Molecular Genetic Studies of Inherited Cystic Kidney Disease in Oman

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Abstract

Inherited kidney diseases are fundamental causes of chronic kidney disease (CKD) and end stage kidney disease (ESKD); accounting for approximately 20% of all CKD cases and up to 10% of adults and over 70% of children reaching ESKD.

Oman is the second largest country in the South East of Arabian Peninsula. Omani population is characterized by large family size, presence of tribal and geographical settlements and higher rates of consanguineous marriages, which facilitate the study of autosomal recessive disorders. Rare genetic disorders create considerable burden on healthcare system in Oman and are major causes of congenital abnormalities and perinatal deaths in hospitals. The prevalence of inherited kidney disease was estimated to be high, but there is a lack for a comprehensive data. Therefore, this study aimed to evaluate the magnitude of inherited kidney disease in this population and identify the molecular genetic causes of inherited cystic kidney diseases in Omani patients. First, I performed a population-based retrospective analysis of ESKD patients commencing RRT from 2001 to 2015 using the national renal replacement therapy (RRT) registry and evaluated the epidemiological and etiological causes of ESKD with focused attention on inherited kidney diseases. Second, I designed a targeted gene panel (49 genes) and used massive parallel sequencing technologies for the molecular genetic diagnosis of cystic kidney disease in 53 patients. An overall molecular genetic diagnostic yield of 75% was achieved; with 46% of detected causative variants were novel genetic findings. Third, I evaluated the utility of molecular genetic testing in patients with autosomal recessive polycystic kidney disease (ARPKD) and described the clinical and genetic profile of this cohort. Finally, whole exome sequencing (WES) was used to determine the genetic causes of CKD in 11 unrelated children suspected with recessively inherited kidney diseases. Definite genetic diagnosis was achieved in 54.5% of cases, reflecting the importance of genomic implications in those with uncertain aetiology causing CKD. This study creates a solid basis reflecting the genotype-phenotype of some inherited kidney diseases in Omani population and reveals the enormous diagnostic power of genomic technologies.

Dedications

This thesis is dedicated to the noblest, dearest and the most beloved of men, to the one who placed education on his most important priority and honoured me with this scholarship, **His Majesty Sultan Qaboos bin Said bin Taimur**, may God rest his soul in peace. Your Majesty, you are the one who pave the way for us to reach the highest levels of knowledge and you deserve to be honoured with these accomplishments.

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Abbreviations

aa	Amino acid
ACMG	American College of Medical Genetics and Genomics
ADPLD	Autosomal dominant polycystic liver disease
ADPKD	Autosomal dominant polycystic kidney disease
ALMS	Alström syndrome
ARPKD	Autosomal recessive polycystic kidney disease
bp	Base pair
BBS	Bardet-Biedl syndrome
BQ	Base quality
CADD	Combined Annotation Dependent Depletion
CAKUT	Congenital anomalies of the kidney and urinary tract
CDG1A	Congenital disorder of glycosylation type 1a
CED	Cranioectodermal dysplasia
CKD	Chronic kidney disease
CMD	Corticomedullary differentiation
CNS	Central nervous system
CNVs	Copy number variants
CTGA	Catalogue for Transmission Genetics in Arabs database
dbSNP	Single nucleotide sequence polymorphism
DDR	DNA damage response
DPM	Ductal plate malformation
DZIP1L	DAZ-interacting zinc finger protein 1-like protein
ECD	Enrichment Control DNA
EDTA	Ethylenediaminetetraacetic acid
ESKD	End stage kidney disease
ESP	Exome sequencing project
EV	Extracellular vesicles
EVC	Ellis-van Creveld syndrome
ExAC	Exome Aggregation Consortium
FC	Fibrocystin
gDNA	Genomic DNA
gnomAD	Genome Aggregation Database
GANAB	α-glucosidase neutral AB
GC	Guanine-cytosine
GERP	Genomic Evolutionary Rate Profiling
GPCRs	G-protein-coupled receptors
GNPs	Granule neurons precursors
GQ	Genotype quality
GRCh37	Genome Reference Consortium Human Build 37
GRCh38	Genome Reference Consortium Human Build 38
HANAC	Hereditary angiopathy with nephropathy, aneurysms and muscle cramps
HGMD	Human Genome Mutation Database

HGP	Human Genome Project
HIPKD	Hyperinsulinemic hypoglycemia and polycystic kidney disease
HNF1B	Hepatocyte nuclear factor 1β
HPO	Human Phenotype Ontology project
hUREC	Human urine-derived renal epithelial cells
IFT	Intraflagellar transport
IEM	Illumina Experiment Manager
IS	Inner segment
JATD	Jeune Asphyxiating Thoracic Dystrophy
JBTS	Joubert syndrome
Kbp	Kilo base pair
KS	Kartagener syndrome
LCA	Leber Congenital Amaurosis
LOVD	Leiden Open Variation Database
LR-	Left-right
LR-PCR	Long range PCR
LRT	Likelihood Ratio Test
MAF	Minor allele frequency
MKS	Meckel–Gruber syndrome
MOC	Microtubule-organizing centre
MOH	Ministry of Health
MOI	Mode of inheritance
MQ	Mapping quality
MRI	Magnetic resonance imaging
MZSDS	Mainzer-Saldino syndrome
NGS	Next generation sequencing
NPHP	Nephronophthisis
NPHP-RC	NPHP-related ciliopathies
NURTuRE	National Unified Renal Translational Research Enterprise
OFD1	Oral-Facial-Digital syndrome Type 1
OMIM	Online Mendelian Inheritance in Men
OS	Outer segment
PBS	Phosphate buffered saline
PC1	Polycystin-1
PC2	Polycystin-2
PCD	Primary ciliary dyskinesia
PCR	Polymerase chain reaction
PD	Peritoneal dialysis
PGD	Preimplantation genetic diagnosis
phyloP	Phylogenetic p-values
PKD	Polycystic kidney disease
PKDB	Polycystic Kidney Disease Mutation Database
PKHD1	Polycystic kidney and hepatic disease 1
PMM2	phosphomannomutase 2
PMP	Per million population

PolyPhen-2	Polymorphism Phenotyping v2
PRs	Photoreceptors
QC	Quality control
RD	Read depth
RGMC	Reduced generation of multiple motile cilia
RPE	Retinal pigmented epithelium
RRT	Renal replacement therapies
RTKs	Receptor tyrosine kinases
Shh	Sonic hedgehog
SIFT	Sorting Intolerent from Tolerant
SLSN	Senior–Loken syndrome
SNVs	Single nucleotide variants
SRPS	Short Rib Polydactyly syndrome
SVs	Structural variations
TSC	Tuberous sclerosis complex
TGF-beta	Transforming growth factor-beta
TZ	Transition zone
US	Ultrasonography
VCF	Variant Call Format
WES	Whole exome sequencing
YLL	Years of life lost

Chapter 1. Introduction

1.1 Cilia and Human Diseases

1.1.1 Cilia structure, classification and functions

On the apical surface of nearly all human body cells, there are microscopic, membrane-covered hair-like structures called cilia. Cilia are evolutionary conserved throughout species from nematodes to protozoa (Arts and Knoers, 2013, Mitchison and Valente, 2017). Structurally, cilium contains three principal parts an anchoring structure called basal body, a transition zone and a cylindrical backbone projecting from the cell surface to the extracellular space called axoneme (Figure 1.1). With such distinctive structural complements, cilia are arguably the most complex cellular organelle (Rohatgi and Snell, 2010).

The axoneme provides support to the ciliary structure, determines cilia elongation and length, acts as pathways for ciliary proteins movement and in some cases facilitates ciliary motion. A cross section of the axoneme shows an arrangement of nine sets of microtubular doublets forming a ring either surrounding a central microtubule pair (9+2) or missing the central pair (9+0). Each doublet consists of a complete microtubule (A-tubule) connected to incomplete microtubule (B-tubule), which are assembled of α- and β- tubulin monomers, respectively (Horani and Ferkol, 2018). The basal body, which is located at the base of cilia, originates from the mother centriole serving as microtubule-organizing centre (MOC). The basal body is constructed from nine sets of short triplet microtubules from which the doublet microtubules of axoneme originate. The distal area of the basal body where the triplet microtubules emerge to the axonemal doublet microtubules defines the transition zone, which serves as a selectivity barrier controlling protein trafficking in and out of the cilium (Figure 1.1). Cilia are surrounded by ciliary membrane that is continuous to the plasma membrane but has a unique complement of proteins and lipids (Rohatgi and Snell, 2010). The ciliary membrane is enriched with various receptors essential for detecting extracellular signals.

Based on their structure and functions, cilia are basically divided into motile and immotile (primary) cilia. With the exception of sperm flagella, motile cilia are usually present as large groups on a cell surface (called multiciliated cells) and beat in a coordinated ‘back

and forth' manner to facilitate the movement of cells and fluids (Davenport and Yoder, 2005). For instance, there are approximately 200 motile cilia on the apical surface of each cell along the trachea, beating closely to clear mucus and dirt and muds out of the lungs (Horani and Ferkol, 2018). Moreover, motile cilia are abundant on the ependymal cells of the brain promoting cerebrospinal fluid movement as well as the cells lining fallopian tube and epididymis of reproductive tracts facilitating the movement of ovum and sperms. The axoneme of motile cilia has a 9+2 configuration (Figure 1.1). The power required for axoneme motility is mediated through the axonemal dynein arms that are motor complexes attached to microtubule doublets forcing them to orient against each other (Heuser et al., 2009). The sliding of microtubules is controlled by an elastic element called nexin, which ties the nearby outer doublets together. Furthermore, radial spokes are T-shaped axonemal protein complex that bond each set of the outer doublets to the central pair and controls dynein arms activity. Together, the central pair, dynein arms and T-spokes are critical for ciliary motion. Motile cilia vary in their length and beating frequencies. For instance, compared to the respiratory cilia, brain ependymal cilia have larger size and faster motion level (O'Callaghan et al., 1999). The normal beating rate of human airway cilia is between 8 and 14 beats/s and any changes in the surrounding environment or the exposure to pollutants may greatly affect ciliary motion (Al-Rawi et al., 1998, O'Callaghan et al., 1999). Notwithstanding, some signalling mechanisms, such as airway nitric oxide, control beating frequency, complete understanding of the impact of the intracellular and intercellular stimuli in regulating cilia motion is not yet achieved (Horani and Ferkol, 2018).

Primary cilia are single, immotile organelles present on the surface of most cell types in nearly all organs and tissues of human body (Lancaster and Gleeson, 2009). The axoneme of primary cilia illustrates some structural differences from that of motile cilia, where it lacks dynein arms and the central microtubule pair, thus has 9+0 microtubule arrangement. Primary cilia are implicated in organ development, such as kidney and limbs, and in neurosensory roles such as hearing, smelling and sight (Yildiz and Khanna, 2012). Thus, they are thought to be key sensory antennae enriched with receptors through which the cell detects changes in the surrounding environment. Based on the cell type, primary cilia act as mechano-, chemo- and photoreceptors. For example, primary cilia on the epithelium of renal tubule function as mechanoreceptor sensing urine flow, while on retina serve as photoreceptors detecting light stimuli (Hsiao et al., 2012). Thus, primary

cilia are involved in activation and transduction of diverse signalling pathways associated with cellular proliferation, differentiation and polarity that are principal for tissues development and homeostasis, including the Sonic hedgehog (Shh), Wnt, calcium, growth factor, receptor tyrosine kinases (RTKs) and G-protein-coupled receptors (GPCRs) pathways (Hsiao et al., 2012, Braun and Hildebrandt, 2017). The reason behind the enhancement of receptors and concentration of signalling pathway on the primary cilia is not fully clear. Along with their ability to receive sensory signals, primary cilia are found to possess bioactive extracellular vesicles (EVs) at their tips and secrete ectosomes and exosomes critical for intercellular communication, suggesting a potential role for primary cilia in EV biogenesis and reception (Wood et al., 2013).

The conventional classification of motile (9+2) and immotile cilia (9+0) fails to highlight the complexity of these different cilia types. For example, this classification is violated by a type of immotile cilia with axonemal configuration of 9+2 presenting on specific olfactory sensory neurons (D'Angelo and Franco, 2009). In addition, nodal cilia, which present on the embryonic node during gastrulation, are solitary organelle with a 9+0 microtubule configuration. However, they differ from primary cilia in the possession of dynein arms that provide the ability to move in a circular clockwise direction. It is postulated that the axonemal central pair is critical for ciliary beating (back-and-forth), thus its absence in nodal cilia creates a rotational motion (D'Angelo and Franco, 2009). Rotation of cilia at an average of 600 rpm makes the flow of the extra-embryonic fluid to move throughout the nodal surface towards the left (Mitchison and Valente, 2017). This directional flow is detected by primary cilia around the nodal cilia and elicits asymmetric gene expression cascade critical for left-right (LR) axis determination, including the major downstream effector gene and key factor in LR-axis development *Nodal*, which is a member of the transforming growth factor-beta (TGF-beta) superfamily (Yoshiba et al., 2012, Pennekamp et al., 2015, Mitchison and Valente, 2017). Whether the response of primary cilia at peripheral site of embryonic node is triggered by mechanical sensing of the flow or involved binding of developmental morphogens to ciliary receptors is not fully clear (Satir and Christensen, 2008).

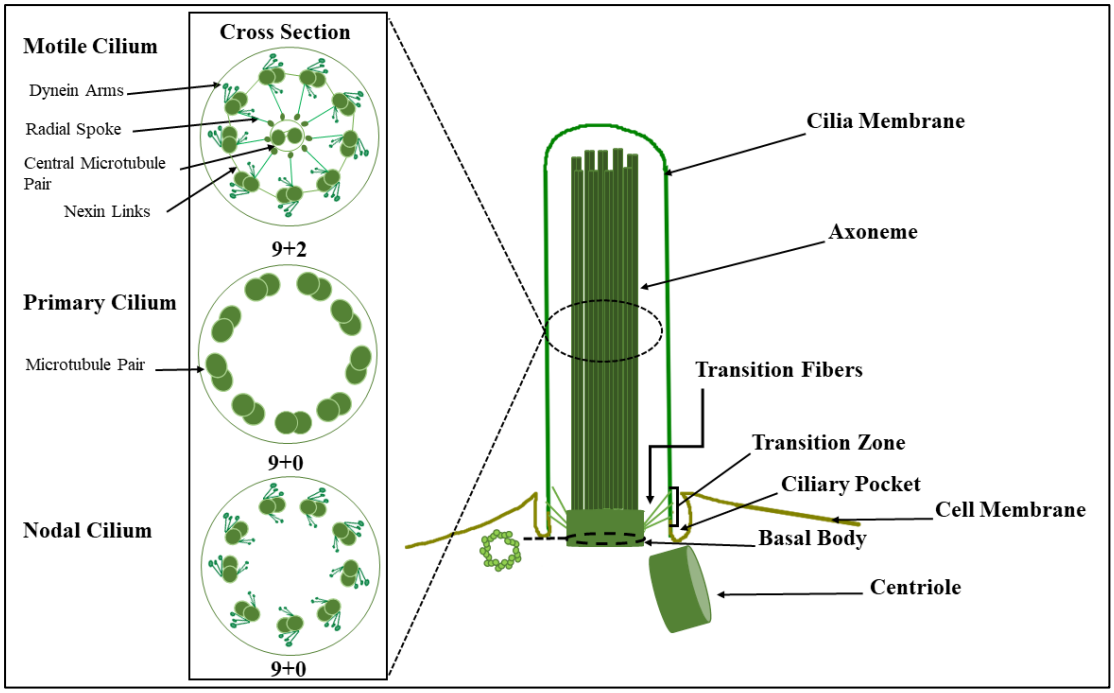

Figure 1.1 Schematic diagram of cilia Structure. The inner core of cilia is composed a cytoskeleton structure of microtubule bundles called axoneme originating from a centriole-derived microtubule structure called basal body. At transition zone (TZ) the triplet microtubules of the basal body derived into doublet microtubules. TZ is important in modulating proteins transportation in and out of the cilium. The axoneme is covered with cilia membrane, which originates from plasma membrane but consists of distinct constituents such as channels and receptors. Additional cross section diagrams of a typical axonemal microtubule structure of the motile cilium (9+2), immotile (primary) cilium (9+0) and the nodal cilium (9+0) are shown.

1.1.2 Dynamics of ciliogenesis

The complexity of cilium raises the query of how this organelle was constructed in a systematic manner. Cilium is a dynamic organelle that assembles during quiescence in a process known as ciliogenesis and disassembles during mitosis (Ishikawa and Marshall, 2011). Despite there are some structural differences among cilia types, ciliogenesis is conserved. Prior entry of G1 phase, basal body originates from the mother centriole or *de novo*, travels to the cell surface and docks onto the cell cortex in proximity to the plasma membrane. Later, fusion of basal body to plasma membrane and association with membrane vesicles generates ciliary membrane partition. The basal body acts as nucleation site for axoneme extending out microtubule bundles protruding from the cell surface towards the extracellular space and hence inducing the outgrowth of cilium. Due to the absence of protein synthesis machine in cilia, all compartment proteins are synthesised in the Golgi apparatus. Consequently, this demanded a selective importing of ciliary proteins from the cytoplasm to the base of the cilium through ciliary gate as well as protein trafficking along ciliary length through a molecular motor-based process called intraflagellar transport (IFT). At the beginning of cell cycle, cilia absorb and the basal body travels to the nucleus to organize centrosomes. Ciliary desorption is thought to acts as novel checkpoint for G_1-S transition and represents intensive procedures including disassembly of axonemal microtubules, obstruction of ciliary reformation and elimination of ciliary membrane (Hsu et al., 2017).

Continued growth of cilia needs active transport through a highly preserved process of IFT. IFT was initially recognized in the ciliogenesis of the unicellular green alga *Chlamydomonas reinhardtii*. IFT is a bidirectional process that involves movement of ciliary proteins, both structural and signaling components, along the axoneme through IFT molecular trains (or motors). Transport of proteins from cytoplasm to ciliary tip is called anterograde, while transport in the opposite direction is called retrograde. IFT trains are classified into long, narrow trains of about 700nm in length and short, condense trains of about 250 nm (Pigino et al., 2009). Anterograde transport occurs through the interactions of IFT long trains with members of kinesin-2 family, while IFT short trains are associated with cytoplasmic dynein 2 in retrograde transport (Figure 1.2*)* (Ishikawa and Marshall, 2011). Connection of cargo proteins to IFT trains is obtained through an association with two IFT protein complexes (A and B). The IFT complex A consists of 6 subunits and facilitates retrograde transport, whereas the IFT complex B composes of 14

subunits proteins and mediates anterograde transport (Bhogaraju et al., 2013). The IFT complex B is also important for cilia formation and preservation as loss of any subunits leads to shortened or lack of cilia (Ishikawa and Marshall, 2011). The BBSome, a complex of Bardet-Biedl syndrome (BBS) proteins, is an additional critical protein complex that is involved in transporting ciliary membrane proteins through IFT trains and controls the turnaround transport from anterograde to retrograde (Wingfield et al., 2018). Remarkably, IFT-associated proteins are also critical in controlling different constituents of the Hedgehog signalling pathway (Goetz and Anderson, 2010). Although a fruitful progression has been made in clarifying how ciliary assembly and disassembly is highly regulated, a complete understanding of the full molecular mechanisms is still inferior. Consequently, there is a demand for integrative models to fill the gaps and illustrate the way the ciliary trafficking components and pathways interact together.

