LSFC</td>
<td><strong>SURF1</strong>: ? involved in biogenesis of the COX complex; <strong>LRPPRC</strong>: ? may play a role in cytoskeletal organization, vesicular transport, or transcriptional regulation of both nuclear and mitochondrial genes; may bind mature mRNA in the nucleus outer membrane.</td>
<td></td>
</tr>
<tr>
<td><strong>TACO1</strong></td>
<td>LS, dystonia and OA</td>
<td>Acts as a translational activator of mitochondrial-encoded cytochrome c oxidase 1.</td>
<td></td>
</tr>
<tr>
<td><strong>TARS2</strong></td>
<td>Axial hypotonia and severe psychomotor delay</td>
<td>Encodes mitochondrial aminoacyl-tRNA synthetase.</td>
<td></td>
</tr>
<tr>
<td><strong>TAZ</strong></td>
<td>Barth syndrome</td>
<td>Some isoforms may be involved in cardiolipin metabolism.</td>
<td></td>
</tr>
<tr>
<td><strong>TK2</strong></td>
<td>Spinal muscular atrophy/infantile myopathy</td>
<td>Phosphorylates thymidine, deoxycytidine &amp; deoxyuridine;</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Clinical Features</td>
<td>Mode of Action</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>TMEM70</strong></td>
<td>HCM, LA, 3-methylglutaconic aciduria; PAH</td>
<td><strong>V</strong> biogenesis of mt ATP synthase</td>
<td></td>
</tr>
<tr>
<td><strong>TRIT1</strong></td>
<td>Microcephaly, myoclonic epilepsy, global neurodevelopmental delay, Diabetes</td>
<td>Combined catalyses the transfer of a dimethylallyl group onto the adenine at position 37 of both cytosolic and mitochondrial tRNAs resulting in formation of N6-(dimethylallyl)adenosine ((6)A)</td>
<td></td>
</tr>
<tr>
<td><strong>TRMU</strong></td>
<td>Death at 3-4 months or reversible hepatopathy, LA (normal copy number)</td>
<td>Catalyses the 2-thiolation of uridine at the wobble position (U34) of mitochondrial tRNA(Lys), tRNA(Glu) and tRNA(Gln); required for the formation of 5-taurinomethyl-2-thiouridine (tm5s2U) of mitochondrial tRNA(Lys), tRNA(Glu), and tRNA(Gln) at the wobble position</td>
<td></td>
</tr>
<tr>
<td><strong>TSFM</strong></td>
<td>Encephalomyopathy, +/- concentric HCM, hepatopathy</td>
<td>CI, III, and IV induces the exchange of GDP to GTP; remains bound to the aminoacyl-tRNA.EF-Tu.GTP complex up to the GTP hydrolysis stage on the ribosome</td>
<td></td>
</tr>
<tr>
<td><strong>TUFM</strong></td>
<td>Leukodystrophy, microcephaly, polymicrogyria</td>
<td>Promotes binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis</td>
<td></td>
</tr>
<tr>
<td><strong>TYMP</strong></td>
<td>MNGIE</td>
<td>Catalyses the reversible phosphorolysis of thymidine.</td>
<td></td>
</tr>
<tr>
<td><strong>UQCRB</strong></td>
<td>Hypokalemia and LA</td>
<td>III component of the ubiquinol-cytochrome c reductase complex</td>
<td></td>
</tr>
<tr>
<td><strong>VARS2</strong></td>
<td>Microcephaly, epilepsy</td>
<td>I or Combined gene expression and tRNA aminoacylation</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: List of current known (and putative) pathogenic nuclear genes related to mitochondrial disease and associated clinical phenotypes.
1.5 DIAGNOSTIC APPROACHES TO MITOCHONDRIAL DISEASE

1.5.i Current diagnostic algorithms

Despite significant advances in our understanding of the molecular basis of mitochondrial diseases, identifying and confirming the diagnosis still remains a formidable challenge. Part of the difficulty is due to the expanding spectrum of clinical phenotypes; and the ever increasing recognition of causative genes.

Once the diagnosis is considered, then at present the investigations are often highly specialized and complex. Initial clinical evaluation documenting the personal and family history, using clinical investigations to document the extent of the phenotype remain pivotal to building the clinical evidence base in support of the diagnosis. This may lead to a specific phenotype or syndrome which implicates a specific gene defect (McFarland et al., 2010).

Often this is not the case, and the clinician must adopt a multidisciplinary approach, linking together information from clinical, histopathological, histochemical and biochemical testing to target molecular genetic analysis. Performance of a muscle biopsy often remains central to diagnostic algorithms of adult mitochondrial disease (McFarland et al., 2010) (Figure 11).
1.5.ii Future Diagnostic Algorithms

Currently, making a comprehensive diagnosis is only possible in approximately two thirds of patients thought to have mitochondrial disease. The implementation of next generation whole exome and whole genome sequencing over the next five years is likely to inordinately modify our diagnostic approach. However, interpreting the immense genetic diversity present in the exome and the genome will be challenging, and a biopsy may still be needed to prove that the underlying pathogenic variants are causative. Also, it is important to remember that, for mitochondrial DNA disorders, the molecular defect may not be detectable in a blood sample. Urinary epithelium and buccal mouth swabs may provide an alternative to an invasive procedure such as a muscle or liver biopsy for mtDNA analysis. However, 25 years after the discovery of the first genetic causes of mitochondrial disease, the diagnostic yield is still critically dependent on the meticulous clinical and biochemical characterisation of patients.
1.6 CURRENT TREATMENT AND PREVENTION STRATEGIES

1.6.i Clinical management

Unfortunately to date, there are few effective treatments and no known cures for mitochondrial diseases. Definitive pharmacological treatment for patients with mitochondrial disease, except for patients with deficiency of coenzyme Q10, is lacking. Non-pharmacological therapies that have been investigated include nutritional supplements, the ketogenic diet and exercise. Preventative strategies have gained much interest recently with the development of pioneering new in vitro fertilization (IVF) techniques.