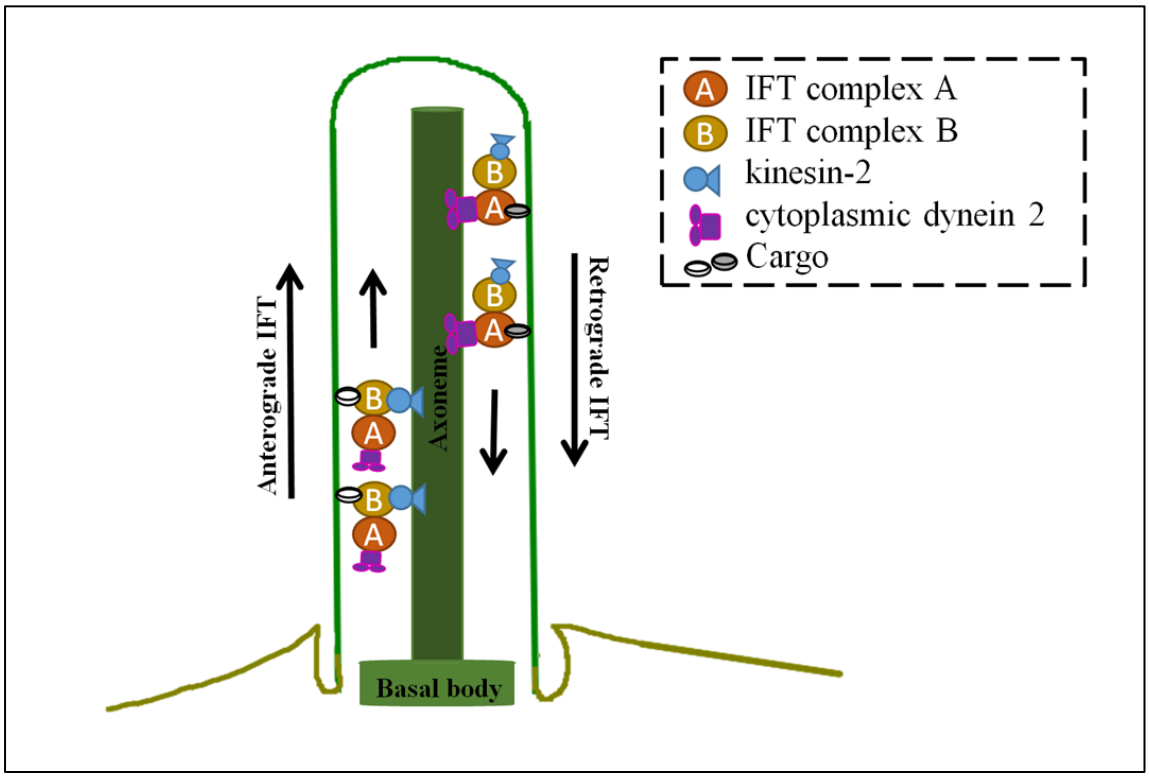

Figure 1.2 Intraflagellar transport (IFT) machinery of cilia. Diagram of a cilium illustrating the IFT. The IFT complex B, which consists of 14 well-established proteins (Ift20, Ift22, Ift25, Ift27, Ift46, Ift52, Ift54, Ift57, Ift70, Ift74/Ift72, Ift80, Ift81, Ift88 and Ift172) along with Kinesin-2 mediate anterograde transport (from ciliary base to the tip) (Bhogaraju et al., 2013). The IFT complex A, which comprises of 6 known proteins (Ift144, Ift140, Ift139, Ift122, Ift121 and Ift43) along with cytoplasmic dynein 2 delivered retrograde transport (from ciliary tip to the base) (Bhogaraju et al., 2013).

1.1.3 Human ciliopathies: history and pathogenesis

Although the first observation of flagella or motile cilia was made in the late 17th century by Anton van Leeuwenhoek, about a century later the primary immotile cilia were observed (Reiter and Leroux, 2017). In 1835, earlier studies of mammalian cilia using light microscopy were documented, where considerable attentions were mainly given to the mechanism and biochemistry of motile cilia (Bloodgood, 2009). Primary cilia on mammalian cells were first described in 1898 by KW Zimmerman, who drew illustrations of solitary cilia-like structure and assumed that this organelle possess some sensory functions (Hua and Ferland, 2018). However, primary cilia was mostly ignored by biologists and considered as evolutionary useless vestige. Later, using the electron microscopy, studies were focused on examining the build-up structure of cilia, therefore new insights in the inner structure of motile and primary cilia was achieved in 1954 and 1956, respectively (Satir and Christensen, 2008). Since then, multiple studies continued to show the presence of primary cilia in various mammalian cells, nevertheless its physiological functions remained mysterious until its pathophysiological significances in kidney cells were proposed in 1995 (Satir and Christensen, 2007). The recognition of the IFT in *C. reinhardtii* led to discovering the association between mutations in the gene encoding the IFT88 protein and polycystic kidney disease (PKD), thus for the first time dysfunction of primary cilia was linked to human disease (Pazour et al., 2000). Further attention was giving to this organelle after discovering that the critical developmental sonic hedgehog (Shh) pathway depends on the primary cilia and IFT system, fascinating scientists from different majors (Huangfu et al., 2003). Following these major discoveries, system biologists focused their research on establishing functional models that demonstrate the similarity between the genetics of mice, zebrafish and human cells in order to understand the molecular mechanism defining cilia role in developmental pathologies and human diseases. A multi-species informative database associated with cilia and associated disorders are found in Cildb (http://cildb.cgm.cnrs-gif.fr/), which serves as a reference for ciliopathy predicted proteins (Arnaiz et al., 2014). Further advances in genetic research have defined most of the molecular basis behind the dysfunctional mechanisms causing cilia-associated disorders, termed ciliopathies.

Since the initial used in 1984, the term ciliopathies is commonly used to describe a variety of devastating multi-systemic human disorders that are caused by genetic defects of the cilia structure, composition or function (Reiter and Leroux, 2017). At the present,

about 35 confirmed inherited disorders are classified as ciliopathies and the list continues to expand (Reiter and Leroux, 2017). It includes primary ciliary dyskinesia (PCD)/ Kartagener syndrome (KS), polycystic kidney diseases (PKD), nephronophthisis (NPHP), Leber congenital amaurosis (LCA), Senior–Loken (SLSN), Alström syndrome (ALMS), Joubert (JBTS), Jeune (or asphyxiating thoracic dystrophy, JATD), short rib polydactyly (SRPS), Ellis-van Creveld syndrome (EVC), oral-facial-digital (OFD) syndromes, Bardet–Biedl (BBS), and and Meckel–Gruber (MKS).

Because both motile and primary cilia share similar core structure and ciliogenesis mechanism, defects in cilia structure or disruption of components associated with cilia construction can impact both motile and primary cilia distinctly or together, thus impairing different physiological functions associated with motility and sensation (Ishikawa and Marshall, 2011). Furthermore, due to extensive distribution and involvement in numerous cellular functions, impairment of cilia can give rise to a broad spectrum of characteristic features ranging from embryonic lethality, through typical organ anomalies to severe loss of function causing early manifesting diseases or moderate loss of function leading to late-onset diseases. These remarkable features are recognized as hallmarks of ciliopathies, affecting main organs such as brain, eyes, respiratory system, kidneys, liver, skeleton and reproductive system (Figure 1.3). Kidney, liver and pancreatic cyst formation, heart anomalies, retinal degeneration, hearing loss, neural tube defects, obesity, mental retardation, skeletal anomalies and defects of the central nervous system (CNS) are the common features associated with ciliopathies, which may occur isolated or part of distinguishable syndromes (Badano et al., 2006). The phenotypic parameters outlining ciliopathies are overlapped nevertheless may aid the diagnosis and treatment of some unrecognized diseases or syndromes with novel features. Polycystic ovarian syndrome and several subcutaneous cysts were reported recently along with the canonical ciliopathies features of kidney failure and liver cirrhosis, which may demonstrate a new rare syndrome (Tan et al., 2018).

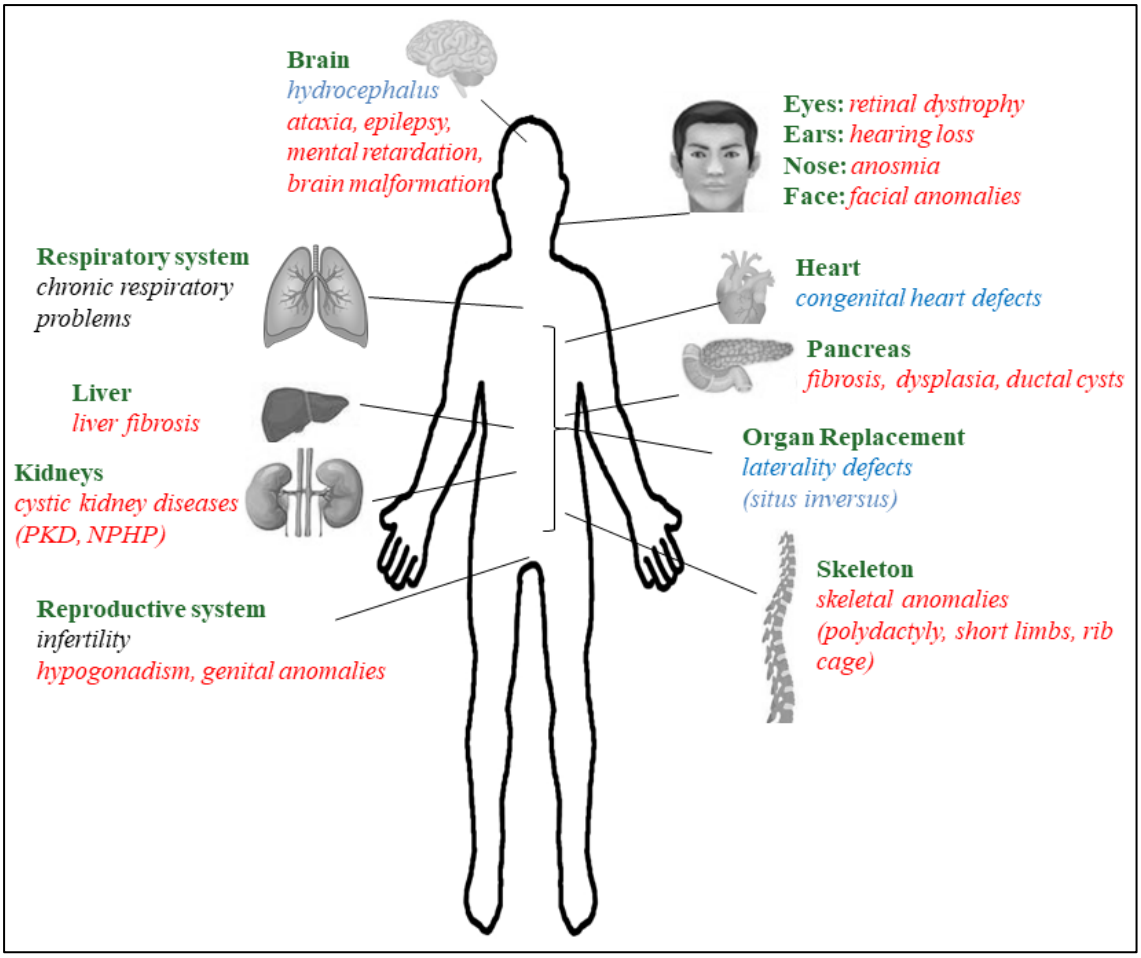

Figure 1.3 Different spectrums of ciliopathy phenotypes in relation to different human organs. Clinical phenotypes caused by motile cilia are indicated by black colour, while those caused by primary non-motile cilia are indicated by red colour. Clinical overlapping features between the two cilia types are associated with laterality defects, congenital heart defects and hydrocephalus (blue colour). NPHP, nephronophthisis; PKD, polycystic kidney disease.

1.1.4 Motile ciliopathies

The disturbance of ciliary motility caused by defect in the assembly or function of dynein arms, dynein regulatory complex or central microtubule pair can entirely lead to motile ciliopathies. Primary ciliary dyskinesia (PCD) is a rare inherited disease of the motile cilia, which was the first and remains the only known motile ciliopathy (Horani and Ferkol, 2018). PCD has an estimated incidence of approximately 1:20,000 live births and accounts for 5% of children with chronic respiratory tract infections (Horani and Ferkol, 2018). Chronic upper and lower respiratory tract disease in the form of sinusitis, bronchiectasis and atelectasis, laterality anomalies, frequent ear infections, and infertility are the major clinical manifestations of PCD. Approximately 50% of PCD patients present with *situs inversus totalis* (Kartagener's syndrome), which is an identical-reversal image of main internal organs, indicating nodal ciliary function defect throughout embryogenesis (Kennedy et al., 2007, Horani and Ferkol, 2018). In rare PCD cases, complex laterality anomalies, such as situs ambiguous or heterotaxy, are reported with congenital heart abnormalities (Kennedy et al., 2007, Horani and Ferkol, 2018). Furthermore, reduced generation of multiple motile cilia (RGMC) is another type of PCD presenting with similar phenotypes, except laterality anomalies, but differs in disease aetiology since it is caused by abnormalities of multiciliogenesis process (Mitchison and Valente, 2017). Particularly, hydrocephalus is noticeably more common in PCD arises from RGMC than in typical PCD patients and the reason is not fully clear (Mitchison and Valente, 2017). PCD often has an autosomal recessive mode of inheritance, although some rare cases of autosomal dominant and X-linked mode, with PCD-like clinical features, have been described (Reiter and Leroux, 2017, Horani and Ferkol, 2018). Motile ciliopathies show a significant genetic heterogeneity, where mutations in up to 37 genes have been reported, possibly demonstrating the complexity of the structures demanded for ciliary motion (Horani and Ferkol, 2018).

1.1.5 Primary ciliopathies

Although motile cilia show some sensory abilities, dysfunction of the primary cilia can give rise to different sensory, physiological and developmental abnormalities causing primary ciliopathies. Different potential molecular aetiologies can lead to primary ciliopathies including defects of primary cilia assembly, maintenance, signaling transduction machinery or protein trafficking components. In contrast to motile ciliopathies, primary ciliopathy disorders show a broad range of phenotypic variability; this can be attributed to the ubiquity of primary cilia. Moreover, a comprehensive overlapping in genetic causes and clinical features is observed across different disorders of primary ciliopathies, revealing the complexity of pathomechanisms underlying diseases. Currently, primary ciliopathies are clinically classified and diagnosed based on the main implicated organ(s).

In brain, primary cilia are key initiators of the Shh pathway, which plays a major role in brain physiology development and implicated in regulating neural stem cells. Therefore, defective Shh signaling caused by mutations in associated genes generates serious neurological developmental defects, including defective closure of neural tube (anencephaly, encephalocele), hydrocephalus, and additional midline abnormalities such as occipital encephalocele, anomalies of corpus callosum and holoprosencephaly, which is a rare genetic disorder associated with a broad range of brain and cranio-facial anomalies (Mitchison and Valente, 2017). Because several components of Shh pathway are distinctly present in the primary cilia at various steps of pathway activation, mutations in numerous genes have been associated with Shh-associated developmental defects that are also part of ciliopathies features. For instance, mutations in different components of the IFT complex in mice, such as *Ift139* and *Ift122*, evidenced the impact of cilia-mediated Shh signals in neural tubes specification and mutations in some basal body proteins, such as *Mks1*, *Ofd1*, *Evc* exhibit morphological defects associated with EVC, JBTS, MKS and OFD (Ruat et al., 2012). Cilia-associated Shh signals have also been implicated in the hippocampal development, which is a complex brain structure mainly involved in learning and memorizing, and in expansion of hippocampal progenitors (Ruat et al., 2012). Furthermore, in cerebellum, cilia-initiated Shh signals have been implicated in different steps of development and are key drivers of proliferation in granule neurons precursors (GNPs), thus dysregulation caused by mutations in associated genes may explain the development of cerebellar dysgenesis and hypoplasia observed JBTS and

BBS (Ruat et al., 2012, Mitchison and Valente, 2017). Additional cilia-mediated pathway that found to be involved in cerebellar development is the canonical Wnt signaling pathway, where mutations in its enhancer Jouberin, encoded by the *AHI1* gene, cause the typical cerebellar vermis hypodysplasia, known as 'molar tooth sign' (MTS), a hallmark of JBTS (Louie et al., 2010).

In the outer layer of neural retina, there are highly specialized neurons involved in the transmission of visual signals called photoreceptors (PRs), which are covered by retinal pigmented epithelium (RPE) a layer of phagocytic cells. There are two types of PRs, rods and cones, each consisting of distinct outer segment (OS) and inner segment (IS). The OS is connected to the IS through a modified primary (9+0) cilium called PRs-connecting cilium that emerges from the basal body in the IS and extends to OS through the axoneme. PRs- connecting cilium through its transition zone plays critical role in controlling protein trafficking in and out of the OS, mainly the disk proteins rhodopsins, through IFT complex. Considering the complexity of PRs connecting cilium, mutations in numerous PRs proteins have been associated with isolated or syndromic retinal dystrophies, the most common ocular phenotype of ciliopathies. For instance, mutations in *ALMS1*, involved in rhodopsins and other proteins transport across axoneme, have been associated with the most severe type of retinal dystrophies LCA and ALSM (Collin et al., 2005), while mutations in *CC2D2A*, modulates the elongation of connecting cilium, was documented in cases of retinitis pigmentosa, JBTS and MKS (Bachmann-Gagescu et al., 2011, Mitchison and Valente, 2017). Notwithstanding, mutations in 120 genes have been associated with retinal dystrophies, not all types are ciliopathies and not all retinal dystrophies-associated genes are implicated in cilia assembly or functioning (Nash et al., 2015). Consistently, some ciliary genes are linked with both isolated and syndromic (ciliopathies) forms RDs, as in *C21ORF2* and *IFT140* genes (Nash et al., 2015, Mitchison and Valente, 2017).

In kidneys of adult, primary cilia serves as key sensors responding to changes in urine flow, composition and osmolality through regulating different intracellular signaling pathways including, Wnt, G-protein signaling, mTOR and even SHH pathway (Mitchison and Valente, 2017). Ciliary dysfunction in the kidneys usually causes the development of cysts, which are fluid-filled sacs of epithelial cells caused by dilation of diverse parts of nephrons and collecting ducts. Cysts may develop at any age and can differ in size, numbers and locations. Cystic kidney diseases, including the two major groups of PKD

and NPHP, were the first group of disorders classified as primary ciliopathies, defining a broad spectrum of renal ciliopathies.

In the liver, primary cilia extend from the epithelium of the biliary ducts, initiating early during development. Primary cilia are involved in the development and function of liver, serving mechano-, chemo-, and osmo-receptor sensing and transducing signals associated with biliary luminal flow (Mitchison and Valente, 2017). Thus, dysfunction of primary cilia has been associated with the ductal plate malformation (DPM) along with abnormal bile ducts that are often enclosed by massive matrix and cystic expansion, leading to congenital hepatic fibrosis, a phenotype that manifests in many primary ciliopathies including PKD, NPHP, BBS, JBTS, and MKS. The growth and functioning of pancreas is another critical role of primary cilia that is maintained through the regulation of different developmental pathways. Ciliary dysfunction can cause characteristic anomalies in pancreas, including fibrosis, dysplasia and ductal cysts, although less common, possibly attributed to the conserved function of exocrine and endocrine. In contrast, pancreatic defects in the form of β-cells defect and diabetes mellitus are typical manifestations of ALMS (Mitchison and Valente, 2017).