1.6.ii Nutritional supplements

Standard doses of vitamin C and K, thiamine, and riboflavin are reported to be of conflicting benefit in isolated cases and open label studies; however ubiquinone (coenzyme Q10) may have a beneficial effect in patients with isolated Q10 deficiency (Horvath, 2012). To date, the largest, therapeutic, randomized, placebo, control-trial of idebenone in patients with LHON was performed by Klopstok and colleagues. Although this study failed to reach its anticipated primary end-points, supportive evidence of the therapeutic benefits of idebenone continues to mount (Klopstock et al., 2011).

1.6.iii Noxious metabolite removal

Several studies have specifically targeted the removal of noxious metabolites. Initial studies performed to buffer lactate with bicarbonate resulted in exacerbation of cerebral dysfunction (De Vivo and DiMauro, 1999). Dichloracetate has also been trialled to reduce lactic acid levels with noted improvement in cerebral metabolic parameters (De Stefano et al., 1995). However a follow-up clinical trial showed unacceptable side effects (a partially irreversible toxic neuropathy) (Kaufmann et al., 2006). For patients with mitochondrial neurogastrointestinal encephalopathy (MNGIE), initial studies of attempting to reduce thymidine by haemolysis proved fruitless (Yavuz et al., 2007). However, allogeneic hematopoietic stem cell transplantation (Schupbach et al., 2009; Halter et al., 2011; Filosto et al., 2012) and TPase enzyme replacement therapy appear more promising (Lara et al., 2006).
1.6.iv Ketogenic diet

The role of ketogenic diet in mitochondrial disease therapeutics remains controversial with limited robust evidence of efficacy. It may have a role in respiratory chain and PDH complex defects but further studies are warranted (Santra et al., 2004; Kang et al., 2007; Martikainen et al., 2012).

1.6.v Exercise and gene shifting

Both endurance and resistance training have been extensively investigated in relation to their postulated, mechanistic roles in provoking heteroplasmy change and gene shifting (Taivassalo et al., 1999; Taivassalo et al., 2003; Jeppesen et al., 2006; Murphy et al., 2008). Variability in responses to ‘shift’ in wild type and mutant mtDNA has been reported and larger studies in relation to exercise safety and dosing are still warranted.

1.6.vi Supportive care and surveillance

The chronicity and heterogeneity (Tein et al., 1993) both clinically and genetically of mitochondrial disease complicate the clinical care pathway of patients with mitochondrial disorders. Frequently patients require surveillance follow-up over their lifetime, which is often characterised by a multispecialty approach (neurologist, cardiologist, endocrinologist, nephrologists and ophthalmologists) and multidisciplinary approach (specialist nurses, physiotherapists, dieticians and speech therapists). This is often dictated by the clinical phenotype of the patient. Several supportive therapies have been investigated including antiepileptic efficacy in Alpers syndrome and POLG related seizures (Tein et al., 1993; Kollberg et al., 2006; Tzoulis et al., 2006) (that concomitantly identified mitochondrial disease as a risk factor for liver failure (Krähenbühl et al., 2000)); levodopa for POLG-related parkinsonism (Luoma et al., 2004), anti-depressants for mood disorders in all forms of mitochondrial disease (DiMauro et al., 2006); blood transfusion for anaemia related to Pearson’s syndrome (DiMauro et al., 2006) and solid organ transplantation related to either cardiac or liver failure (Bonnet et al., 2001; Santorelli et al., 2002; Bhati et al., 2005; Dimmock et al., 2008a) with variable efficacy.
1.6.vii Prevention of disease transmission

MtDNA is exclusively maternally-inherited and is present in multiple copies in all cells (Taylor and Turnbull, 2005b). The multicopy nature means that mtDNA mutations can either be homoplasmic (when all copies of mtDNA carry the mutation) or heteroplasmic (varying proportions of mutated and wild-type mtDNA) (Taylor and Turnbull, 2005a). All offspring of a woman with homoplasmic mtDNA mutation will unequivocally carry the mtDNA mutation. However, the situation with women carrying heteroplasmic mtDNA mutations is much more complicated (Grady et al., 2014). During the development of the oocyte there is a genetic bottleneck with a marked reduction in the copy number of mtDNA followed by a rapid expansion of mtDNA copy number during oocyte formation (Cree et al., 2008; Wai et al., 2008; Freyer et al., 2012). This means that some heteroplasmic mtDNA mutations (e.g. single, large scale mtDNA deletions) are rarely transmitted due to purifying selection (Stewart et al., 2008). Other heteroplasmic mtDNA mutations (e.g. the m.3243A>G MELAS and m.8344A>G MERRF mutations) are transmitted, but the developmental genetic bottleneck can lead to offspring being born with a range of different mtDNA mutation levels.