Impairment of the IFT components leading to dysregulation of Hedgehog pathways is mostly associated with at least 16 diverse skeletal genetic disorders called skeletal-ciliopathies that are primarily characterized by long narrow chests, short ribs and limbs, polydactyly as well as dwarfism. Skeletal ciliopathies can be categorized into four main groups: cranioectodermal dysplasia (CED also known as Sensenbrenner syndrome), Ellis-van Creveld syndrome (EVC), short-rib thoracic dysplasia (SRTD) and oral-facial-digital syndrome (OFDS) (Ishikawa and Marshall, 2011, Zhang et al., 2018) (Table 1.1). The SRTD family contains different rare skeletal associated syndrome such as short-rib polydactyly syndrome (SRPS), Jeune asphyxiating thoracic dysplasia (JATD), and Mainzer-Saldino syndrome (MZSDS) (Oud et al., 2017). The severity of these anomalies ranges from the non-lethal phenotypes of EVC and CED, through the more severe JATD, to the perinatal lethal SRPS. Each skeletal ciliopathies can also occasionally present with anomalies of different organs and tissues including brain, heart, eyes, kidneys, liver, pancreas, intestine and genitalia (Ishikawa and Marshall, 2011, Oud et al., 2017) (Table 1.1). For example, along with skeletal anomalies JATD and MZSDZ patients can also manifest with NPHP, blindness, liver fibrosis, as well as intellectual disability in JATD and pancreatic anomalies in MZSDS (Oud et al., 2017). Furthermore, congenital heart

abnormalities are frequently found in EVC patients (Table 1.1). There are up to 15 different type of OFD syndrome, but the X-linked OFD type 1 (OFD1) is the most recognized type. OFD1 is featured by the typical defects of oral cavity (split-tongue, oral frenula and dental anomalies), facial (cleft lip/palate) and digits (syndactyly and polydactyly) in combination to PKD, and CNS anomalies (Table 1.1). All types of these ciliopathies, except OFD1, have an autosomal recessive mode of inheritance, where mutations in at least 23 separate loci have been associated with these disorders (Zhang et al., 2018). Along with clinical and genetic heterogeneity, skeletal ciliopathies is characterized by genetic overlapping, which is frequently recognized between CED, JATD, SRPS and MZSDS (Oud et al., 2017, Reiter and Leroux, 2017).

Ciliopathy	Clinical features
Cranioectodermal dysplasia (CED) (Sensenbrenner syndrome)	Cranioectodermal dysplasia; narrow thorax, dental abnormalities, hepatic and kidney involvement
Ellis van Creveld syndrome (EVC)	Skeletal dysplasia; congenital heart disease; polydactyly; ectodermal dysplasia
Short rib polydactyly (SRPS)	Lethal skeletal dysplasia, polydactyly, several congenital anomalies
short-rib thoracic dysplasia (SRTD)	Skeletal dysplasia; thoracic malformations; polydactyly; kidney cysts; retinitis pigmentosa
Oral-facial-digital syndrome type I (OFD1)	Oral cavity, face, and digit abnormalities; CNS abnormalities; cystic kidney disease; X-linked with male lethality

Table 1.1 Clinical characteristics of skeletal ciliopathies. CNS, central nervous system.

1.1.6 Association between motile and primary ciliopathies

A considerable overlapping in clinical features of motile and primary ciliopathies has been documented in terms of laterality anomalies, infertility and hydrocephalus (Mitchison and Valente, 2017). The associations are not clear, but detection of sensory receptors on motile cilia raises the query of whether sensory functions can be assigned to both motile and primary cilia. In fact, the impact of mechanosensory signals on nodal cilia along with the mixture of motile and primary cilia at the LR organiser make laterality anomalies part of the overlapping spectrum in both motile and primary ciliopathies. Clinical studies reported partial and complete *situs inversus* in cases of JBTS, NPHP and skeletal ciliopathies (Jeune syndrome) (Moalem et al., 2013). Excitingly, the spectrum of ciliopathy was even broaden following the reporting of PCD in a fetus with NPHP and *situs inversus* caused by a homozygous mutation in the *NPHP2* gene, which usually associated with infantile NPHP (Moalem et al., 2013). Furthermore, complex X-linked syndromic types of motile cilia disorders have been reported in combination with retinitis pigmentosa caused by mutation in *RPGR* gene and OFD type1 syndrome, although the cause of motility dysfunction is not clear (Budny et al., 2006, Moalem et al., 2013).

1.1.7 Genetic complexity of ciliopathies

While ciliopathies are mainly caused by defects in ciliary proteins, dysfunction of some non-ciliary proteins appears also to have impact on cilia assembly or function, hence causes ciliopathies (Reiter and Leroux, 2017). XPNPEP3, for instance, is an enzyme that located in the mitochondria of renal tubule cells. Mutations in the *XPNPEP3* gene may modulate ciliary function by proteolytic cleavage of ciliary proteins and hence involved in NPHP-like pathogenesis (O'Toole et al., 2010). One the other hand, many ciliary proteins have been observed at other cellular (extra-ciliary) sites, such as nucleus, Golgi apparatus, cytoplasm or even immune synapses of T-cells, and possess non-ciliary function, therefore attention is required when allocating biological defects to cilia (Hua and Ferland, 2018). Involvement in cell cycle regulation and controlling cytoskeletal and protein-trafficking are main extraciliary functions (Reiter and Leroux, 2017).

Several genes can be causative for a particular ciliopathy disorder; nonetheless, mutations in the same gene may lead to distinct ciliopathy disorders in different individuals. For instance, mutations in the *MKS1* gene are traditionally believed to cause the severe type of MKS, however recent findings of *MKS1* mutations in JBTS patients have make the borders defining these two syndromes less distinct. Similarly, mutations in *CSPP1* gene can lead to isolated JBTS or JBTS associated with JATD, at which *CSPP1*-linked JBTS is often associated with mild phenotype and efforts to correlate clinical outcome with *CSPP1* were ineffective (Hua and Ferland, 2018).

Through functional studies, remarkable associations have been generated between the function and ciliary domain of mutated protein and the related clinical features. Several proteins were shown to localized in clusters and complexes involving in specific roles within cilium. For instance, the vast majority of skeletal dysplasia-associated ciliopathies and BBS linked with mutations in BBS genes are consequences of the dysfunction IFT components (Mitchison and Valente, 2017). In addition, mutations of most proteins located in the transition zone, which are key regulators of ciliary signaling cascades and protein trafficking, are associated with JBTS, MKS and NPHP (Mitchison and Valente, 2017).

Some ciliopathy-related genes seem to be very tissue- and organ-specific and consequently associated with distinct clinical phenotypes, while others are less selective but still reflect some organ preferential. Some examples are *ARL13B* gene, which is

purely associated with neurological features and lead to classical JBTS, and *IFT80* and *DYNC2H1*, at which mutations are associated merely with isolated short-rib polydactyly phenotype (Cantagrel et al., 2008). On the other hand, mutations in *TMEM67* are consistently implicated with liver manifestations and congenital hepatic fibrosis, whereas mutations in *C5orf42* were identified in patients with OFD and JBTS more frequently associated with polydactyly phenotype (Brancati et al., 2009, Lopez et al., 2014). At the end of spectrum, there are pleiotropic ciliopathy-genes that are mutated in a broad range of ciliopathies, such as *CEP290*, which has been associated with a wide varieties of ciliopathy phenotypes related to defects in retina, kidney, liver and CNS, thus has been documented in SLSN, NPHP, JBTS, BBS and MKS (Coppieters et al., 2010).

Most ciliopathies have autosomal recessive mode of inheritance, with exception of ADPKD and OFD1 that have dominant and X-linked dominant inheritance, respectively. However, the inheritance of BBS in some families has complicated by oligogenic pattern in order to modify the penetrance of the disease, at which recessive mutations in a BBS gene is combined to third heterozygous mutation in a different allele (Lindstrand et al., 2016, Mitchison and Valente, 2017). Although true oligogenic inheritance has not been documented in other ciliopathy, *trans* modifier alleles, also termed genetic phenotypic modifiers, contributing to differential expressivity have been observed across the ciliopathy spectrum (Lindstrand et al., 2016, Mitchison and Valente, 2017). A positive correlation has been made between a specific heterozygous mutation in distinct alleles and the occurrence of retinal manifestations, such as (p.Ala229Thr) mutation in *RPGRIP1L* was implicated with retinitis pigmentosa in ciliopathies and the *AHI1* (p.Arg830Trp) mutation and the progress of retinal degeneration in patients with homozygous *NPHP1* deletions (Khanna et al., 2009, Louie et al., 2010). However, as these are merely sporadic observations, further confirmation in larger, independent cohort is demanded to confirm the true influences of these modifiers.

1.2 Renal Ciliopathies

Renal ciliopathies are human genetic kidney disorders that are characterized by PKD, NPHP and renal cystic dysplasia. Essentially, renal ciliopathies are often linked with extrarenal clinical manifestations associated with ciliary disruption in other organs mainly retina, CNS, liver and bones (Badano et al., 2006). Autosomal dominant and recessive polycystic kidney disease (ADPKD and ARPKD, respectively), nephronophthisis (NPHP) and the NPHP-related ciliopathies (NPHP-RC) are the most recognized inherited renal ciliopathies (Table 1.2). The formation of cystic kidneys is the most prevalent clinical feature manifesting in these disorders (Tobin and Beales, 2008). Cystic kidneys phenotype is also a common feature in syndromic ciliopathies, such as JBTS, BBS, MKS and the OFD1. Together, the overall estimated prevalence of renal ciliopathies is about 1:2,000 live births (Kagan et al., 2017).

Some of renal ciliopathies, such as PKD and NPHP, often lead to chronic kidney disease (CKD), which is a common condition characterized by irreversible kidney damage that constantly progress to end stage kidney disease (ESKD), hence renal replacement therapies (RRT), including haemodialysis, peritoneal dialysis (PD) or kidney transplant are demanded for survival.

Cystic kidney lesions are frequently seen by the radiologist in people older than 50 years, where the majority of these lesions are benign and simple, even though it is also common to found complex and multifocal cystic kidney lesions. Cystic kidney diseases are generally assessed using Ultra Sonography (US), computed tomography (CT), and magnetic resonance imaging (MRI), with MRI been the best for the characteriztion of number and nature of cystic lessions. Cystic kidney diseases consist of a broad spectrum of conditions that are categorized based on their pathogenesis into inherited, acquired and developmental disease (Katabathina et al., 2010). While inherited conditions are associated with different genes implicated in the formation and functioning of the kidney primary cilia, non-inherited cystic diseases are developed due to obstructive, stromal-epithelial interactions and neoplastic mechanisms (Katabathina et al., 2010). Inherited cystic kidney diseases include ADPKD or ARPKD, nephronophthisis, tuberous sclerosis (TS), von Hippel-Lindau disease, medullary cystic kidney disease (MCKD), BBS, OFD syndrome, and MKS syndrome. These diseases will be discussed in more details later on this chapter. Different options for treatment of some of the cystic kidney diseases have

been developed following detailed knowledge of the molecular mechanisms that underlie their pathophysiology.

Acquired cystic kidney diseases are defined as the occurrence of multiple kidney cysts (≥ 3 / kidney) in patients with ESKD, especially when commencing dialysis (Katabathina et al., 2010). It is estimated that at least 50% of patients received dialysis have acquired cystic kidneys, although the percentage differs in agreement with the duration of dialysis since it is found in almost 100% of patients following 10 years of dialysis (Katabathina et al., 2010). Bleeding cysts, cyst infection, ureteral stones and kidney malignancy are the main complications of acquired cystic kidney diseases.

Developmental cystic kidney diseases include medullary sponge kidneys, multicystic dysplastic kidney as well as segmental / localized cystic kidney disease. Medullary sponge kidney, which has an estimated prevalence of 1:5000, is a congenital developmental abnormality categorized by ectasia and cystic dilatation of the kidney medullary collecting ducts (Katabathina et al., 2010). The precise pathogenesis mechanism of this condition is not fully clear and the majority of cases are sporadic, although familial cases have also been reported (Katabathina et al., 2010). Different conditions have been linked with medullary sponge kidney including inborn hemihypertrophy, Beckwith-Wiedemann syndrome, Caroli syndrome, Wilm's tumor as well as horseshoe kidney (Katabathina et al., 2010). Multicystic dysplastic kidney disease is caused by abnormal metanephric differentiation of the kidney during embryogenesis. The majority of multicystic dysplastic kidney disease cases are sporadic; nevertheless familial forms have been reported. Localized kidney cystic disease, which is also described as segmental and unilateral kidney cystic disease, is a rare, non-progressive condition that is categorized by multiple cysts in only one kidney. Localized kidney cystic disease is generally a benign condition that needs frequent follow-up with functional imaging evaluations.

Autosomal dominant polycystic kidney disease (ADPKD)

ADPKD is the most common type of PKD affecting over 10 million individuals worldwide, with an estimated prevalence of 1:400 to 1:1,000 live births, representing significant public health burden (Harris and Torres, 2009, Bergmann et al., 2018). The onset of kidney function deterioration is widely variable among ADPKD patients ranging from as early as first decade to as late as eighth decade (Cornec-Le Gall et al., 2018). Furthermore, disease progression is found to be variable. Approximately 75% of ADPKD patients developed ESKD by age of 70 years, thus requiring RRT modalities (Neumann et al., 2013). Moreover, 7-15% of the prevalent ESKD population in developed countries has ADPKD (Akoh, 2015). The major leading causes of death in ADPKD receiving RRT are cardiac diseases and infections (Harris and Torres, 2009, Bergmann et al., 2018).

Enlarged kidneys with multiple bilateral cysts are the typical characteristic features of ADPKD (Table 1.2). Since ADPKD is a multisystem disorder, other clinical symptoms, such as congenital hepatic fibrosis, formation of extrarenal cysts in liver, pancreas, seminal vesicles and arachnoid membrane as well as cardiovascular-associated anomalies including hypertension, hypertrophy or failure of left ventricular, heart valve defects and intracranial arterial aneurysms, are often featured (Dillman et al., 2017). Occasionally, a small proportion of ADPKD are reported with severe polycystic liver disease (PLD) presenting with a massively enlarged cystic liver, thus necessitating surgical interventions (Cornec-Le Gall et al., 2018). Interestingly, the total number of children with early manifesting ADPKD might be similar to those with ARPKD, at which ADPKD not necessarily associate with severe prognosis (Bergmann et al., 2018, Cornec-Le Gall et al., 2018). Thus, the overall estimated prevalence of PKD in children is approximately 1 in 10,000 (Bergmann et al., 2018).

Diagnosis	MOI	Gene(s)	Extrarenal manifestations	Renal ultrasound	Median age of ESKD (years)
ADPKD	AD	*PKD1* *PKD2* *GANAB* *DNAJB11*	intracranial aneurysms, polycystic liver, cysts in pancreas and spleen, diverticular disease and hernias	Polycystic kidneys	58.1 years (*PKD1*) and 79.9 years (*PKD2*)
ARPKD	AR	*PKHD1* *DZIP1L*	Congenital hepatic fibrosis	Polycystic kidneys	Variable
NPHP	AR	*NPHP* genes (*NPHP1-20*), *XPNPEP3*, *SLC41A1*, *TRAF3IP1*, *AH11* and *CC2D2A*	Retinal degeneration, cerebellar vermis aplasia, liver fibrosis, situs inversus and skeletal anomalies	normal-sized or small/ shrunken, hyperechogenic kidneys, corticomedullary cysts and poor CMD (except infantile NPHP)	Usually before 30 years

Table 1.2 Comparison between Autosomal dominant and recessive polycystic kidney disease (ADPKD and ARPKD, respectively) and nephronophthisis (NPHP). AD, autosomal dominant; AR, autosomal recessive; CMD, corticomedullary differentiation; ESKD, end stage kidney disease; MOI, mode of inheritance.

ADPKD is genetically a heterogeneous disease mainly caused by mutations in *PKD1* gene (located in 16p13.3) or *PKD2* gene (located in 4q22.1), leading to alterations of the transmembrane proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively (Figure 1.4) (Ma et al., 2017). *PKD1* is characterized by a large transcript size consisting of 46 exons and its position on a complex genomic region that is duplicated six times throughout chromosome 16, thus sharing high level of similarity with these pseudogenes (*PKD1P1-PKD1P6*), whereas *PKD2* is a shorter gene composing of 15 exons (Harris and Torres, 2009). *PKD1* mutations are responsible for up to 77% of ADPKD patients, whereas mutations in *PKD2* account for up to 13% (Audrezet et al., 2012, Heyer et al., 2016b). Both genes illustrate high level of allelic heterogeneity, where up to 1,273 and 202 pathogenic variants were reported in *PKD1* and *PKD2* gene, respectively, in the Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB) (URL:http://pkdb.pkdcure.org).

Although mutations in *PKD1* and *PKD2* are the major causes of ADPKD, 5-10% of pedigrees remain as either genetically unsolved or harbour rare mutations in other genes causing ADPKD-like phenotype, such as α-glucosidase neutral AB (*GANAB*), *DNAJB11* or hepatocyte nuclear factor 1β (*HNF1B*) gene (Heyer et al., 2016a, Porath et al., 2016, Bergmann et al., 2018). Mutations of the gene *GANAB*, which is implicated in protein folding and encoding for glucosidase Ila subunit, have been described in some pedigrees with mild ADPKD and autosomal dominant polycystic liver disease (ADPLD) (Porath et al., 2016). Furthermore, some dominant mutations in genes mostly linked to ADPLD (e.g. *PRKCSH*, *SEC63*, *LRP5*, *ALG8* and *SEC61B*) or autosomal dominant tubulointerstitial kidney disease (ADTKD) (e.g. *MUC1*, *UMOD*, *REN* or *SEC61A*) can phenocopy ADPKD despite clinical differences (Zaucke et al., 2010, Bergmann et al., 2018, Cornec-Le Gall et al., 2018). Though distinctions of extrarenal features may be made, hereditary angiopathy with nephropathy, aneurysms and muscle cramps (HANAC) syndrome caused by mutations in *COL4A1* gene may lead to renal cysts that mistaken with ADPKD (Bergmann et al., 2018, Cornec-Le Gall et al., 2018). Occasionally, renal presentations in some syndromic forms of PKD, such as the X-linked OFD1 and hereditary cancer syndromes von Hippel–Lindau syndrome and Tuberous sclerosis complex (TSC) due to mutations in *TSC1* and *TSC2* genes were described indistinguishable from that of ADPKD, especially with the lack or mild extrarenal phenotypes (Prattichizzo et al., 2008, Cornec-Le Gall et al., 2018). Two possible explanations can be made for patients

remained genetically unsolved: (1) undetermined missense, deep intronic splicing, promoter, or even mosaic *de novo* variants in the known genes or (2) additional PKD-associated genes encoding proteins interfere with biogenesis of PC1 and PC2. Therefore, genetic analysis through massive parallel sequencing of a panel of PKD associated genes can provide clearer picture of full aetiology and direct diagnosis of ADPKD-like cases.

Several efforts were performed to correlate disease severity with patients' genotype and stronger indications of an allelic influence were evidence in relation to ADPKD-*PKD1*. *PKD1* mutations have been mostly associated with early onset and more severe forms of the disease, although perinatal deaths in some rare severely affected *PKD2* patients were also described (Bergmann et al., 2008). Overall, the severity of ADPKD associated with *PKD1* can be explained by larger kidneys caused from the development of more number of cysts at earlier ages (about 20 years earlier), lower glomerular filtration rate (GFR), earlier decline in kidney function and hence earlier onset of ESKD (Harris et al., 2006). The median age of developing ESKD is 58.1 years in *PKD1* patients compared to 79.9 years in *PKD2* patients (Bergmann et al., 2018). Several studies made useful clinically prognostic value for type and position of *PKD1* mutations, where protein-truncating mutations were associated with severe renal features and impact patient survival (Rossetti et al., 2007, Hwang et al., 2016). However, more recent large-population studies have reported no clear difference among mutation types in relation to either gene and uncertain impact of *PKD1* mutation position have been proposed (Heyer et al., 2016a). Less allelic influences were correlated with the development of sever PLD and vascular anomalies, which have been attributed to mutations in both genes (Harris et al., 2006). Independent of mutation or allelic affect, ADPKD is more sever in males, although over 80% of patients are females, postulating the strong impact of environmental factors and hormonal variations in renal disease outcomes (Heyer et al., 2016a).