Current options available in the UK include ovum donation, pre-implantation genetic diagnosis (PGD) and prenatal diagnosis. The advantage of ovum donation is that there is no risk of transmitting the mtDNA disease. Disadvantages may include the shortage of available oocytes and the lack of maternal genetic relationship of any offspring; important issues to be considered by women harbouring potentially pathogenic mtDNA mutations. PGD and prenatal genetic diagnosis are currently offered in the UK to women with heteroplasmic mtDNA mutations and have the advantage of reducing the risk of severely affected offspring (Steffann et al., 2006). For prenatal diagnosis there is always the potentially difficult decision around termination of pregnancy and neither technique is likely to be of benefit for women with high levels of heteroplasmic mtDNA mutations, and of no benefit for women with homoplasmic mtDNA mutations.

1.7 Phenotyping

Phenotype (from the Greek phainein meaning to ‘show’ and typo, meaning ‘type’) is a composite of individual characteristic traits that result from the individual’s genotypic interaction with its environment (Wanscher, 1975; Arnold and Mayr, 1982). Despite major advances in our understanding of the molecular basis of mitochondrial diseases, making a definitive diagnosis still remains a formidable challenge. Part of this difficulty is identifying
patients with milder phenotypes; in addition to the expanding phenotypic spectrum. Once a diagnosis of mitochondrial disease is considered, meticulous documentation of the personal and family history, use of clinical investigations to document the extent of the phenotype are paramount to building the clinical evidence base in support of the diagnosis. The importance of this clinically, is that this may lead to a specific phenotype or syndrome that implicates a specific gene defect. If this is not the case, a systematic approach is employed, that, if appropriate, involves a biopsy of the affected tissue. This then leads to the histochemical and biochemical evaluation of mitochondrial function that can help target the genetic investigations. The implementation of next generation whole exome and whole genome sequencing will undoubtedly modify the diagnostic approach, in an outpatients setting. However, interpreting the huge genetic diversity in the exome and genome may prove challenging and diagnostic yield will still remain critically dependent on the fastidious clinical and biochemical characterisation of individuals with mitochondrial disease; aspects pertinent to several studies in this thesis.

1.8 Discussion

Mitochondrial diseases are a group of genetic disorders that may give rise to a conspicuous spectrum of clinical symptoms, in any organ or tissue, at any age, and with any mode of inheritance. Despite significant advances in our understanding of the molecular basis of mitochondrial diseases, definitive diagnosis remains a major challenge. This is in part due to the expanding phenotypic spectrum of such disorders, genotypic variability and the complexity of diagnostic investigations. To date, a definite diagnosis is only possible in approximately two thirds of patients with suspected mitochondrial disease. The advent of next generation sequencing proposes to revolutionise the diagnostic algorithm of mitochondrial disease; however targeted mutational analysis remains our current mainstay approach. Almost 25 years after the discovery of the first genetic causes of mitochondrial disease, diagnostic yield remains dependent on the meticulous clinical and biochemical characterisation of patients. Understanding the mechanistic relationship between genotype and phenotype may also prove pivotal to discovering an effective treatment; in a group of disorders with currently few effective treatments and no known cures.

In the studies presented here, I sought to further define the clinical and molecular understanding of mitochondrial DNA and nuclear DNA mutations in a well-characterised cohort of adult patients with suspected mitochondrial disease. I reviewed seminal papers that formed and shaped the evolution of Mitochondrial Medicine, investigated the pattern of
genotypic and phenotypic expression of defects of both the mitochondrial and nuclear genomes and evaluated aspects pertinent to the success of future therapeutic studies including appreciation of disease frequency and assessing patient-centred symptom severity and outcomes.

In the first study I was invited to co-author a book chapter in a forthcoming book entitled ‘Landmark papers in Neurology’, in which my co-author and I were asked to select and critique ten seminal papers that we thought epitomised mitochondrial medicine, since its inception. Our given remit was that the chapter and chosen manuscripts were to be clinically focussed and targeted toward the general neurologist. Firstly, we chronicled the discovery of the morphology and function of mitochondria, and the increasing recognition of human diseases associated with primary and secondary mitochondrial dysfunction. We reviewed the initial description of mitochondrial disease, so-called, Luft’s disease (1962) (Luft et al., 1962), in a 35 year old woman with hypermetabolic syndrome that heralded the advent of mitochondrial medicine. Over five decades later, despite the expeditious advances in genetic analysis, the molecular basis of this disease remains elusive. Over the ensuing decades, there was an exponential growth in the number of reports of multisystem patient syndromes, purportedly related to mitochondrial dysfunction that coincided with the rapid expansion of human chemical pathology and the complete sequencing of human mitochondrial DNA (Anderson et al., 1981). These advances defined the premolecular era of mitochondrial medicine and culminated in the publication of our second selected seminal manuscript. In 1986, John Morgan Hughes and colleagues, in London, meticulously described a large case series of patients with mitochondrial disease. This exacting characterization of the clinical features of 66 patients with histologically-defined mitochondrial disease supported the concept of a varied, overlapping spectrum of diseases as opposed to distinct clinical entities only, which 35 years later, has been vindicated by the molecular portrayal of mitochondrial disorders (Petty et al., 1986). We then recount pivotal steps in the evolution of the molecular era, including the discovery of, the first pathogenic mutation in mitochondrial DNA (Holt et al., 1988a); the first pathogenic mtDNA point mutation (Wallace et al., 1988a) and the first description of Mendelian inheritance of a mitochondrial disorder, in relation to a new group of mtDNA maintenance disorders characterized by muscle restricted mtDNA deletions (Zeviani et al., 1989). Furthermore we detailed the identification of the genetic basis of the most common form of mitochondrial disease, namely a heterogenic point mutation in the tRNA\textsubscript{Leu(UUR)} gene, in mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) (Goto et al., 1990); followed by the identification of the most common nuclear-
mitochondrial disease gene encoding mitochondrial polymerase gamma (POLG) (Van Goethem et al., 2001b).