Current studies of human and mice showed that genetic inactivation of both *PKD1* or *PKD2* alleles due to full inactivating mutations is embryonically lethal (Ong and Harris, 2015, Bergmann et al., 2018). In contrast, patients with biallelic mutations with at least one incompletely penetrant (hypomorphic) allele reported to survive, illustrating the impact of hypomorphic alleles in reducing the expression and activities of polycystin proteins and proposing a threshold or dosage mechanism in cyst formation (Hopp et al., 2012, Ong and Harris, 2015). The severity of biallelic hypomorphic *PKD1* or *PKD2* mutations can range from typical to severe form of ADPKD. For instance, neonatal-onset

ADPKD was reported in a patient with incompletely penetrant homozygous *PKD2* mutation caused by uniparental disomy as well as two incompletely penetrant *PKD1* alleles were reported in sever ARPKD-like patients (Rossetti et al., 2009, Vujic et al., 2010, Losekoot et al., 2012). Furthermore, the coinheritance of inactivating allele *in trans* with a second hypomorphic allele can also result in severe form of ADPKD detected in utero (Rossetti et al., 2009, Bergmann et al., 2018). On the other hand, monoallelic hypomorphic mutations alone may result in mild cystic kidney disease where patients develop fewer numbers of cysts presenting later in adulthood and their kidney functions are unlikely to deteriorated (Cornec-Le Gall et al., 2018).

Although ADPKD is mostly a late-onset disease, rare severe cases manifesting in utero with massive cystic kidneys and oligohydramnios leading to fetal death were also reported (Rossetti et al., 2009, Vujic et al., 2010). In fact, up to 3% of children with mutations in PKD genes show very-early onset and severe unusual fast progressing form of ADPKD (Gimpel et al., 2019). This make the total incidence of symptomatic ADPKD in children greater than that expected for other serious paediatric kidney diseases, including ARPKD, nephrotic syndrome and haemolytic uraemic syndrome (Gimpel et al., 2019). Different types of genetic alterations have been reported in severely affected ADPKD children. Rarely, patients with digenic disease involving bilineal inheritance of both *PKD1* and *PKD2* mutations had been observed with more severe form than that of single gene-linked phenotype (Pei et al., 2001). In addition, coinheritance of pathogenic allele in other cystogenes, such as *HNF1B* along with a pathogenic *PKD1* or *PKD2* mutation has been connected with very early-onset ADPKD cases (Bergmann et al., 2011). Deletions of the geomic region of *PKD1* and the neighbouring tuberous seclerosis complex gene *TSC2* was linked with more severe childhood ADPKD often leading to kidney failure a combined with clinical symptoms of tuberous seclerosis, also known as a contiguous gene syndrome (CGS) (Sampson et al., 1997, Rossetti et al., 2009). Of note, milder typical ADPKD renal phenotypes with TSC were also reported in patients with mosaic deletions CGS (Sampson et al., 1997, Cornec-Le Gall et al., 2018).

Clinically, ADPKD is most frequently determined thorough abdominal imaging-based diagnosis using the most available inexpensive radiological method ultrasonography (US) or the more sensitive magnetic resonance imaging (MRI). There are age-dependent cyst number criteria that are widely used in clinics for the diagnosis of suspected or in-risk individuals with a positive family history (Gimpel et al., 2019). Although, ADPKD is

typically transmitted as dominant trait throughout generations, approximately 10-25% of cases occur sporadic without a recognisable family history, reflecting diagnostic challenge (Iliuta et al., 2017). Lack of parental medical records, sporadic *de novo* mutations, germline or somatic mosaicism with lower dosage in leukocyte DNA or mild disease due to hypomorphic *PKD1* or *PKD2* alleles are the possible explanations for absence of familial history (Iliuta et al., 2017). Moreover, significant clinical variability in disease severity among twins, siblings and patients generating inter- and intra-familial variability proposed a critical role for other genetic, epigenetic or even environmental factors that are still unclear (Fain et al., 2005, Bergmann et al., 2018).

The PC1 protein is a large (4303 amino acid (aa)) integral membrane protein with a receptor-like structure comprising 11 transmembrane domains and an extracellular region containing different domains that are critical for cell-to-cell and cell-to-matrix interactions (Figure 1.4) (Ma et al., 2017). In contrast, PC2 (968 aa) is a large conductance nonselective cation channel that serve calcium transport (Figure 1.4) (Ma et al., 2017). Numerous evidences propose that PC1 and PC2 are localized at the plasma membrane and the primary cilium mediating fluid flow sensation and may function in the same mechanotransduction pathways (Ong and Harris, 2015). The present model preferred by researchers is that PC1 acts as a mechanosensor (reacts to alterations in mechanical stimuli) or a chemosensor (reacts to chemical signals), and that PC1 modulates the activity of the PC2 calcium channel based on signaling responses (Ma et al., 2017). Both PC1 and PC2 interact with each other via their coiled-coil motifs in their cytoplasmic region constructing a heterodimeric polycystin complex (Ong and Harris, 2015). Different studies showed that the development of clinical phenotypes associated with ADPKD, including renal and hepatic cysts as well as the development of cardiovascular defects is correlated with the reduced expression (haploinsufficiency) or loss of PC proteins function in the related cells as evidenced in haploinsufficient and hypomorphic models of ADPKD (Ong and Harris, 2015). Along with PCs dosage effect, it is commonly believed that a second hit hypothesis, where further acquired somatic mutation is necessary for the initiation of cystogenesis, is critical in the pathogenesis of ADPKD (Harris and Torres, 2009).

The process of cyst growth in ADPKD is gradual causing massive cystic kidneys that are characterized by fluid-filled cysts distributing all over the renal parenchyma (Bastos and Onuchic, 2011). Cysts differ in their sizes and cyst fluid found to be clear, cloudy or even

dark due to bleeding, watery or thick. Although cysts in ADPKD may developed from all segments, those emerged from the collecting ducts are larger in sizes and in enormous numbers than those derived from other origins (Bastos and Onuchic, 2011). The majority of cysts in ADPKD are lined by a particular layer of poorly distinguished epithelial cells, despite the minority are lined by a remarkable hyperplastic epithelium (Bastos and Onuchic, 2011). In progressive ADPKD kidneys, interstitial fibrosis may result in cyst-surrounding fibrosis.

Cellular and molecular studies of DNA samples isolated from the epithelial cells of affected kidneys showed that even though ADPKD has dominant mode of inheritance, the mechanism of cystogenesis is recessive. The process of cyst formation according to the two-hit model involves the germline mutation in *PKD* genes, which initiates the first hit, along with a further somatic mutation in the originally normal allele, which creates the second hit. The mechanism of two-hit model, which involves cyst formation in kidneys and liver, was supported by a wide range of genetically manipulated orthologous mouse models of this disease (Bastos and Onuchic, 2011). However, the initially anticipated two-hit model has been further extended following the observation that the biological effect of *PKD1* genes inactivation on cyst formation depends on the inactivation time, where early gene inactivation causes rapid and diffuse formation of the renal cysts (Piontek et al., 2007). Such observations highlighted the biological consequences of *PKD1* inactivation that are regulated by developmental switch defining the completion of the kidney maturation process (Piontek et al., 2007). Several studies showed that the *PKD* genes inactivation induced in adult life would not be sufficient to establish the necessary cell proliferation for rapid cyst growth (Bastos and Onuchic, 2011). Therefore, it has been hypothesized that a third-hit model is demanded along with the inactivation of both *PKD* alleles for significant cyst formation in the mature kidneys (Takakura et al., 2009). Kidney injuries, such as ischemic or toxic damages, which elicit a repair response, may represent a third-hit stimulating a rapid cellular proliferation that may be a precondition for the occurrence of rapid cyst growth following somatic mutation or in the presence of reduced polycystin expression (Takakura et al., 2009). Thus, a third-hit stimuli may explain the late occurrence of disease long after the original genetic defect (Takakura et al., 2009). Recently, Torres et al. (2019) proposed that ischemic or toxic renal injuries are rare events in human, and hypothesized that cystogenesis in PKD may be enhanced by the frequently happening renal crystal deposition.

In ADPKD, high levels of proliferation and apoptosis, abnormalities in cell differentiation and planar cell polarity, alterations in salt and fluid transport from reabsorptive to a secretory behaviour and extracellular matrix alterations are the main characteristic phenotypes of the cyst-surrounding epithelial cells (Bastos and Onuchic, 2011). These abnormal cellular features are accompanied by reduction in PC1 and PC2 expression (dosage) below critical ranges, highlighting the crucial role of polycystins in regulating cell proliferations and in preserving a characteristic phenotype of renal tubular epithelium. The molecular basis behind these cellular alterations is not fully clear; however some primary clues have been emerged from the detection of altered signaling pathways in the disease. The mechanism of polycystins dosage seems to cause cystogenesis by disturbing cell homeostasis associated signaling pathways, including Ca^{2+}, cAMP, mammalian target for rapamycin (mTOR) and Wnt signalling (Bastos and Onuchic, 2011). At cell adherens junctions, PC1 is found to complex with E-cadherin and α-, β- and γ-catenins, however, in ADPKD the PC1/E-cadherin complex is interrupted since the reduction in Ca^{2+} maintain PC1 and E-cadherin in the cytoplasm, reflecting the role of PC1 in controlling the development of proper adherens junctions (Bastos and Onuchic, 2011). Furthermore, the detected endothelial dysfunction and the decreased in nitric oxide formation in ADPKD patients support the expression of PC1 and PC2 in endothelium and vascular muscle cells and play a complex function in the vascular integrity conservation (Qian et al., 2007).

The PC1-PC2 complex functions as a sensor in the primary cilium, facilitating signal transduction by Ca^{2+} signaling through PC2, which regulates critical cellular processes including cell proliferation, differentiation, apoptosis, and gene expression (Bastos and Onuchic, 2011). PC2 is also critical component that contributes in intracellular Ca^{2+} homeostasis in the ER. Therefore, in an absence, reduction or overexpression of PC2, as in ADPKD cyst cells, the release of Ca^{2+} from intracellular and endoplasmic reticulum stores is decreased and the ciliary fluid-flow is bent leading to defective Ca^{2+} signaling, which apparently is responsible for intracellular accumulation of cAMP (Bastos and Onuchic, 2011). In normal kidney epithelial cells, cAMP inhibits proliferation, while in ADPKD cells abnormal cell proliferation occurs in response to cAMP accumulation, which is followed by the enrolment and phosphorylation of different varieties of proteins and activation of mitogen-activated protein kinase pathway

stimulating proliferation and Cl^- and fluid secretion contributed on cyst growth (Bastos and Onuchic, 2011).

Cell cycle dysregulation is another important feature of PKD and different studies showed a direct association between polycystins and regulation of cell cycle. For instance, PC1 were found to have a significant inhibitory impact on cell proliferation through activation of JAK2 and mediating G0/G1 arrest (Bastos and Onuchic, 2011). In ADPKD, the observed changed in the activity of AP-1 transcription factor, which is activated by PC1, may contribute to the detected alterations in cell differentiation, proliferation and apoptosis (Parnell et al., 2002). Additionally, the interaction of PC1 with the tuberin, *TSC2* gene product, at the plasma membrane level, stops its phosphorylation thus stabilizing the tuberin-hamartin complex, which in turn inhibits the mammalian target for rapamycin (mTOR) (Bastos and Onuchic, 2011). However, in ADPKD, the disturbance of this mechanism activates mTOR and stimulates cell proliferation (Bastos and Onuchic, 2011). On the other hand, PC2 can reduce cell proliferation by stimulating pancreatic extracellular signal-regulated protein kinase-dependent phosphorylation of the translation initiation factor 2 alpha (eIF2α) in the ER (Liang et al., 2008).

Anomalies of planner cell polarity are an important factor that contributes to ADPKD cyst expansion by disturbing the oriented epithelial cell division along the longitudinal axis, which is critical for tubule formation and elongation (Bastos and Onuchic, 2011). Studies of animal models showed that the disoriented cell division is not the main event eliciting or initiate cystogenesis (Bastos and Onuchic, 2011). In fact, it was found that together canonical and non-canonical Wnt signaling pathways are associated with cyst growth in ADPKD, as the disruption of *PKD1* can activate the Wnt/β-catenin signaling pathway (Bastos and Onuchic, 2011).

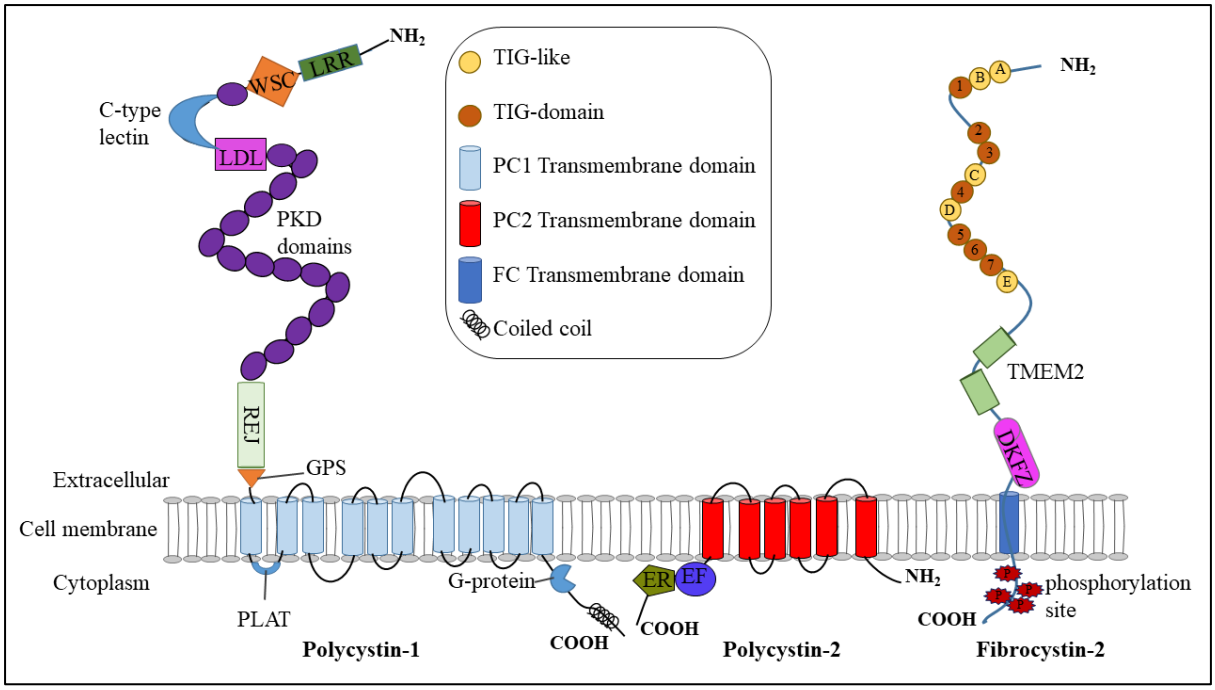

Figure 1.4 Schematic representations of the predicted molecular structure of the ADPKD proteins polycystin-1 (PC1) and polycystin-2 (PC2) and the ARPKD protein fibrocystin (FC). PC1 is an 11-segment integral membrane protein that consists of a long N-terminal (NH_2) extracellular region, which composed of 16 copies of PKD domain and other critical domains, and a short intracellular C-terminus (COOH). PC2 is a large non-selective cation channel with high permeability for calcium. PC2 consists of six transmembrane domains and both terminal ends are intracellular or inside cell organelles. Together, PC1 and PC2 facilitate calcium entry into cells. FC consists of a large N-terminal extracellular domain containing various glycosylation sites, a single transmembrane segment and a short C-terminus with four potential protein kinase A phosphorylation sites. Protein motifs and domains found in the schematic structure are described below. PC1 contains the following motifs and binding domains: G-protein: G protein binding; GPS: G protein–coupled receptor proteolysis site; LDL: low-density lipoprotein–like region; LRR: leucine-rich repeat; PKD domains: polycystic kidney disease domain repeat; PLAT: PC1-lipoxygenase, alpha (α) toxin; REJ: receptor for egg jelly domain; WSC: cell wall integrity and stress-response component. PC2 contains the following domains: EF: calcium-binding helix-loop-helix motif comprising of two helixes, E and F. ER: ER retention signal. FC contains the following domains: DKFZ: DKFZ Homology;TMEM2: Transmembrane protein-2 homology.

1.2.1 *Autosomal recessive polycystic kidney disease (ARPKD)*

ARPKD is one of the most prevalent inherited PKD in infants and children with an estimated incidence of 1:20,000 to 1:40,000 live births, resultant in a carrier frequency of approximately 1:70 in non-isolated population (Zerres et al., 1998, Bergmann et al., 2018). Higher incidence of ARPKD is predicted in isolated or inbred populations with more frequencies of consanguineous marriages, such as the described incidence of 1:8,000 in Finland (Kaariainen, 1987).

Classically, ARPKD is characterized by bilateral enlarged kidneys with multiple cysts mostly developed in distal tubules and collecting ducts, consequently leading to impairment of kidney function (Table 1.2). Congenital hepatic fibrosis due to ductal plate malformation is another typical feature of ARPKD that lead to serious complications including portal hypertension, hypersplenism and oesophageal varices (Bergmann et al., 2018) (Table 1.2). ARPKD often manifest much earlier than ADPKD, either prenatally displaying Potter's features or postnatally afterbirth, during childhood or at early adolescence. However, some rare cases of elderly ARPKD patients occurring with mild features were reported (Bergmann et al., 2018). Approximately 50% of ARPKD patients manifest with their renal complications as neonates and are already born with two massively enlarged kidneys (Capisonda et al., 2003). After birth, respiratory deficiency caused by pulmonary hypoplasia is the major cause of death in approximately 30-50% of infants, while kidney failure is rarely causing neonatal death (Bergmann et al., 2005). An optimistic long-term prognosis is predicted for those surviving the neonatal period, where 82% suspected to survive for 10 years, and rarely survived beyond the age of 60 years (Fonck et al., 2001, Bergmann et al., 2018). Nevertheless, ARPKD is still recognized as a severe leading cause of serious renal and liver-associated morbidity in children, which sometimes requires a kidney-, liver- or combined kidney and liver transplantation (Bergmann et al., 2018).

ARPKD disease is caused by mutations in polycystic kidney and hepatic disease 1 (*PKHD1*) gene (located 6p12.3-p12.2), which is one of the largest human genes encompassing at least 86 exons. The longest open reading frame transcript (NM_138694.4) consists of 67 exons and predicted to encode a 4074 aa receptor-like protein called fibrocystin (FC), also known as polyductin (Harris, 2009).

Illustrating a high level of *PKHD1* allelic heterogeneity, at least 700 distinct mutations were described throughout the gene in ARPKD/*PKHD1* database (http://www.humgen.rwth-aachen.de/). Despite allelic complexity, different efforts to examine genotype-phenotype correlation of this disease were conducted, where the type of mutations rather than its location on the *PKHD1* determine disease outcome (Bergmann et al., 2003, Bergmann et al., 2004a). Two truncating mutations are described to be lethal, although few exceptional cases were reported, and the influence of some missense mutations is found to be similar to truncating mutations. Major inter- and intra-familial clinical differences were documented among ARPKD patients, suggesting the impact of additional genetic and environmental factors on disease severity (Bergmann et al., 2005a).