These rapid developments in our understanding of the genetic basis of mitochondrial disorders, by the year 2000, had paved the way for several studies that attempted to estimate the prevalence of disease; no mean feat given the clinical heterogeneity of these disorders. We chose to critique two cardinal papers evaluating the prevalence of mitochondrial diseases; which may reflect our groups’ biases, in terms of historical research interests (Majamaa et al., 1998; Chinnery et al., 2000a). Accurate mitochondrial disease epidemiology encounters many additional challenges including variable genotype-phenotype correlates, expanding and diverse clinical features, the complex nature of referral pathways, population founder effects and genetic bottlenecks that may result in under or over representation of specific mtDNA (Macmillan et al., 1998) or nuclear disorders (Skladal et al., 2003). The first epidemiological studies were of a single pathogenic mtDNA mutation associated with discrete clinical phenotypes. These studies reported a widely variable frequency of m.3243A>G in diabetes ranging from 0.13% to 60%; with such discrepancies most likely due to study design and population under investigation (Kadowaki et al., 1994; Newkirk et al., 1997). In 1998, the first population based study of a single pathogenic mutation was carried out northern Ostrobothnia in Finland (Majamaa et al., 1998). Majamaa et al. estimated a minimum point prevalence of 16.3/100,000 (95% CI=11.3–21.4/100,000) (equivalent to one in 6135). This was soon followed two years later by a population based study of all forms of mitochondrial DNA disorders (Chinnery et al., 2000a). This main study estimated that 12.48/100,000 (equivalent to one in 8013) either had mitochondrial disease or were at risk of developing mitochondrial disease. The discrepancies between both studies may have, in part, been due to study design and, in part, due to the makeup of the study populations. Subsequent work by this group (Schaefer et al., 2008), and a study presented in this thesis (Gorman et al., 2015e), would suggest the original prevalence figures were a gross under estimation, with a revised adult mitochondrial disease prevalence closer to one in 5,000 for mtDNA disease and one in 4300 when including pathogenic mutations of both the mitochondrial and nuclear genomes.

We then sought to appraise, the first large, therapeutic, randomized, placebo, control-trial of idebenone in patients with LHON performed by Klopstok and colleagues (Klopstock et al., 2011). Lessons gleaned from this study included review of the validity and appropriateness of the peremptory, primary end-points, which this study failed to achieve in the first instance and which only achieved statistical validity following post hoc interaction analysis of all secondary end-points. Given the inherent spurious nature and serious limitations of statistical post-hoc
analysis, we would advise caution in the authors’ interpretation that patients with discordant visual acuities at baseline, showed a difference in response to Idebenone.

We finalised this book chapter by highlighting another pivotal paper that has framed our current understanding of the genetic basis of mitochondrial disease with the advent of new molecular techniques that hold potential to revolutionize the diagnosis of mitochondrial disorders, from a genetic perspective (Calvo et al., 2012). However, reminiscent of the premolecular era, we would suggest that irrespective of the diagnostic yield of these new technologies, the accepted diagnostic algorithm is still intimately dependent on the meticulous clinical and molecular phenotyping of patients with mitochondrial disease.

In several studies presented in this thesis, I provide additional evidence of the benefits of deep phenotyping of patients on diagnostic yield. In summary, I have shown that a targeted gene approach may be implemented in the diagnosis of adult patients with mtDNA maintenance disorders manifesting with progressive external ophthalmoplegia (PEO) and additional clinical features. Of the known maintenance genes, ten had been associated with PEO (POLG, POLG2, SLC25A4, C10orf2, RRM2B, TK2, MFN2, OPA1, MGME1 and DNA2), at the outset of this thesis. To better understand the phenotypic and genotypic heterogeneity of adult-onset PEO associated with disordered mtDNA maintenance, I firstly evaluated the distinct clinical and molecular characteristics of mutations in the nuclear-encoded mitochondrial maintenance gene RRM2B in 31 adult patients with mitochondrial disease causing PEO, characterised by a mitochondrial DNA maintenance defect in muscle (Pitceathly et al., 2012). Despite notable clinical overlap between the mitochondrial maintenance genes, I identified salient clinical features including hearing loss, bulbar dysfunction and gastrointestinal disturbance that may help prioritise genetic analysis towards the RRM2B gene. These findings were prompted by a previous short report (Fratter et al., 2011) from a collaboration between Newcastle and Oxford, and subsequent review publications that I performed critiquing the clinical spectrum of RRM2B-related mitochondrial disease (Gorman and Taylor, 2014). In a further two studies, after excluding known mitochondrial maintenance gene defects, a range of sequencing and molecular biological techniques were employed to study a large cohort of 68 adult patients with PEO either with and without multiple mtDNA deletions in skeletal muscle. This led to the identification of autosomal recessive (nine patients) and autosomal dominant (six patients) mutations in the SPG7 gene encoding paraplegin (Pfeffer et al., 2014) and novel autosomal dominant mutations in the AFG3L2 gene, typically associated with spinocerebellar ataxia type 28 (SCA28) (two patients) (Gorman et al., 2015d). These findings combined, suggested the emergence of clinical features that in addition to careful documentation of family history, may help formulate a simple diagnostic algorithm (Figure 12) in adult-onset Mendelian PEO
associated with mitochondrial disease. The presence of optic atrophy and early onset visual loss would prioritize genetic testing towards OPA1 analysis; prominent features of parkinsonism and/or sensory neuronopathy would prioritize genetic testing towards POLG analysis; prominence of hearing loss, bulbar dysfunction and gastrointestinal disturbance would prioritize genetic testing towards RRM2B analysis; isolated PEO with or without mild proximal myopathy and exercise intolerance would prioritize genetic testing towards PEO1 analysis; a dystrophic muscle phenotype with prominence of respiratory failure would prioritize genetic testing towards TK2 analysis; whilst a complex neurological phenotype characterised by spastic ataxia would prioritize genetic testing towards either SPG7 or AFG3L2 analyses.

**Figure 12**: Diagnostic algorithm in adult-onset Mendelian PEO associated with mitochondrial disease

*RRM2B* is a gene involved not only in mtDNA replication but also in nuclear DNA repair. During external review of one of the studies presented in this thesis that chronicled the distinct clinical and molecular characteristics of adults with *RRM2B*-related mitochondrial disease (Pitceathly et al., 2012), we were asked by one of the reviewers to comment on any clues suggesting abnormalities in DNA repair in the RRM2B mutant patients. We suspected that the reviewer was alluding to the association between p53R2/RRM2B and cancer and revised the
final manuscript accordingly, to take account of this point raised (Discussion, paragraph 3 of the manuscript). Notably, two patients developed solid tumours: oral and breast carcinoma. We concede our numbers were small and no mutational analysis of tumour tissue was performed, hence no definitive causal association could be inferred. Similarly, we endeavoured to highlight the conspicuous absence of proximal renal tubulopathy in adults with RRM2B-related mitochondrial disease, a common finding in children with RRM2B mutations and mtDNA depletion. In these adult-onset cases, the nature of renal involvement was primarily glomerular in nature.

Isolated human complex I (CI) deficiency is the most frequent respiratory chain defect reported in mitochondrial disease, exhibiting conspicuous genotypic and phenotypic heterogeneity. In less than half of patients, the genetic basis of the enzyme deficiency is known. I describe the detailed clinical, physiological, biochemical and molecular characterisation of two patients, who presented only in their twenties and whose clinical pictures were dominated by progressive exercise intolerance and severe isolated mitochondrial complex I deficiency in muscle, which I show are due to novel heteroplasmic mutations in the mitochondrial DNA-encoded MTND1 gene. I present their distinctive clinical features that exemplify the importance of serum lactate testing in cases of persistent unexplained exertional weakness or dyspnoea. In addition, together with colleagues, I characterise their VO2 kinetics during graded cardiopulmonary exercise testing, assess in vivo mitochondrial function using phosphorous spectroscopy and evaluate the molecular mechanisms underlying this purely muscular phenotype to elucidate the structural consequences of both mutations on complex I biogenesis. We demonstrate for the first time, that mitochondrial supercomplex reorganisation occurs as a response to a compensatory mechanism to extricate the clinical phenotype involving upregulation of complex I assembly factors; a phenomenon not previously reported with MTND1 mutations (Gorman et al., 2015a).

Following a previous study describing the phenotypic heterogeneity of the 8344A>G mtDNA "MERRF" mutation (Mancuso et al., 2013), I present a fascinating case, expanding the clinical spectrum associated with this gene (Blakely et al., 2014). The patient presented with marked facial and distal muscle weakness and respiratory failure, with a muscle biopsy showing evidence of severe COX deficiency. The marked mitochondrial histochemical abnormalities with a phenotype strongly suggestive of a limb girdle muscular dystrophy, were more suggestive of a rare, mild mt-tRNA mutation, exhibiting high mutation threshold hence initial genetic testing was directed to whole mtDNA genome sequencing in preference to targeted m.8344A>G mutation analysis. This case illustrates that the m.8344A>G mutation can cause
indolent distal weakness with respiratory failure, with marked histochemical defects in muscle and hence extends the evolving phenotypic spectrum attributable to the m.8344A>G “MERRF” mutation.

Now with the increasing shift in emphasis from diagnosis to the development of therapeutic pharmacological and prevention strategies, I sought to assess important aspects pertinent to this. Recent advances in diagnostic techniques, streamlining of referral pathways in the United Kingdom and fastidious family tracing have permitted, for the first time, recording of the minimum prevalence of adult mitochondrial disease, to include pathogenic mutations in both mitochondrial and nuclear DNA. The included prevalence paper (Gorman et al., 2015e) describes the minimum point prevalence for all mitochondrial DNA mutations as 1 in 5,000; consistent with our previously published and highly cited work in Annals of Neurology. However, in addition, I have now evaluated the prevalence of nuclear mutations to be responsible for overt mitochondrial disease with a minimum point prevalence of 2.9 in 100,000 adults. Combined these data suggest that mitochondrial disorders, including pathogenic mutations of both the mitochondrial and nuclear genomes, are a common form of inherited neuromuscular disease ($\approx$ one in 4300). I propose that these findings are fundamental to assessing current interventions, providing evidence-based health policies and planning future services. This is perhaps most pertinent at present because of the development of new IVF based techniques (pronuclear and metaphase II spindle transfer) that hold potential to prevent the transmission of mitochondrial DNA disease and thus significantly reduce patient and societal disease burden.