Despite been a typical infantile-onset disease, ARPKD may symptom later in life with ADPKD-like mild renal features in addition to congenital hepatic fibrosis instead of PLD that should orient the diagnosis (Heyer et al., 2016a). Notably, in about 15% of ARPKD patients who carry *PKHD1* monoallelic mutations hepatorenal findings were reported including enlarged kidney echogenicity and the development of many tiny liver cysts (phenocoping ADPLD), suggesting the impact of other genetic and/or environmental factors must be demanded to manifest monoalleic cystic phenotype (Gunay-Aygun et al., 2011).

In some ARPKD patients with moderate renal phenotype and were not linked to *PKHD1*, mutations in the *DZIP1L* gene (located chr3q22.3) were detected (Lu et al., 2017). *DZIP1L* encode for DAZ-interacting zinc finger protein 1-like protein (DZIP1L), which present in the centrioles and the distal ends of basal bodies (Lu et al., 2017). Since DZIP1L interacts with septin2, a transition zone protein that involved in maintenance of periciliary diffusion barrier, impairment of diffusion barrier caused by *DZIP1L* mutation compromised the localization of PC1 and PC2 proteins (Lu et al., 2017). A correlation of type of *DZIP1L* mutations in disease severity is not yet defined, which will be enhanced by the evaluation of more *DZIP1L*-associated families.

Remarkably, ADPKD can mimic the phenotype of ARPKD, where mutations in *PKD1* and *PKD2* can show recessive mode of inheritance (Bergmann et al., 2018). To be precise, the inheritance of either one inactivating in combination to one hypomorphic or two hypomorphic *PKD1* and *PKD2* were detected in severely affected ADPKD patient

with typical ARPKD-like clinical presentation and without obvious family history of PKD. The genetic spectrum of ARPKD is further expanded following linking phosphomannomutase 2 (*PMM2*) gene to a combined hyperinsulinemic hypoglycemia (HI) and polycystic kidney disease (HIPKD) disorder (Cabezas et al., 2017). Recently, a promoter mutations in *PMM2* have been detected in patients with ARPKD-like and HI clinical features, although this gene was previously associated with congenital disorder of glycosylation type 1a (CDG1A), a severe multi-organ disorder characterized by various neurological anomalies, highlighting *PMM2* pleiotropy (Cabezas et al., 2017). Of note, *PMM2* enzyme is critical in N-glycosylation and impaired gycosylation has been linked with PKD (Cabezas et al., 2017).

FC is an integral membrane protein consisting of single transmembrane domain, a broad extracellular N-terminal domain and a short C-terminal cytoplasmic tail (Figure 1.4). Higher levels of expression of FC are found in adults' kidneys, liver and pancreas (Bergmann et al., 2018). Generally, FC is located to apical plasma membrane and the primary cilia (mainly basal bodies) in renal tubular and biliary epithelial cells (Zhang et al., 2004; Wang et al., 2007). Through a special motif in its C-terminal tail, FC is targeted to the cilia membrane. FC is believed to experience Notch-like processing, where it is proteolytically cleaved to release cytoplasmic tail, which travels to the nucleus at which it assumed to modulates the expression of downstream genes involved in cyst formation (Kaimori et al., 2007). FC is found to interact with PC2 in the PC1-PC2 complex, thus FC is hypothesized to control renal tubular cysts formation and progression by modifying PC2 expression, highlighting a common cystogenesis mechanism modulating the two diseases (Zhang et al., 2004, Kim et al., 2008). Recently, FC was also localized to mitotic spindles and mitotic impairment caused by loss of function of FC is proposed to underlie cyst formation in ARPKD (Zhang et al., 2010). Despite these observations, complete understanding of detailed-cystogenesis mechanisms in ARPKD is still lack.

1.2.2 *Nephronophthisis (NPHP) and related disorders*

Nephronophthisis (NPHP) is an autosomal recessive inherited kidney disease that constitutes the most prevalent monogenic causes of ESKD in the first 3 decades of life, responsible for 2.4-15% of paediatric patients with ESKD (Hildebrandt et al., 2009, Luo and Tao, 2018). In Netherland, an estimated annual incidence of 1 to 5 children with NPHP developed ESKD (Stokman et al., 2018). The incidence of NPHP differ worldwide ranging between 1:50,000 live-births in Finland and Canada to 1:1,000,000 in the USA (Luo and Tao, 2018, Srivastava and Sayer, 2014).

Literally, the term NPHP is derived from the Greek and means 'disappearance of the kidney', which related to smaller kidney size with advancing kidney disease. The declined ability of kidneys to concentrate urine, chronic tubulointerstitial nephropathy and CKD are the major characteristic features of NPHP. The typical renal histological changes present with NPHP are similar to that of medullary cystic kidney disease (MCKD), including disruption of tubular basement membrane, interstitial fibrosis, tubular atrophy and dilated tubules with or without cyst (Hurd and Hildebrandt, 2011). Initially ultrasonography of NPHP patients does not show any specific changes, however, later with disease progression individuals are characterised by normal or reduced-size hyperechogenic kidneys with small corticomedullary cysts (1.5 cm) and poor CMD (Hurd and Hildebrandt, 2011, Srivastava and Sayer, 2014).

Polyuria, polydispsia and secondary enuresis caused by impairment of distal tubular function are the typical clinical features of NPHP. Urine analysis of NPHP patients generally does not illustrate any characteristic anomalies, thus proteinuria and haematuria often found at later stages, where proteinuria may advance into glomerulorsclerosis (Luo and Tao, 2018). Arterial hypertension, severe anaemia and growth retardation are usually present later after kidney failure (Wolf, 2015). Such mild and non-specific symptoms may delay the diagnosis of the disease by an average of 3.5 years (Soliman et al., 2012).

NPHP is generally associated with progressive deterioration of kidney function leading to ESKD before the age 30 years. Thus, on the basis of the median age of ESKD development, NPHP is clinically distinguished into three subgroups: infantile, juvenile and adolescent (Table 1.3) (Hildebrandt et al., 2009). The classical and most common form, juvenile NPHP, which was initially introduced in 1951 by Fanconi et al, is characterized by ESKD at a mean age of 13 years and symptoms manifesting within first

decade of life (Hildebrandt et al., 2009). Infantile NPHP is a very rare type with sever phenotypes at which the ESKD usually developed during first years of age, while the third type adolescent NPHP has a median age of ESKD of 19 years (Wolf, 2015). Beside this historical classification, different reports of late-onset NPHP have been described, where patients developed ESKD beyond third decade of life (23 and 56 years), extending the age of ESKD from birth to 60 years and highlighting the important of considering NPHP diagnosis in adults with ESKD with unclear causes(Georges et al., 2000, Wang et al., 2019).

	Infantile NPHP	Juvenile NPHP	Adolescent NPHP
Median age of ESKD (years)	1 year	13 years	19 years
Clinical features	Antenatal oligohydramnios sequence and severe hypertension	Polyuria, polydipsia, secondary enuresis, CKD, severe anemia, growth retardation, proteinuria (later stages), normal blood pressure	Similar to juvenile NPHP
Radiological features	Large kidneys with large cortical microcysts	Normal-sized or shrunken, hyperechogenic kidneys with corticomedullary cysts and poor CMD	Similar to juvenile NPHP
Extrarenal associations	Liver fibrosis, cardiac anomalies (*situs inversus* and ventricular septal defects), frequent respiratory tract infections	Retinitis pigmentosa, cerebellar vermis aplasia/hypoplasia, liver fibrosis and skeletal anomalies	Similar to juvenile NPHP
Genes	Typically: *INVS and NPHP3.* *NEK8*, *TTC21B*, *ZNF423*, *CEP83*	All genes except *INVS*	*NPHP3, NPHP4, NEK8*

Table 1.3 Clinical, histological and genetic characteristics of nephronophthisis (NPHP) subtypes. CKD, chronic kidney disease; CMD, corticomedullary differentiation; ESKD, end stage kidney disease.

NPHP may present as an isolated disorder limiting the phenotypes to the kidneys or as a part of multisystem disease associated with extrarenal organ involvements, such as retina, CNS, liver and bones, presenting in up to 20% of NPHP cases (Luo and Tao, 2018). Therefore, NPHP is a main clinical feature found in several well described complex syndromes, so called NPHP-related ciliopathies (NPHP-RC), such as SLSN, JBTS, MKS and Jeune syndrome. The syndromic association of NPHP with retinopathy is very common presenting in approximately 10-15% of cases and showing broad pathological spectrum (Table 1.4) (Srivastava and Sayer, 2014). Moreover, NPHP can be accompanied by neurological anomalies, such as cerebellar vermis hypoplasia, encephalocele and hypopituitarism, by hepatic fibrosis, as well various skeletal defects, such as short ribs , cone-shaped epiphysis and postaxial polydactyly (Table 1.4) (Braun and Hildebrandt, 2017). In rare cases, additional manifestations including laterality defects, congenital heart disease, ulcerative colitis and potential lung involvement are associated with NPHP (Table 1.4) (Wolf, 2015, Braun and Hildebrandt, 2017). The severity and overlapping of clinical presentations with other ciliopathies may complicate an earlier diagnosis of NPHP-RC, though clinical diagnosis may be confimed by genetic testing.

NPHP illustrates excessive phenotypic and genetic heterogeneity with at least 25 different recessive genes have been linked with the disease (Luo and Tao, 2018). These are *NPHP1*, *NPHP2*/*INVS*, *NPHP3*, *NPHP4*, *NPHP5*/*IQCB1*, *NPHP6*/*CEP290*, *NPHP7*/*GLIS2*, *NPHP8*/*RPGRIP1L*/*MKS5*, *NPHP9*/*NEK8*, *NPHP10*/*SDCCAG8*/*SLSN7*, *NPHP11*/*TMEM67*/*MKS3*, *NPHP12*/*TTC21B*/*JBTS11*, *NPHP13*/*WDR19*, *NPHP14* /*ZNF423*, *NPHP15*/*CEP164*, *NPHP16*/*ANKS6*, *NPHP17*/*IFT172*, *NPHP18*/*CEP83*, *NPHP19*/*DCDC2*, *NPHP20*/*MAPKBP1*, *NPHP1L*/*XPNPEP3*, *NPHP2L*/*SLC41A1*, *TRAF3IP1*, *AH11*/*JBTS3* and *CC2D2A*/*MKS6* (Luo and Tao, 2018). Mutations in NPHP genes can explain only up to one-third of cases and around 60% of cases remain genetically unsolved, proposing further genes have yet to be revealed (Konig et al., 2017, Luo and Tao, 2018). The most common genetic cause of NPHP is mutations in *NPHP1* gene explaining up 20% of cases, while mutations in each of the remaining genes possibly contribute up to 1% of cases (Srivastava and Sayer, 2014, Luo and Tao, 2018). A large homozygous deletion of the whole *NPHP1* gene (290 kb, chromosome 2q13) was the first genetically identified cause of NPHP and the most frequent *NPHP1* defect causally associated with isolated-NPHP (Srivastava et al., 2017). Recent study showed that homozygous deletion of the same genomic region was not only associated with

typical kidney presentations, but also was able to present extrarenal manifestations, such as retinal degeneration associated with SLS and neurologic phenotype associated with JBTS (Konig et al., 2017). Remarkably, mutations and copy number variants (CNVs) of *NPHP1* have also been reported with BBS, suggesting rare principal cause of this disorder (Lindstrand et al., 2016).

The genotype-phenotype correlation in NPHP-RC appears to be affected by gene and variants heterogeneity as well as modifier genes (Chaki et al., 2011). Mutations in a particular gene may result in an enormous spectrum of clinical phenotypes ranging from isolated NPHP, NPHP-RC to a potentially severe neonatal disease associated with MKS (Srivastava et al., 2017). For instance, mutations in *CEP290* can lead to NPHP, SLNS, JBS or MKS and mutations in *AHI1* can lead to NPHP or JBTS (Luo and Tao, 2018). Genotype-phenotype correlation studies have indicated some association between the type of mutations in some genes and the severity of the disease, where truncating mutations in *CC2D2A* and *TMEM67* have been implicated with potentially severe clinical presentations compared to missense mutations (Mougou-Zerelli et al., 2009, Srivastava et al., 2017). Despite that, difference in phenotypic heterogeneity in NPHP may inefficiently be clarified by single locus allelism and only digenic inheritance and triallism may provide some clarifications (Luo and Tao, 2018). For example, NPHP accompanied by severe neurological symptoms by the inheritance of a heterozygous mutation in NPHP6 or *AHI1* (p.Arg830Trp) in combination to biallelic NPHP1 mutations (Tory et al., 2007). Additionally, oligogenic inheritance have been documented in some patients with mutations in several NPHP genes, including *NPHP1*, *NPHP5*, *NPHP6*, *NPHP8*, *NPHP9*, *NPHP11* and *TTC21B*, suggesting a possibility of an epistatic interaction of NPHP mutations and modifier impacts of other causative variants (Penchev et al., 2017, Luo and Tao, 2018).

Almost all NPHP genes encode nephrocystins that localize to transition zone (*NPHP1, NPHP4, CEP290, RPGRIP1L, TMEM67* and *CC2D2A)*, inversin compartment (*INVS*, *NEK8*, *ANKS6*) components of IFT complexes (*TTC21B*, *WDR19*, *IFT172*), or other locations within primary cilia (Srivastava et al., 2017, Luo and Tao, 2018). However, NPHP-like genes such as *XPNPEP3* and *SLC41A1* with protein products located to mitochondria and *GLIS2* gene with protein product located to nucleus have also been implicated with NPHP, highlighting that NPHP-associated genes might not entirely be ciliary (O'Toole et al., 2010). Recently, on the basis of their location and functions,

protein interaction studies grouped the NPHP proteins into four different nephrocystin modules: the NPHP1-4-8 (NPHP1, NPHP4, and RPGRIP1L) module, NPHP2-3-9-ANKS6 (INVS, NPHP3, NEK8 and ANKS6) module, NPHP5-6 (IQCB1 and CEP290) module and the MKS module (MKS1, CC2D2A, and TCTN2). These modules are associated with different ciliary signaling pathways including Hedgehog, Wnt, cAMP signaling pathways as well as mTOR pathway. Additionally, some NPHP proteins (such as NEK8, CEP164, ZNF423, SDCCAG8 and CEP290) have been associated with the nuclear DNA damage response (DDR) signaling pathways that is believed to be critical in the origination and development of NPHP disease (Slaats and Giles, 2015).

Extraarenal manifestations	Syndrome
Retinitis pigmentosa (RP)	Senior-Løken syndrome (SLSN)
	Arima syndrome (cerebro-oculo-hepato-renal syndrome)
	Alström (RP, obesity, DM type 2, hearing impairment)
	RHYNS (RP, hypopituitarism, skeletal dysplasia)
Oculomotor apraxia	Cogan syndrome
Nystagmus	Joubert syndrome/Joubert syndrome related disorders
Coloboma	Joubert syndrome/Joubert syndrome related disorders
Encephalocele	Meckel-Gruber syndrome (occipital encephalocele, NPHP)
Vermis aplasia	Joubert syndrome/Joubert syndrome related disorders
	COACH
Hypopituitarism	RHYNS (RP, hypopituitarism, NPHP, skeletal dysplasia)
Liver fibrosis	Boichis syndrome
	Meckel-Gruber syndrome (occipital encephaolocele, NPHP)
	Arima syndrome (cerebro-oculo-hepato-renal syndrome)
	Joubert syndrome/Joubert syndrome related disorders
Postaxial polydactyly	Joubert syndrome/Joubert syndrome related disorders
	Bardet-Biedl syndrome (NPHP, RP, obesity, deafness)
	Ellis van Creveld
Skeletal abnormalities	Jeune syndrome/asphyxiating thoracic dystrophy
	Mainzer-Saldino syndrome
	Sensenbrenner syndrome/cranioectodermal dysplasia
	Ellis van Creveld
Situs inversus, cardiac anomalies, bronchiectasis	infantile NPHP

Table 1.4 Extrarenal characteristics of nephronophthisis (NPHP) and the associated syndromes. RP, retinitis pigmentosa.

1.2.3 *Syndromic ciliopathies*

JBTS is an autosomal recessive disease with an estimated prevalence of 1:80,000 and 1:100,000 (Parisi, 2019). It is characterized by neurological anomalies of cerebellar vermis and brainstem defects on cranial views by MRI known as the "molar tooth sign". Typical clinical features of JBS include episodic breathing abnormalities, hypotonia that developed into ataxia, and developmental delay or cognitive impairment. Some JBTS patients exhibit neuropathological features exclusively, while other present with multisystem organ features including liver and kidney fibrosis, retinal pigmentosa, occipital encephalocele, and polydactyly (Vilboux et al., 2017). Amongst 23–32% of patients with JS have renal disease either as NPHP or cystic kidney disease resembling ARPKD, and kidney failure is the leading cause of death in those over 5 years of age (Parisi, 2019). By now, 35 causative genes have been associated with JBTS , most of which can explain less than 10% of cases while some of them contribute to only one or counted cases (Parisi, 2019). These include *CEP104*, *NPHP1*, *TMEM237*, *ARMC9*, *PDE6D*, *ARL13B*, *CC2D2A*, *CPLANE1*, *CEP120*, *AHI1*, *CEP41*, *CSPP1*, *TMEM67*, *INPP5E*, *TCTN3*, *SUFU*, *ARL3*, *TMEM138*, *TMEM216*, *CEP290*, *POC1B*, *TCTN1*, *TCTN2*, *PIBF1*, *KIAA0586*, *KIF7*, *KATNIP*, *ZNF423, RPGRIP1L*, *TMEM231*, *TMEM107*, *B9D1*, *MKS1*, *B9D2* and *OFD1*.

BBS is a rare complex multisystem syndromic ciliopathy characterised by multiple clinical manifestations including mental retardation, behavioural anomalies and obesity, retinitis pigmentosa, cystic kidney disease, polydactyly and gonadal anomalies. Premature death occurs frequently on individuals with BBS due to kidney failure (Tobin and Beales, 2009). The worldwide prevalence of BBS differs significantly from 1:160,000 newborns in northern European populations to 1:13,500 in the Bedouins of Kuwait and 1:17,500 in Newfoundland where the communities are isolated and characterized by higher levels of consanguinity (Forsythe and Beales, 2013). Typically, BBS has an autosomal recessive mode of inheritance, although oligogenic hereditary has also been described sporadically (Lindstrand et al., 2016). To date, mutations in 20 monogenic genes (*BBS1* to *BBS20*) have been associated with BBS, displaying extensive genetic heterogeneity (Braun and Hildebrandt, 2017, Priya et al., 2016).