The Wellcome Trust Centre for Mitochondrial Research in Newcastle has pioneered some of these techniques, however, before these techniques could be used in the UK, a change in the Human Fertilisation and Embryology Act was required. At the time of writing this thesis, the UK Parliament was due to debate these regulations and if passed this would represent the first legislation of its kind in Europe. Central to the parliamentary debate was how many women could potentially benefit from the development of either pronuclear or metaphase II spindle transfer. This was a challenging question because of the unique genetics of mitochondrial DNA disease and the clinical heterogeneity of these diseases. I undertook an observational cohort study (Gorman et al., 2015c) to address this issue and estimated that there is a minimum of 2,472 women with pathogenic mitochondrial DNA mutations in the UK of childbearing age that are at risk of transmitting serious mitochondrial disease to their offspring, which equates to 152 pregnancies per year. These figures translate to 12,423 women of childbearing age with pathogenic mitochondrial DNA mutations and 778 estimated live births per year involving potential transmission of serious disease in America. Our findings have considerable
implications not only for the UK and US, but also for other jurisdictions throughout Europe, and indeed Australia that are considering these techniques, pending the UK parliamentary decision. These data will facilitate projection of future averted healthcare costs, in these countries, in terms of determining the number of women requiring provision of, or reproductive advice on, these new IVF techniques offsetting the cumulative lifetime, healthcare costs of managing such debilitating genetic disorders.

The molecular basis of many of the common mitochondrial disorders has been elucidated over the last decade and although there is a vast spectrum of phenotypic expression throughout different genotypes, common symptoms are reported. Perceived fatigue is a prominent symptom in patients with mitochondrial disease but to date, its prevalence, severity and aetiology is poorly understood. My aim was to determine the prevalence and nature of perceived fatigue in a large, genetically heterogeneous group of patients with mitochondrial disease and systematically assess potential covariates of fatigue compared to healthy controls and patients with Myalgic Encephalopathy /Chronic Fatigue Syndrome (Gorman et al., 2015b). I demonstrate for the first time, that clinically relevant fatigue is common and often severe in patients with mitochondrial disease irrespective of age, gender or genotype. Sleep impairment can readily be distinguished from perceived fatigue arising as a primary manifestation of mitochondrial disease whilst there is a more complex association between perceived fatigue and mood disorders, warranting further assessment. The challenge now, is to identify causal factors that may help direct tailored pharmacological and non-pharmacological symptomatic therapeutic strategies; with potential for a shared therapeutic paradigm with patients with other chronic neurological disorders, exhibiting clinically relevant fatigue.

Health related quality of life (HRQOL) is important for understanding the impact and progression of chronic disease. However, there is a need to develop disease-specific HRQOL measures that focus on the characteristic symptoms of a certain disease or condition and their impact. We sought to present the conceptualisation, development and preliminary psychometric assessment of a mitochondrial disease-specific health related quality of life measure: the Newcastle Mitochondrial Quality of life measure (NMQ) (Elson et al., 2013). The item validation processes resulted in the removal of 40 items, including three whole domains that included stroke, seizures and work. The final questionnaire consisted of 63 items within 16 unidimensional domains. As many patients with severe clinical phenotypes of mitochondrial diseases suffer from intractable seizures or recurrent strokes or are unable to work due to their disease, it was surprising to know that the three domains (stroke, seizures and work domains) of the initial pilot questionnaire did not show adequate construct validity or internal consistency reliability and were therefore excluded from NMQ. Although the initial bias was to
include domains that were considered from a physician-centred perspective as relevant to a patient’s quality of life such as stroke and seizures; repeatedly these aspects were not considered important to the patient group as a whole. This may reflect the low prevalence of strokes (8%) and seizures (12%) within the cohort and the genotypic-phenotypic specificity of such symptoms; aspects that are critiqued in the final manuscript. This study is, to my knowledge, the first to devise a disease-specific, patient-centred quality of life tool. We propose to perform further psychometric assessment and revision of NMQ, with a follow-up multicentre analysis, that is currently underway.

Conclusion

Studies presented in this thesis sought to further define our clinical and genetic understanding of mtDNA and nDNA mutations in adult patients with suspected mitochondrial disease. I have demonstrated a simple diagnostic algorithm that may help prioritise gene analysis in cases of adult–onset PEO delineated by skeletal muscle restricted multiple mtDNA deletions. I concede that evaluations of the underlying molecular mechanisms, as presented are extremely preliminary and further in-depth analysis is warranted, particularly in relation to mutations in both the SPG7 and AG3FL2 genes. I present two further studies that exemplify the diversity of clinical expression of firstly a relatively common mtDNA mutation that extends the clinical phenotypic spectrum of m.8344A>G-related mitochondrial disease; and secondly another study of two patients with novel mutations in MTND1 with unique clinical symptoms characteristic of marked exercise intolerance related to complex I deficiency. I have shown that fatigue is highly prevalent and debilitating in patients with mitochondrial disease irrespective of genotype and have developed a disease specific quality of life scale that demonstrates good internal reliability and construct validity. Such detailed deep clinical and symptomatic phenotypic profiling will serve to improve more timely and accurate diagnosis; will aid development of a stratified selection approach at targeting the most appropriate patients and patient-centred outcome measures in future studies particularly therapeutic interventions.

Future work proposed includes revisiting the clinical phenotypes of other nuclear genes including C10orf2-related mitochondrial disease, as a multicentre collaboration; further evaluation of the underlying compensatory mechanisms in one of the patient’s with complex I deficiency following a pharmacological trial of an ‘off-label’ medication combined with an exercise intervention program; further deep clinical phenotyping of organ specific systems (including cardiac and gastrointestinal) in patients harbouring the m.3243A>G mutation and evaluation and validation of other patient-centred outcome measures that may serve useful in
future intervention studies, as the Wellcome Trust Centre for Mitochondrial Research prepares a programme for the next phase of Mitochondrial Medicine, that involves the evaluation and discovery of effective therapeutic strategies.
Chapter 2. Aims and scope
2.1 Aims and scope

The overall aims of this thesis were to further define the clinical and molecular understanding of mtDNA and nDNA disorders by deep phenotyping well-characterized cohorts of adult patients with suspected mitochondrial disease; to revisit measures of disease frequency and burden; and due to the lack of relevant, consistently applied functional outcome measures in trials involving this patient population, we sought to devise a disease-specific quality of life scale (Newcastle Mitochondrial Quality of life measure (NMQ)) and validate an outcome measure that is relevant to both clinicians and patients (Fatigue Impact Scale) as part of a ‘trial readiness’ programme (Figure 13).

Figure 13: Diagrammatic algorithm of thesis overview.
2.2 Seminal papers in Mitochondrial Medicine

I first critiqued seminal papers, (co-authored with Professor Patrick Chinnery) that personify landmark discoveries in the development of Mitochondrial Medicine, from the initial reports of a mitochondrial disorder, to the present day, with the advent of next generation sequencing. In this body of work, I sought to evaluate the merits of fastidious clinical and biochemical characterization of patients in the molecular era; and their contribution to current diagnostic yield.

2.3 Pathogenic nuclear DNA mutations in adult mitochondrial disease

I initially endeavoured to meticulously collate the molecular and clinical characteristics of adult patients with both genetically determined and suspected mitochondrial disease (genetically undetermined). These patients had either attended our specialist clinic or been referred for opinion to our diagnostic laboratory in Newcastle. This facilitated collaboration with other research centres to evaluate mutation frequency, clinico-pathological features and identification of known genes not previously associated with mitochondrial disease.

Firstly, recent advances in the identification of nuclear genes associated with both paediatric and adult-onset mitochondrial disease afforded me the opportunity to review the clinical spectrum of disease associated with mutations in the nuclear-encoded mitochondrial maintenance gene, Ribonucleotide reductase M2B (TP53 inducible) (*RRM2B*). Clinical manifestations of *RRM2B* mutations were recognised to range from a rapidly fatal infantile neuromuscular syndrome with renal tubular insufficiency (Bourdon *et al.*, 2007; Bornstein *et al.*, 2008; Kollberg *et al.*, 2009) to a progressive external ophthalmoplegia presenting in the second decade or adult life (Tyynismaa *et al.*, 2009). In a multicentre study, we sought to clearly define, for the first time, the clinico-pathological characteristics of adults with *RRM2B*-related mitochondrial disease and establish genotype–phenotype correlations. These findings formed the basis of two subsequent reviews on *RRM2B*-related mitochondrial disease.

Secondly, with the implementation of new diagnostic approaches, and after excluding known maintenance disorder genes, whole exome sequencing, followed by targeted Sanger sequencing and multiplex ligation-dependent probe amplification analysis were used to revisit a well characterised cohort of adult patients with progressive external ophthalmoplegia (PEO)
either with or without multiple mitochondrial DNA deletions in skeletal muscle, presenting in mid-adult life. This led to the identification of two novel genetic causes of PEO associated with muscle-restricted multiple mtDNA mutations and facilitated the interrogation of disease mechanisms.

2.4 Clinical and molecular correlates of mtDNA-related mitochondrial disease

Whole mtDNA genome sequencing in preference to targeted gene approach was implemented in three fascinating cases, to determine the genetic basis of the mitochondrial disorder. The first patient presented with marked facial and distal muscle weakness and respiratory failure, with a muscle biopsy showing evidence of severe COX deficiency. The marked mitochondrial histochemical abnormalities with a phenotype strongly suggestive of a limb girdle muscular dystrophy were more suggestive of a rare, mild tRNA mutation, exhibiting high mutation threshold hence initial genetic testing was directed to whole mtDNA genome sequencing. Our findings expand the clinical spectrum associated with a known causative gene (m.8344A>G) and further define our clinical and molecular understanding of mtDNA-related disease.

We then sought to detail the clinical, physiological, biochemical and molecular characterisation of two patients whose clinical pictures were dominated by progressive exercise intolerance and severe isolated mitochondrial complex I deficiency in muscle due to novel heteroplasmic mutations in the mitochondrial DNA-encoded MTND1 gene. We characterise VO$_2$ kinetics during graded aerobic exercise, assess in vivo mitochondrial function using phosphorous spectroscopy and evaluated the molecular mechanisms underlying this purely muscular phenotype to elucidate the structural consequences of both mutations on complex I biogenesis.