At the end of ciliopathy spectrum MKS represents the most severe clinical features of human ciliopathies involving all organ systems. It is an autosomal recessive congenital

developmental disease characterized by high phenotypic pleiotropy and extreme genetic heterogeneity, (Hildebrandt et al., 2011). The global incidence of MKS varies between 1:13,250 and 1:140,000 live births (Hartill et al., 2017). Classical symptoms of this syndrome are the malformations of the CNS that seem to have diverse presentation, including Dandy–Walker malformation (hydrocephalus, microcephalus and most lethal complete anencephaly) and occipital encephalocele (Hildebrandt et al., 2011, Hartill et al., 2017). Affected patients frequently feature cystic-dysplastic kidney disease, liver association, congenital heart anomalies, pulmonary hypoplasia, dysmorphic features and skeletal anomalies mainly postaxial polydactyly, occipital encephalocele, and frequently congenital liver fibrosis (Braun and Hildebrandt, 2017). Because of the potentially severe developmental malformations, embryonic or perinatal death is constantly reported in MKS patients (Braun and Hildebrandt, 2017). The pathogenesis of MKS has been associated with at least 14 different genes(*MKS1*, *TMEM216*, *TMEM67*, *CEP290*, *RPGRIP1L*, *CC2D2A*, *NPHP3*, *TCTN2*, *B9D1*, *B9D2*, *TMEM231*, *C5orf42*, *CSPP1*, *KIF14*, *TMEM107*, *TXNDC15* and *CEP55*), most of which are mutated in NPHP, JBTS and BBS (Braun and Hildebrandt, 2017, Hartill et al., 2017). Mutations in these genes contribute to 50-60% of MKS cases and genotype-phenotype correlations suggested association between type of mutation and severity of developmental phenotypes (Braun and Hildebrandt, 2017, Hartill et al., 2017). Hypomorphic mutations are predicted to have degenerative impact with limited organ specific disease compared to truncating mutations that predispose to severe developmental disease (Braun and Hildebrandt, 2017).

1.3 Epidemiology of Kidney Disease in Oman

1.3.1 Demographics of Oman

Oman is the second largest country in the South East of Arabian Peninsula with surface area of 309,500 square kilometres (National Centre for Statistics and Information, 2017) (Figure 1.5). It is bounded by the Arabian Sea and the Gulf of Oman on the east, the Arabian Gulf (Persian Gulf) on the northwest and the desert of Rub' al Khali (Empty Quarter) of Saudi Arabia on the west (Figure 1.5). Oman is neighbour by the United Arab Emirates (UAE) on the north, Saudi Arabia on the west and Yemen on the southwest (Figure 1.5). The total population is estimated to be 4,414,051, of which 56% are Omani natives and 44% are non-Omani (National Centre for Statistics and Information, 2017). Oman has a relatively young population, where about 36.1% of the population is under 15 years of age, while only 5.9% are in the sixth decade and over (Ministry of Health Annual Health Report, 2016). The rate of annual population growth in Oman is considered high with approximately 33.7 per 1,000 total population (Ministry of Health Annual Health Report, 2016), compared to the UK, for example, which has 11.1 live births per 1,000 total population (66.4 million) in 2018 (Office for National Statistics (ONS), 2019). Omani population had gender ratio of 102 males for every 100 females and the life expectancy at birth has increased by 19.1 years since 1980 (57.8 years) to currently stand at 76.9 years (Ministry of Health Annual Health Report, 2016).

In Oman, family size is often large with an average of eight offspring per family (National Centre for Statistics and Information, 2017). Omani population has a unique structure consisting of varieties of tribal communities occupying a definite territory that is still conserved over generations despite modernization. Similar to other Muslim and Arab communities, the custom of consanguineous marriages is extremely conserved in Omani community, due to social, cultural, geographic and economic factors (Rajab et al., 2013). The percentage of consanguineous marriages is estimated to be high (56.3%) (Tadmouri et al., 2009), where first cousins marriages are the most favoured (16.8%), followed by second cousins (11.8%), double first cousins (4.8%) and first cousins once removed (2.6%) (Rajab and Patton, 2000). Furthermore, the tradition of within-tribe (endogamous) marriages is still favoured and preserved in this community, accounting for 20.4% of total marriages (Rajab and Patton, 2000).

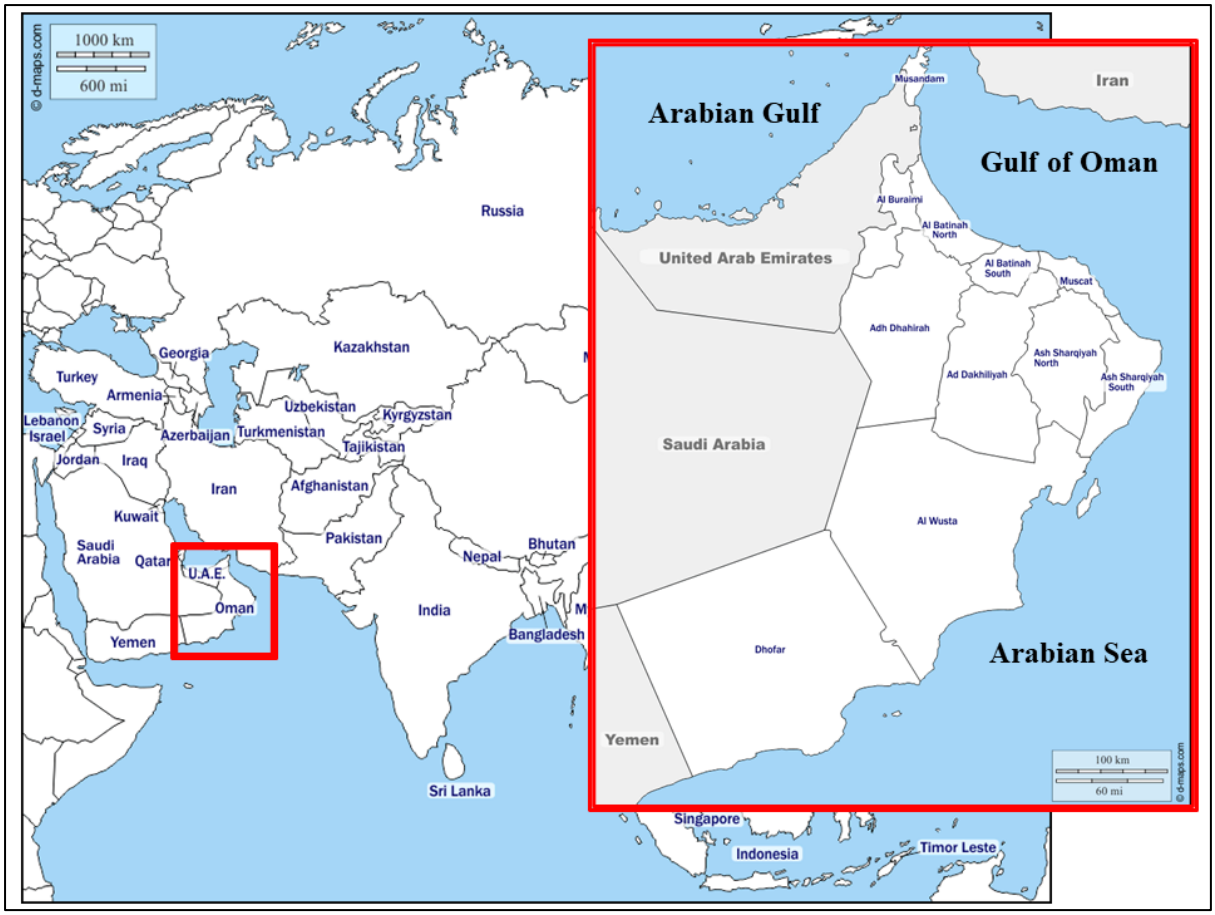

Figure 1.5 The geographical location of Oman. Maps adapted with modification from the d-maps.com (https://d-maps.com/carte.php?num_car=5160&lang=en).

1.3.2 Genetic disorders in Oman

Genetic data of Omani population has been published in different studies and can also be obtained through the Ministry of Health (MOH) information system (Al-Gazali et al., 2006, Rajab et al., 2013). Over 600 genetic diseases have been detected in Oman, of which recessive diseases are the most common category causing childhood mortality and morbidity (Rajab et al., 2013). Based on the MOH report in 2008, 39% of perinatal deaths in hospitals are associated with congenital malformations and genetic disorders, constituting a serious healthcare burden (Rajab et al., 2013). According to the WHO data, 17-43% of infants deaths in Europe recorded between 2005 and 2009 was assigned to congenital anomalies, with the highest rates were reported in Malta (43%) and Ireland (42%), while in the UK it accounts for 23% of infants deaths (Boyle et al., 2018).

The database of the Catalogue for Transmission Genetics in Arabs (CTGA) has shown that the number of disorders following autosomal recessive mode of inheritance in Arab countries, including Oman, is greater than that of autosomal dominant (Al-Gazali et al., 2006). The extended family and tribal structures tend to create exceptional patterns of genetic diseases, making rare disorders more frequent in Omani community (Tadmouri et al., 2009). Moreover, consanguineous and endogamous marriages are known to increase the risk of birth defects, congenital abnormalities, mental retardation and diverse recessive disorders. Higher frequencies of parental consanguinity (73%) were detected among Omani new-borns with major congenital malformations (Sawardekar, 2005).

The geography of Oman, its settlement and the preservation of unique population structure have produced a useful and advantageous population for the investigation of genetic diseases. The rapid growth rate of the population, large families and close family ties facilitate the study of large families with autosomal dominant diseases and provide significant statistical advantages. The presence of genetic isolates in the form of tribes and geographical settlement and the higher rate of consanguineous marriages facilitate the investigation of autosomal recessive diseases, where diseases can be linked with the tribes (Rajab et al., 2013). Therefore, different recessive clinical phenotypes and gene mapping had been described from Omani families, such as Robinow syndrome (Afzal et al., 2000) and Escobar syndrome (Rajab et al., 2005). Genetic studies of Omani families with different recessive diseases had led to detection of novel mutations in this population (Rajab et al., 2015).

1.3.3 Chronic kidney disease (CKD) in Oman

In Oman, CKD is a major health concern causing years of life lost (YLLs) due to premature mortality (Al Alawi et al., 2017b). The prevalence of ESKD patients receiving RRT in Oman at the end of 2013 was 655.8 per million population (PMP), with an incidence of 120 PMP (United States Renal Data System, 2014). Figure 1.6 illustrates the annual increase in the prevalence and incidence of ESKD in Omani from 2008 to 2013.

The epidemiology of CKD in Oman remains unexamined for a long period except from several single centre studies describing the clinical information of patients (Al-Lawati, 2013; Rajab *et al*., 2005). Only, recently, Al Ismaili et al., (2017) examined the epidemiological data of ESKD population (1983-2013) and illustrated that diabetic nephropathy (46%) was the major cause of kidney failure. Furthermore, Al Riyami *et al*., (2019) studied the epidemiology of CKD in Omani children (<14 years of age) of CKD stage (II-V) and showed that congenital anomalies of the kidney and urinary tract (CAKUT) (52.9%), hereditary kidney diseases (39%) and chronic glomerulonephritis (8.2%) are the leading causes of CKD. ARPKD (12%), primary hyperoxaluria (7%), familial focal segmental glomerular sclerosis (5%), congenital nephrotic syndrome (4%) and juvenile NPHP (3.4%) are the main inherited kidney disease observed the study cohort (Al Riyami et al., 2019).

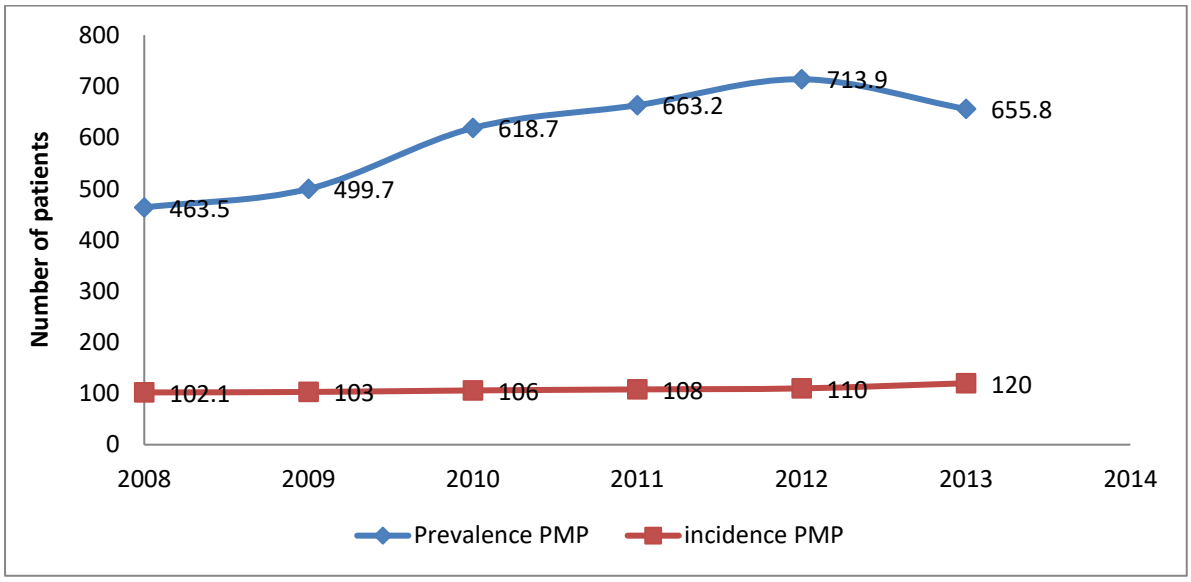

Figure 1.6 Prevalence and incidence of end stage kidney disease (ESKD) in Omani population from 2008 to 2013. PMP, per million population.

Some of hereditary kidney diseases are found to be more prevalent in Oman compared to worldwide figures, presumably due to high rates of consanguinity. An example, cystinuria, which is a rare genetic disease causing kidney stones, has prevalence of 4% compared to the worldwide prevalence of 1% (Al-Marhoon et al., 2015). In a hospital-based study evaluating the observed birth incidence of recessive genetic disorders from 1993 to 2002, autosomal recessive polycystic kidney disease (ARPKD) was the third most frequent disease with an incidence of 1 in 12,000 births, which is higher than the estimated prevalence for non-isolated population (Rajab et al., 2005).

A number of novel OMIM genes associated with inherited kidney disease and different mutations have been mapped for the first time in families from Oman (Rajab et al., 2015). For example, *MKS3* gene causing MKS was mapped for the first time in families from Oman and Pakistan (Khaddour et al., 2007). Furthermore, using autozygosity mapping of a large Omani BBS consanguineous family, White et al. (2007) confirmed that *FLJ23560* is the *BBS10* gene located at 12q21.2 and these families tend to have more severe clinical phenotypes compared to previously described features on the literature (White *et al*., 2007). Although inherited kidney diseases are major health concern in Oman, there is a limitation in the published studies covering the genetic molecular spectrum of these disorders.

1.4 Molecular Diagnosis of Inherited Cystic Kidney Disease

1.4.1 Sequencing technologies

From a diagnostic perspective, determination of causal genetic defect in individuals and families with inherited disease is crucial in order to consider accurate clinical diagnosis as well as to enhance medical treatment and supportive strategies. In addition, identifying causal mutation is important from genetic counselling view in terms of assessing the recurrence risk, screening at-risk individuals and recommending reproductive possibilities such as pre-implantation and prenatal diagnosis.

Following the completion of Human Genome Project (HGP) in 2003, the standard technique for causative mutation searching involved the amplification of the protein coding segments in the suspected causative genes by polymerase chain reaction (PCR) followed by Sanger sequencing (Steyaert et al., 2018). However, since its first emergence a decade ago, high throughput sequencing technologies, which also known as next generation sequencing (NGS), have revolutionized medical genetics research, increased sequencing reliabilities in routine genetic diagnosis and speed up the discovery of novel disease-causative genes. This is because NGS is by far quicker, demand less DNA amount, more accurate, much cheaper and less time consuming compared to Sanger sequencing. NGS provides massive genomic sequencing data through rapid parallel sequencing of selected disease-associated genes, all protein-coding regions (whole-exomes) or even entire human genome.

Generally, all NGS technologies follow a similar principle, but differ in the methodologies used to generate genomic templates and the way to read these sequences (Rizzo and Buck, 2012). The experimental process consists of two basic steps: (1) preparation of sequencing libraries and (2) massive parallel sequencing. Following DNA extraction, libraries preparation is the first step in NGS approach, which comprises the ligation of DNA fragments to certain oligonucleotide adapters specific for the NGS platform used and then clonally amplified these fragments.

Different types of NGS platforms have been developed, but the Ion Torrent sequencers (Life Technologies) and the Illumina sequencers (Illumina) are the most frequently utilized benchtop instruments in the biomedical laboratories for diagnostic purposes. Ion Torrent is based on emulsion PCR, where fragment amplification is performed on a mixture of oil-aqueous emulsion, whereas amplification in Illumina platforms is

performed on isothermal bridge amplification through the channels of the flow cells (Quail *et al*., 2012). For reading the bases in a nucleotide sequence, Illumina is based on the Solexa sequencing by synthesis chemistry, fluorescent detection of incorporated nucleotides, while Ion Torrent is based on the native dNTP chemistry, depending on pH measurement caused by hydrogen ions during base integration (Merriman and Rothberg, 2012; Quail *et al*., 2012). Despite differences of these two platforms, their effect on the genomics, transcriptomics and epigenomics is impressive due to the numerous amount of sequence data produced within an effective time and cost manner.

Targeted gene panel is a term used to describe the targeted sequencing of a subset of genes or certain genomic regions for evaluating the pathogenesis of specific group of diseases, at which some commercial disease-specific gene panels might be found or a customized panel with certain genes of interest might be designed. Targeted gene panel is a good starting diagnostic strategy for inherited disorders providing low costs, rational resources and manageable sequencing data for analysis (Sikkema-Raddatz et al., 2013). For the inclusion of genes to a clinical panel, the guidelines and recommendations of the American College of Medical Genetics and Genomics (ACMG) suggested the inclusion of only genes with enough scientific evidence for role in disease (Rehm *et al*., 2013). In targeted NGS approach, sequencing reads are distributed at a particular genomic areas facilitating higher sequencing representation and depth of coverage, and hence enabling precise variants identification. Prior sequencing, targeted DNA regions of interest are enriched using capture strategies such as hybridization and PCR amplification.

Whole exome sequencing (WES) is a technique that was developed to particularly capture and sequence the coding regions, or exons, of all known protein-coding genes, which collectively constitutes about 2% of the human genome (Clamp et al., 2007). The use of WES is based on the hypothesis that 85 % of the disease-associated alterations are confined in exons (Majewski *et al*., 2011). The early clinical application of WES was in examining rare Mendelian diseases, where the knowledge of responsible genes is not priori required and hence leading to discovery of novel genes. To overcome the limitation of interpretation an extensive list of genetic variants, researchers combined homozygosity mapping with WES to obtain an efficient and non-invasive technique for accurate diagnosis of renal cystic disease (Gee *et al*., 2014).

1.4.2 Variant annotation and prioritization

Sequencing platforms create millions of raw unmapped sequence reads and to address causal mutation detection, a comprehensive workflow can be applied to analyse the generated data. Compared to early days of NGS, substantial progress is made with respect to sequencing technology platforms, software and analysis pipelines. However, these data can still composed of potential errors caused by technological and biological biases as well as mechanical errors that can significantly affect downstream analyses (Taub et al., 2010). Errors can be generated from biases in target capture, base-calling sequence faults, doubts in read alignments, platform-specific technical errors and artificial chimeric reads (Taub et al., 2010). Therefore, establishing standards and guidelines for prioritizing the potential causality of genetic variant in human disease is a critical demand for clinical diagnostic setup (Richards et al., 2015).

After a proper quality evaluation, which mainly involved assessing FASTQ data, trimming of low quality reads and removal of adaptors and marking PCR duplicates, analysis starts by aligning sequence reads to a reference genome sequence in a process called read mapping. Subsequently, a variant calling algorithm is applied to detect differences between the sequencing reads and a reference genome, at which genomic variants in the form of single nucleotide variants (SNVs) or insertion/deletion (indel) can be detected. Raw variants are attained as Variant Call Format (VCF) and are ready for downstream analysis, which involved variants annotation, interpretation, filtration and prioritization. This process involves exclusion of enormous number of variants in order to conclude with one or limited candidate variants clarifying the phenotype of study patient.

Several functional annotation tools are used for variant annotation step that mainly involved adding important supplementary metadata and knowledge in order to improve evaluation of variants likely to influence function. The annotation process includes (1) citing the fundamental genomic locations such as affected gene(s), transcript(s) and other essential features such as exons, introns, and splice sites, (2) association with known human diseases and phenotypes (Online Mendelian Inheritance in Men (OMIM), ClinVar, Human Genome Mutation Database (HGMD), UniProt), (3) comparison to variants already established in variant population databases to determine known allele frequencies and (4) evaluating the effect on protein sequence and conservation.

In variant filtration, thresholds for quality metrics such as genotype quality (GQ) and depth of reads are applied for filtrating variants with erroneous variant call (Carson et al., 2014). Coverage of reads is generally used as an indicator of the accuracy of NGS experiment and is defined as the number of unique reads that contain a particular nucleotide. Read coverage is influenced by the accuracy of alignment algorithm used and mapping of the raw reads to the reference, which in turn affects variant calling accuracy (Steyaert et al., 2018). Notably, GQ score is calculated for each of the detected alteration stating the likelihood of the observed allele, which can also be used as a useful parameter for filtering those variants generated by technical errors (Steyaert et al., 2018).

Once a set of variants is selected after quality threshold filtering, further filtering based on an inheritance pattern and patient's clinical phenotype can be performed and used as strong genetic support for allocating likely pathogenicity to new gene variants detected by WES or WGS (MacArthur et al., 2014). The pattern in disorders with an autosomal dominant mode of inheritance is that candidate-variants are heterozygous in all affected patient within a family, while non-affected are homozygous for the reference allele. In contrast, in an autosomal recessive mode of inheritance, candidate-variants are usually inherited as two different compound heterozygous or less common one pathogenic homozygous in case of consanguineous parents, while non-affected siblings within a family are heterozygous or homozygous for the reference allele and un affected parents are heterozygous for one of the candidate variants. In routine clinical genetic diagnostics of presumed monogenic-disease cases, focus is made first on variants of gene previously implicated in similar human phenotypes, also known as Mendeliome, at which 3209 distinctive genes are linked to 4550 monogenic rare disorders (Steyaert et al., 2018). However, variants in other potential new genes can be suspected as causative in certain circumstances, at which a comprehensive experimental support is required including both functional and bioinformatics studies. Moreover, gathering several families with defects in the same gene and similar clinical presentation is fundamental to confirm confidently the effect of new candidate gene on phenotype (MacArthur et al., 2014). Notably, the scenario of spontaneous or *de novo*, where the candidate-allele in the affected patient is not found in either parent, should always be taken into consideration, where the accuracy depends on the availability of samples of unaffected family members (Jalali Sefid Dashti and Gamieldien, 2017). Furthermore, late onset, incomplete penetrance, and/or differential expressivity of the clinical phenotype are fundamental scenarios that should

be taken into consideration in case of no clear causative candidates remain after filtering prior to terminate the effort of searching for causal variants (Jalali Sefid Dashti and Gamieldien, 2017).

Additional filtration can be performed using the genomic location interpretations and variant type, where potential missense, nonsense, stop-loss, frameshift and splicing variants are predicted to have potential effect on protein function. Nonsense, splicing and frameshift variants are theoretically of high cellular and systemic impact since causing loss or unstable protein function, compared to missense variants that produce non- or incompletely functional protein (Jalali Sefid Dashti and Gamieldien, 2017). Notably, further consideration, such as high frequencies of false positives caused by sequencing error, is demanded prior processing these as causal candidate for further assessment, although some level of confidence may achieved by the occurrence of the same variant in multiple samples (MacArthur et al., 2012).

Since rarity is one of the key criteria used in predicting the likely functional effect of candidates on the encoded protein, rare nonsense and missense variants are anticipated to have much higher functional effect than commonly occurring one (Jalali Sefid Dashti and Gamieldien, 2017). A minor allele frequency (MAF) ≥1% is frequently used as a first point for filtering out and decrease the list of possible candidate variants in disease studies (Jalali Sefid Dashti and Gamieldien, 2017). The population frequency databases (e.g. single nucleotide sequence polymorphism (dbSNP), 1000 Genomes Project, Exome Aggregation Consortium (ExAC), Genome Aggregation Database (gnomAD)) provide allele frequencies in different ethnicity groups. Nonetheless, keeping into consideration the possibility of incomplete penetrance of some disease variants and/or different expression levels of the clinical phenotype, there is a substantial possibility that carriers are labelled as healthy controls in population data sets (Lazarin et al., 2013). In his large-scale study of individuals for routine recessive disease carrier screening of large ethnicity samples (total of 23,453 individuals), Lazarin et al. (2013) showed that 24% of 5700 asymptomatic screened individuals were recognized as carriers for at least one of severe disease while 5.2% were carriers for several severe disorders with carrier frequencies ranging from 0.006% to 7.6%.

Furthermore, the true missense variants (SNVs) can be further annotated and filtered using the functional impact prediction scores in conjugation with evolutionary

conservation scores provided by the following tools: Sorting Intolerent from Tolerant (SIFT) (Ng and Henikoff, 2003), Polymorphism Phenotyping v2 (PolyPhen-2) (Adzhubei et al., 2010), MutationTaster (Schwarz et al., 2010), Likelihood Ratio Test (LRT) (Chun and Fay, 2009), Genomic Evolutionary Rate Profiling (GERP++) (Davydov et al., 2010), phylogenetic p-values (phyloP)(Pollard et al., 2010), Combined Annotation Dependent Depletion (CADD) (Rentzsch et al., 2019). A combination of several functional-impact prediction algorithms are recommended to be used together in filtering pipeline to avoid removing candidates because of false negatives (Jalali Sefid Dashti and Gamieldien, 2017). Automatic removal of variants that are outside the ranges of conservation threshold is not recommended, keeping in consideration the phenomenon of compensated pathogenic deviation, where a substantial proportion of disease causing pathogenic variants in human are fixed and neutrals in other species genomes (Jordan et al., 2015).

Although rare, in-frame insertion and deletions (*indel*) may be deleterious and should be annotated with caution, where rarity in the general population as well as segregation with affected individuals should be taken into account. SIFT-*indel* tool (http://sift.bii.a-star.edu.sg/www/SIFT_indels2.html) may be used to evaluate functional effect of in-frame *indel*. Moreover, InterProScan (www.ebi.ac.uk/interpro/search/sequence-search) can be used to define the impact of a candidate variant on the translated protein and its critical domain.

Although non-coding variants, which present in introns, up- or downstream of coding regions (3′ and 5′ UTRs) and intergenic regions, constitute the majority of variants in the human genome, their potential interpretation is challenging. The functional impact of non-coding variants on the structure and regulatory properties of a protein can be evaluated using several algorithms such as CADD, FATHMM-MKL (http://fathmm.biocompute.org.uk/) and Genome Wide Annotation of Variants (GWAVA) (www.sanger.ac.uk/sanger/StatGen_Gwava).

Additional knowledge about the effect of genetic variant on gene expression is a fundamental factor that should be considered in decision making for prioritizing potential disease-causative variant. There are different tools that can help evaluating the expression of a gene in the tissue or organ of interest, such as Gene Expression Omnibus profiles (www.ncbi.nlm.nih.gov/geoprofiles), the Expression Atlas (www.ebi.ac.uk/gxa), and The Genotype-Tissue Expression (GTEx) project. The association of gene product pathway

with the disease of interest is another important factor to consider in variant prioritization, where online KEGG pathways (www.genome.jp/kegg/) or REACTOME pathways (www.reactome.org/) may serve this function. Moreover, knowledge about the effect of mutation or animal knockout of the gene on the disease or the characteristic feature of the disease using Mouse Genome Informatics database (www.informatics.jax.org/humanDisease.shtml) is also important in evaluating and prioritizing causative-variants and disease-associated genes. Human Phenotype Ontology (HPO) project (http://human-phenotype-ontology.org) can also be utilized for genotype to phenotype mapping for wide areas of disorders. It is important to considered that independent confirmation using Sanger sequencing is advised even if candidate-variant proceeded downstream prioritization (Jalali Sefid Dashti and Gamieldien, 2017).

1.4.3 Technical limitations

Although NGS technologies have led to huge improvement in the current knowledge of genetic basis of inherited diseases, and regardless of the continuous improvement in the performance of these technologies to solve earlier technical limitations, there are a number of technical issues continue to be unsolved. First, the computational power demand for storage and interpretation of massive quantities of genomic data is still an open obstacle. Second, there is a limitation in obtaining sufficient coverage for all targets, where up to 20% of targets in WES and WGS are inadequately covered (Dewey et al., 2014, Steyaert et al., 2018). There are complex genomic regions containing pseudogenes, high guanine-cytosine (GC) content, repetitive sequences and homologous regions. These regions are found difficult to capture, sequence and thereafter mapped to reference genome, thus significantly affect variant calling accuracy and in turn variant annotation accuracy.

Another limitation of these technologies is their failure to precisely determine heterozygous insertions or deletions (indel) ranging from 1 to 4 exons (Steyaert et al., 2018). Numerous NGS-based methods have been proposed to detect structural variations (SVs), which are large-scale genomic alterations of size more than fifty nucleotides causing deletions, insertions, duplications, inversions or translocations, making use of NGS low cost and capability of unbiased detection through base pair resolution (Guan and Sung, 2016). However, because these methods relay on different SVs features and library features, sensitivity and specificity of SVs callers remains a challenge and

different caller produces different inconsistent predictions (Guan and Sung, 2016). Additionally, the specificity of SVs detection was obstructed by the presence of the noise in the data generated by platform-technical errors or false chimeric reads which potentially increase the rate of false positive and false negative (Guan and Sung, 2016).

Although the majority of inherited diseases caused by genetic defects of protein-coding regions, which constitute only up to 2% of the human genome, it is considered as a limitation to sequence only this smaller proportion of the genome (Steyaert et al., 2018). Moreover, incomplete representation and coverage of all protein-coding regions caused by the existence of certain human genomic regions that are not completely annotated yet and the absence of potential non-coding elements, such as untranslated regions (UTRs), enhancers, and long-noncoding RNAs, are other limitations of these technologies. These limitations are expected to be resolve with the use of third-generation sequencing technologies, which are not yet arranged for clinical diagnostics usage (Steyaert et al., 2018).

It is important to consider that in a large-scale genomic examination through WES or WGS there is a potential for detection of secondary and incidental findings (Steyaert et al., 2018). While secondary findings involved variants that are dynamically hunted for but their occurrence is not directly associated with disease of interest, incidental findings involve accidental discoveries of non-matching paternity or variants in non-curable disease. There is a list of 59 medically actionable genes linked to highly penetrant genetic diseases prepared by the ACMG and recommended to be reviewed for pathogenic or likely pathogenic variants unless the patient decided not to obtain secondary findings results (Green et al., 2013). These recommendations aimed to decrease morbidity and mortality without demanding further test (Green et al., 2013). Because these secondary findings have some medical values and social implications to the patient and biologically related members, it is critical to report only those very likely pathogenic variants to prevent unnecessary discomfort and costs for patients without any medical advantages (Steyaert et al., 2018).

1.5 Project Aim and Objectives

There are many known and unknown genes causing the pathogenesis of inherited cystic kidney disease and renal ciliopathies. Genetic study of patients and families from Oman, where the population is characterised by high level of consanguinity as well as genetic and geographic isolates, is postulated to identify novel genetic causes of these diseases. This study aimed to evaluate the magnitude of inherited kidney disease in this population and identify the molecular genetic causes of inherited cystic kidney diseases in Omani patients.

The objectives of this project are:

1. Epidemiological review of kidney disease in Oman through:
    a. Retrospective review of Renal Replacement Therapy register in Oman
    b. Comparison with other countries/registries
2. Set up target NGS panel for diagnostics of inherited cystic kidney ciliopathies
    a. *In silico* design of customized targeted kidney panel (49 genes)
    b. Testing and validation of panel
    c. Use of panel for renal diagnostics
3. Description of the clinical and genetic profile of ARPKD cohort from Oman.
4. Diagnose novel forms of inherited renal ciliopathies for patients who are not solved by the targeted-NGS panel by WES technologies.

Chapter 2. Materials and Methods

2.1 Ethical Approvals

This study was approved by the Research and Ethical Review and Approval Committee of the Ministry of Health (MOH) in Oman (MH/DGP/R&S/PROPOSAL_APPROVED/18/ 2014) and the Ethics Committee of the College of Medicine and Health Sciences in the Sultan Qaboos University, Oman (MREC #1096). All study participants provided written informed consent according to the Declaration of the MOH.

2.2 Retrospective Analysis of End Stage Kidney Disease (ESKD) data

Data from newly registered Omani patients with ESKD commencing RRT from 2001 to 2015 was analyzed using the national renal replacement therapy register in Oman. The registry contains baseline characteristics of patients at initiation of RRT and updates until patient death. The baseline characteristic information included is gender, comorbid conditions (including diabetes mellitus, hypertension, ischemic heart disease, cerebrovascular disease, and respiratory disease), family history of disease, initial hypertension medications, and initial pre-RRT BMI, serum albumin, and creatinine. Haemodialysis data, peritoneal dialysis data, and renal transplantation data were also included in the registry. This data is completed by nephrologists in all renal dialysis units throughout the country once the patient reached ESKD using a standardized form and sent to the main renal dialysis unit in Muscat, where the RRT registry is maintained in a comprehensive database (Al Ismaili et al., 2017). The registry has been established and standardized to enable significant comparisons to other countries registries. The collected dataset is similar to that of the United States Renal Data System (USRDS; form 2728) (Al Ismaili et al., 2017).

All potentially congenital, genetic, or hereditary causes of kidney diseases were extracted from the registry within the study period according to the coding system of this registry. Cases registered and classified under the code Hereditary Familial Renal Disease were reviewed, which include the majority of inherited kidney diseases including ADPKD, ARPKD, Alport syndrome, primary hyperoxaluria, cystic dysplastic kidney,

nephronophthisis, Bartter syndrome, and inherited renal tubular acidosis. The proportion of inherited kidney disease among those commencing RRT was calculated.

2.3 DNA Extraction

DNA isolation was performed in the Molecular Genetics Laboratory in the National Genetic Centre (Oman). Genomic DNA (gDNA) was extracted manually from the peripheral blood samples collected in EDTA (Ethylenediaminetetraacetic acid) tubes and uncultured amniotic fluid collected in sterile containers using a DNAeasy Blood & Tissue kit (Qiagen, Germany) according to manufacturer's instructions. Initially, some preparations were performed for the amniotic fluid sample before carrying extraction, which include (1) centrifugation at 5000xg for 5 min and removing supernatant, (2) washing the pellet with 500ul of phosphate buffered saline (PBS) and centrifugation at 5000xg for 5 min and (3) removing supernatant and re-suspending in 200 µl PBS.

First of all, proteinase K (50 µl) and lysis buffer AL (500 µl) were added to 500 µl of blood and 200 µl of amniotic fluid cells, followed by vortex (5–10 sec) and incubation at 56°C for 10 min. For DNA binding, 200 µl of 100% ethanol was added and the lysate of each sample was transferred into the mini spin column allocated in a 2 ml collection tube. Samples were centrifuged at 6000xg (8000 rpm) for 1 min allowing the DNA to selectively bind to the silica membrane and contaminants to pass through to collection tubes. Two wash steps were performed that involved the addition of Buffer AW1 (500 µl) and centrifugation at 20,000xg (14,000 rpm) for 3 min followed by Buffer AW2 (500 µl) and centrifugation at 20,000xg (14,000 rpm) for 3 min. Finally, DNA is eluted in water or TE buffer (Tris-EDTA; 10mM Tris base, 0.1mM EDTA).

Initial gDNA concentration (ng / µL) and absorption ratio (260 nm / 280 nm) were measured using a full-spectrum Nanodrop 2000 spectrophotometer (Thermo Scientific™). From each extracted sample, an adequate amount of DNA (≥ 10 µg) with high quality (A260 / A280: 1.8-2.0) was achieved. DNA samples were stored at 4 ^{o}C for further studies.

2.4 Targeted Next Generation Sequencing (NGS) Panel

All target-NGS procedures were performed in the Molecular Genetics Laboratory in the National Genetic Centre in Oman. Figure 2.1 presents an overall workflow of the targeted NGS panel protocol performed in this study.

2.4.1 Patient Recruitment and Inclusion Criteria

The cohort comprised of 53 prospective samples from patients with presumed inherited cystic kidney disease from paediatric and adult nephrology services. All patients are Omani from different regions across the country. Patients were classified according to their age group into prenatal, paediatric (birth -13 years of age) and teenage / adult (>13 years of age). Inclusion criteria were renal US scan findings of: increased kidney echogenicity or loss of corticomedullary differentiation or abnormal kidney size or presence of kidney cysts and a possible family history of kidney disease. Family pedigrees were drawn using invitae online tool (https://familyhistory.invitae.com).

2.4.2 Target-capture Panel Design

A customized target sequence gene panel associated with inherited cystic kidney disease was designed using 49 genes to create a capture library (Table 2.1). Genes were chosen based on their known association with cystic kidney disease phenotypes and their known or predicted frequency with an emphasis on autosomal recessive causes of cystic kidney disease, given the high rates of consanguinity. The number of genes was limited due to number of exons / amplicons required per gene and the overall target capture size. Using online Agilent SureDesign Tool (https://earray.chem.agilent.com/suredesign), capture probes were designed to capture target gene-set regions of 243165 bp in size by generating a total of 11712 amplicons (Agilent Technologies, Santa Clara, California, USA). The February 2009 human reference sequence (GRCh37/Hg19) was used to generate set of probes for capturing the targeted regions. More details of the designed panel and the expected genomic coverage of probes are shown in Appendix A1.