2.5 Epidemiology of adult mitochondrial disease

Recent advances in diagnostic techniques, streamlining of referral pathways in the United Kingdom and fastidious family tracing may facilitate recording of the total prevalence of adult mitochondrial disease, including pathogenic mutations of both the mitochondrial and nuclear genomes.
In the first instance, I identified adult cases (>16 years old) with suspected mitochondrial disease following referral to a single specialist mitochondrial centre in a defined geographical region. Those adults with pathogenic mtDNA or nuclear DNA mutations, or pathological multiple mtDNA deletions evident in muscle by at least two techniques (long range PCR, Southern or real-time PCR) and no evidence of other muscle pathology; and in whom clinical and biochemical features (>4% COX deficient fibres) were consistent with mitochondrial disease and alive at the mid-year period of 2011, were included. Comprehensive pedigree tracing from all affected individuals, to define individuals at risk for development of mitochondrial disease was undertaken, allowing me, for the first time, to record the minimum prevalence of adult mitochondrial disease, to include pathogenic mutations in both mitochondrial and nuclear DNA. These figures will hold important implications for the evaluation of therapeutic interventions and provision of future services.

Employing similar methodological techniques of case ascertainment in the same defined geographical region, I sought to establish the number of children born each year in the UK with potentially serious mitochondrial disease to women of child bearing age harbouring pathogenic mtDNA mutations. Using fertility data (live births per 1,000 person-years) obtained from the MRC Mitochondrial Disease Cohort UK, we assessed whether fertility is affected in pathogenic mtDNA mutation carriers. Additionally, we estimated the national prevalence of women with potentially inheritable mtDNA mutations, and used these data together with the most recent national total fertility rate (2013) to estimate the number of pregnancies per year. These findings are particularly relevant at present with the development of new IVF based techniques proposed to prevent the transmission of serious maternally-inherited mtDNA disease and with potential to shape legislative change.

### 2.6 Disease impact and patient reported outcomes

Although there is a vast spectrum of phenotypic expression throughout different genotypes, common symptoms are reported. Consistently, in the clinical setting and during patient focus group workshops, fatigue has been ranked the most common, debilitating symptom amongst our patients with mitochondrial disease, and is reported as an often neglected aspect of the disorder. I sought to determine the magnitude and severity of self-perceived fatigue in patients with mitochondrial disease, whilst evaluating putative biological mechanisms that have been recognised in other neurological disorders and chronic disease states. These findings will have important implications for future prioritisation of the evaluation of patient-centred therapies.
and targeting of pharmacological interventions in a condition with few effective treatments and no known cure.

Lastly, health related quality of life (HRQOL) is increasingly recognised as a fundamental patient-based outcome measure in both clinical and research settings. Generic outcome measures such as the SF-36 have been extensively validated to assess HRQOL across populations and different disease states. However, it is acknowledged that not all relevant aspects of a specific disorder may be captured, due to their inclusive construct. Hence there is a need to develop disease-specific HRQOL measures that focuses on the characteristic symptoms and impact of a specific disease. I sought to undertake the initial conceptualisation, development and preliminary psychometric assessment of a mitochondrial disease-specific HRQOL measure (Newcastle Mitochondrial-Quality of life measure (NMQ)).
Chapter 3. Introduction to Mitochondrial Medicine
3.1 Landmark Papers in Mitochondrial Medicine

Chapter 4. Clinical and molecular aspects of adult mitochondrial disease, due to pathogenic mutations in nuclear DNA
4.1 **Adults with RRM2B-related mitochondrial disease have distinct clinical and molecular characteristics**


4.2 **Mitochondrial disorders caused by Nuclear Genes; RRM2B-Related Mitochondrial Disease**


4.3 **GeneReview: RRM2B-Related Mitochondrial Disease**


4.4 **Clonal expansion of mtDNA mutations modulate SCA28 phenotype**

4.5 Mutations in *SPG7* cause chronic progressive external ophthalmoplegia through disordered mtDNA maintenance


4.6 Adult-onset Mendelian PEO Associated with Mitochondrial Disease

Chapter 5. Clinical and molecular aspects of

mtDNA-related mitochondrial disease
5.1 **Distal weakness with respiratory failure caused by the m.8344A>G “MERRF”**


5.2 **Novel MTND1 mutations cause isolated exercise intolerance, complex I deficiency and increased assembly factor expression**

**Gorman, Grainne S.**, Emma L. Blakely, Hue-Tran Hornig-Do, Helen AL Tuppen, Laura C. Greaves, Langping He, Angela Baker et al. "Novel MTND1 mutations cause isolated exercise intolerance, complex I deficiency and increased assembly factor expression. **Clinical Science**. 2015; DOI:10.1042/CS20140705
Chapter 6. Prevalence of adult mitochondrial disease
6.1 Prevalence of nuclear and mtDNA mutations related to adult mitochondrial disease


6.2 Mitochondrial Donation: How many women could benefit?

Chapter 7. Impact of mitochondrial disease
7.1 Prevalence and causal factors of perceived fatigue in mitochondrial disease


7.2 Initial development and validation of a Mitochondrial Disease quality of life scale

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