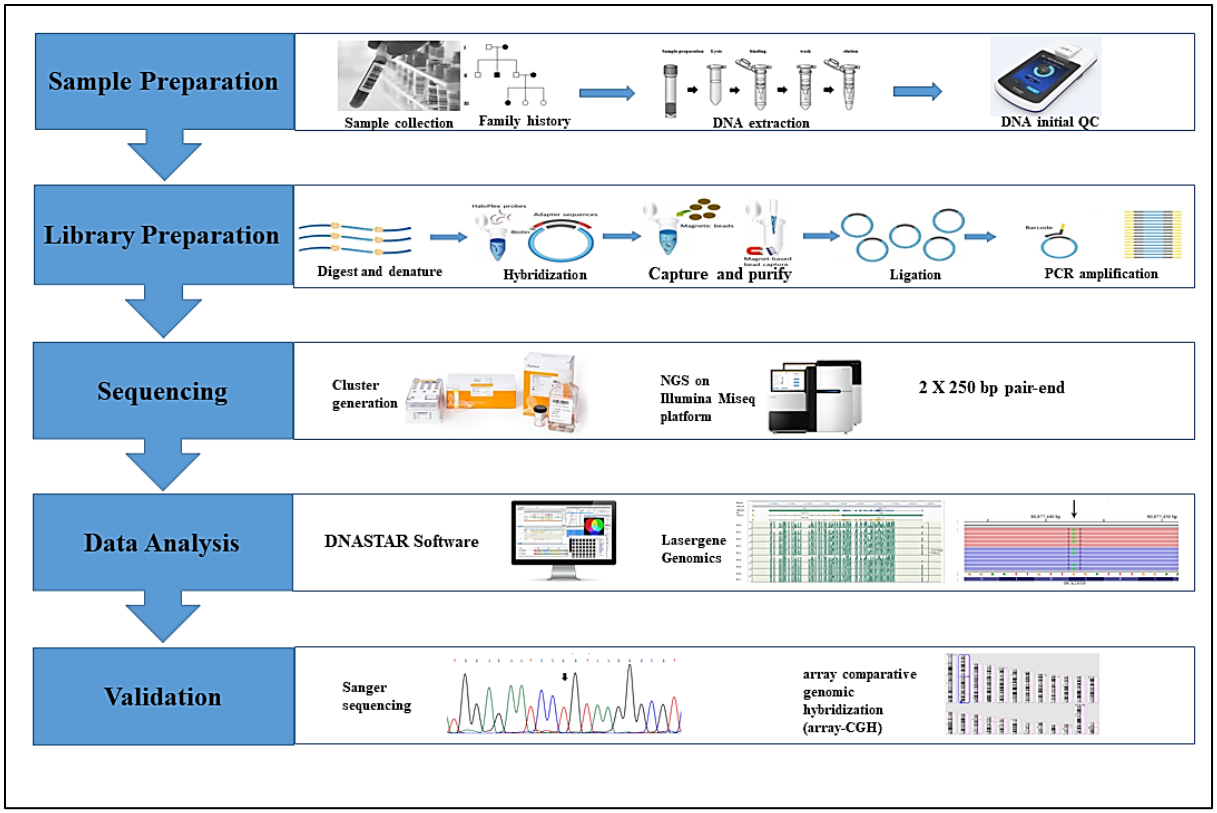

Figure 2.1 Schematic presentation of an overall target NGS workflow. Sample preparation stage consists of DNA samples collection from affected patients and their families (as possible), collection of pedigrees and family history data, DNA extraction of blood samples, quality control (QC) assessment of DNA quality and quantity. Library preparation step consists of DNA samples digestion (12 samples per run), hybridization, capture of target regions, purification using magnetic beads, ligation and PCR amplification of enriched libraries. NGS sequencing on Illumina MiSeq platform involves cluster densities generation on flow cells. DNASTAR lasergene software was used for raw data analysis and depth of coverage of target genes in each sample was checked for QC assurance. Based on the type of detected causative variants, validation was obtained through Sanger sequencing and comparative genomic array. bp, base pair; PCR, polymerase chain reaction; QC, quality control.

Diseases	MOI	Gene
Autosomal dominant polycystic kidney disease (ADPKD)	AD	*PKD1, PKD2*
Autosomal recessive polycystic kidney disease (ARPKD)	AR	*PKHD1*
Autosomal dominant tubulo interstitial kidney disease (ADTKD)	AD	*HNF1B, REN, UMOD*
Nephronophthisis (NPHP)	AR	*NPHP1, INVS, NPHP3, NPHP4, GLIS2, NEK8, TMEM67, TTC21B, WDR19, ZNF423, CEP164, ANKS6, CEP83, DCDC2, SDCCAG8 & CEP290*
Medullary cystic kidney disease	AD	*UMOD, MUC1*
Meckel-Gruber syndrome (MKS)	AR	*MKS1, TMEM67, CEP260, NPHP3 & RPGRIP1L*
Bardet-Biedl syndrome (BBS)	AR	*BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, MKS1, CEP290, WDPCP, SDCCAG8, LZTFL1, BBIP1, IFT27*
Autosomal dominant Polycystic liver disease (ADPLD)	AD	*PRKCSH, SEC63*
Joubert syndrome (JBTS)	AR	*AHI1, NPHP1, CEP290, TMEM67, RPGRIP1L & TTC21B*
Renal cysts and diabetes syndrome	AD	*HNF1B*
Renal dysplasia, cystic susceptibility	AD	*BICC1*

Table 2.1 Disease categories and genes selected for targeted NGS panel for cystic kidney disease. MOI: mode of inheritance. AD: autosomal dominant. AR: autosomal recessive.

2.4.3 DNA Libraries Construction

Prior to library preparation, DNA qualification and quantitation were verified using the following:

(1) 1.0 % of agarose gel (Thermo Fisher Scientific), as per standard electrophoresis techniques (Figure 2.2) to eliminate DNA degradation or contamination.

(2) a fluorometry-based DNA quantitation technique using Qubit® 3.0 Fluorometer (Thermo Fisher Scientific), was used to verify DNA quality and obtain the required concentration (1.8 ng / µl) using Qubit® DNA Assay Kit, according to the manufacturer guidelines.

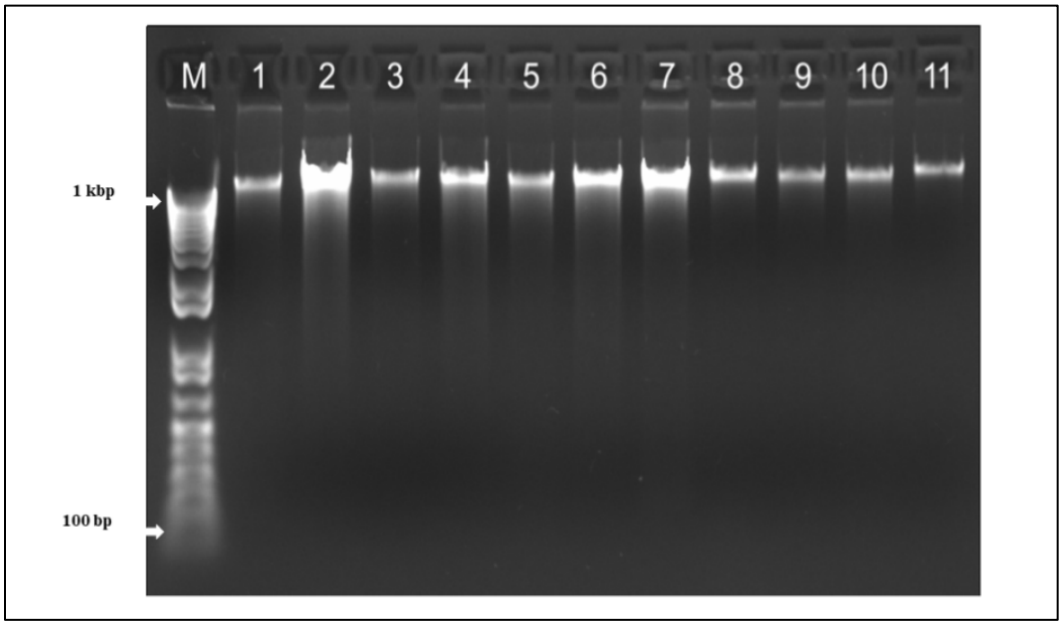

Figure 2.2 Agarose gel (1%) electrophoresis to allow genomic DNA qualification. A size distribution of 2.5 kilo base pair (kbp) without smearing is an indicator of high quality DNA without any degradation. M: marker DNA ladder with molecular sizes of 1 Kbp and 100 bp indicated. Lanes 1-11 are different DNA samples.

The DNA libraries were constructed using the HaloPlex HS Target Enrichment System® for Illumina sequencing kit (Agilent Technologies, Inc.), according to the manufacturer's protocol. This kit provides in-solution capture-based target enrichment of customized amplicons through labeling each DNA sample with a unique molecular barcode. The workflow consists of four main phases: 1) digestion, 2) hybridization, 3) ligation and capturing, 4) PCR amplification (Figure 2.2). A batch size of 12-samples was performed, which composed of 11 different DNA samples along with one Enrichment Control DNA (ECD) provided in the kit.

Firstly, gDNA (50 ng) of each sample was digested using a total of 16 diverse restriction enzymes divided between eight double-reactions (A to H) and heating up on the thermal cycler at 37 °C for 30 minutes. DNA fragments obtained from the digestion were validated by microfluidic electrophoretic analysis of the eight-reactions of ECD using Agilent 2100 Bioanalyzer Electrophoresis system (Agilent Technologies, Inc.). The validation was performed by the use of High Sensitivity DNA Kit (Agilent Technologies, Inc.), where gel-dye mix (9µl), ladder (1µl) and samples (1µl) were prepared and loaded on the chip according to the reagent kit guide. Later, the chip was run on the Bioanalyzer Electrophoresis system and samples were quantified by the equipped 2100 Expert Software (version B.02.07).

Secondly, the digested fragments were hybridized to the customized Haloplex probes, which are designed to hybridize to the target genomic regions and manage circularization of the fragments, using reagents and thermal cycler conditions stated in Table 2.2. For each sample to be multiplexed, a unique molecular barcodes and Illumina sequencing indexes were integrated through proper HaloPlex HS Indexing Primer solutions. A mixture of Hybridization Stop Solution and consistent suspension of AMPure XP bead (Beckman Coulter Genomics) were used to stop hybridization reaction followed by using 70% ethanol to remove the hybridization buffer.

Thirdly, the circularized hybridized fragments were ligated using DNA ligation reagents in Table 2.2. These fragments, which contain biotin from previous step, were captured using Dynabeads MyOne Streptavidin T1 magnetic beads (Thermo Fisher Scientific).

Finally, the captured libraries were amplified by multiplex PCR using reagents and conditions reported in Table 2.2 and 2.3, respectively. After that, Agencourt AMPure XP beads were utilized to cleanse the amplified libraries along with two times 70% ethanol washes.

Before pooling targeted libraries, the 2100 Bioanalyzer was used to validate enrichment and determine the size of enriched DNA libraries (Figure 2.3). The amplified products varied in their fragment size distribution; therefore, by incorporating electropherogram peak between 175 and 625 bp, the concentration was identified, which determines the volume to be pooled from each library. The DNA concentration was converted from ng / µl to nM via the following formula. Thereafter, the differentially indexed libraries with similar concentration (10pM) were pooled together into a single tube and were purified using Agencourt AMPure XP beads (Beckman Coulter Genomics).

$$\text{concentration (nM)} = \frac{\text{concentration (ng/µl)}}{\text{[660 g/mol X average library size (bp)]}} \times 10^6$$

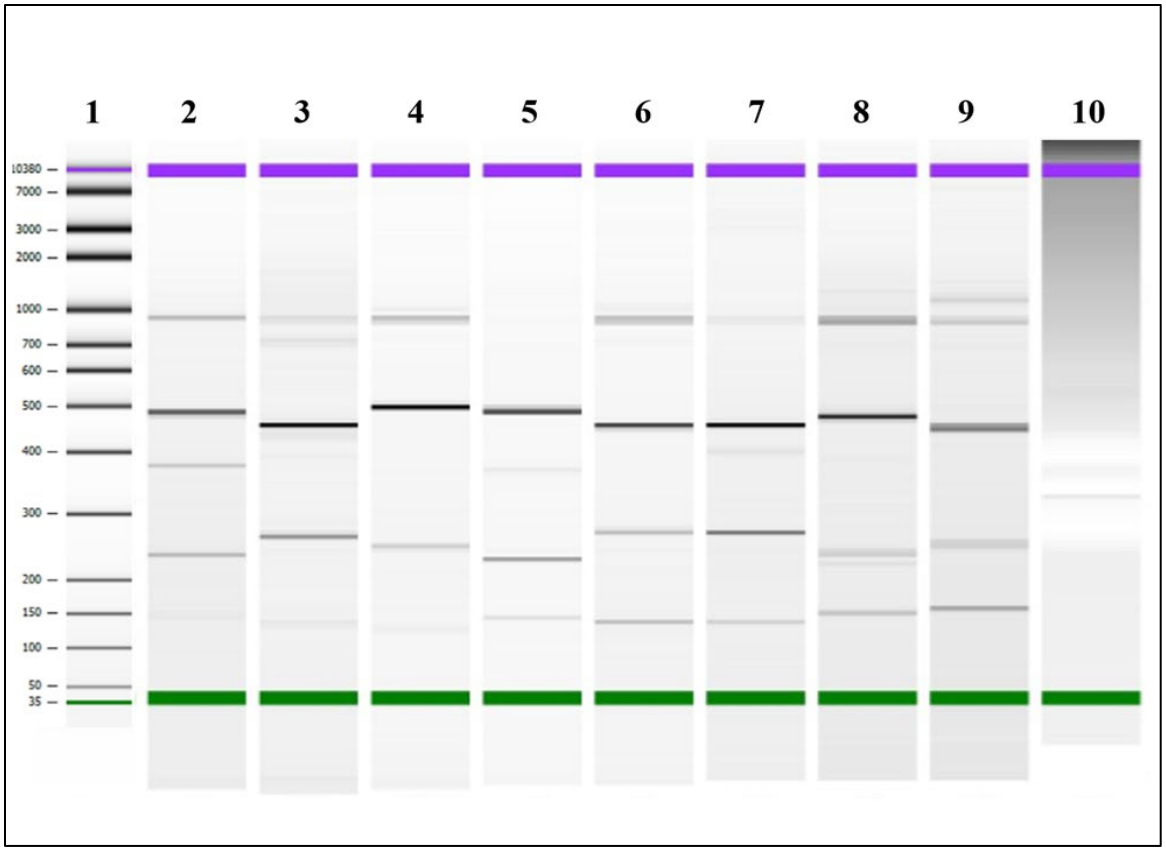

Figure 2.3 Validation of digestion reaction using 2100 Bioanalyzer system analysis. Lane 1: 50-bp DNA ladder, Lanes 2-9: ECD digestions (8 different digests A→H), Lane 10: Undigested ECD. The ECD sample comprises of a mixture of gDNA and 800-bp PCR product enclosing restriction sites for the digested enzymes utilized. During validation, the undigested ECD (lane 10) contains bands at >2.5 kb and 800 bp corresponds to gDNA and PCR product, respectively. A smear of gDNA restriction fragments appeared in all of the eight digested ECD samples, with three main bands at approximately 125, 225 and 450 bp, which correspond to the 800-bp PCR product digested fragments.

Reagent	Volume (µl)
Hybridization:	
Hybridization Solution	442
HaloPlex HS Probe	65
Total	**507**
Ligation:	
HS Ligation Solution	130
1 mM rATP	7.8
Nuclease-free water	512.2
Total	**650**
PCR amplification:	
Nuclease-free water	691.6
Herculase II Reaction Buffer	390
dNTPs (100 mM, 25 mM for each dNTP)	10.4
Primer 1	52
Primer 2	104
Herculase II Fusion DNA Polymerase	52
Total	**1300**

Table 2.2 Master mixes used in hybridization, ligation and PCR amplification reactions.

	Steps	Number of Cycles	Tem. (°C)	Time
Digestion	1	1	37	30 min
	2	1	4	Hold
Hybridization	1	1	95	5 min
	2	1	58	2 hrs
PCR amplification	**1**	1	98	2 min
	2	24	98	30 sec
			60	30 sec
			72	1 min
	3	1	72	10 min
	4	1	8	Hold

Table 2.3 Thermal cycler program used throughout different phases of libraries preparation. hrs, hours; min, minutes; sec, seconds; Tem, temperature.

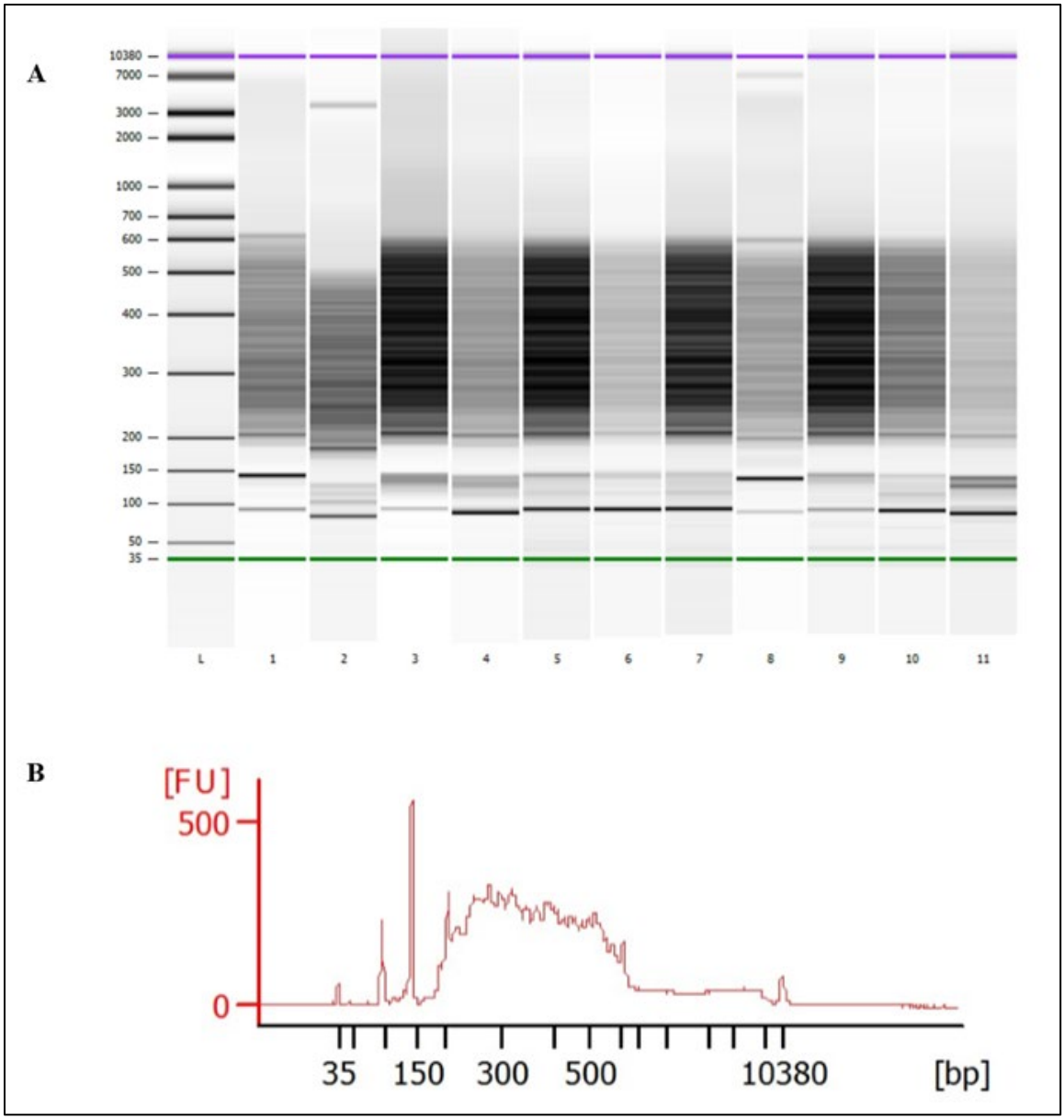

Figure 2.4 Validation and quantification of the HaloPlex HS enrichment libraries by 2100 Bioanalyzer system analysis. (A) Bioanalyzer electrophoresis results; Lane 1: 50-bp DNA ladder, Lanes 2-12: enriched library samples. The majorities of amplified products have size range between approximately 225 to 540 bp. (B) Sample of electropherogram for enriched library. To quantify enriched target DNA concentration, a constant size range of 175 to 625 bp was used. Due to the design, a peak at about 140 bp size is noted, which is a sign of an adapter-dimer product, thus additional round of AMPure purification is suggested following sample pooling. bp, base pair; FU, fluorescence unit.

2.4.4 High Throughput Parallel Sequencing

The final multiplexed library was denatured using 0.2 N NaOH and then diluted to proper loading concentration indicated by the MiSeq reagent kit (12.5pM) using prechilled HT1 hybridization buffer (Illumina, Inc). The denatured diluted library was combined with a 5% denatured and diluted positive control sequencing library (Illumnia PhiX control, 12.5pM), according to Illumina instructions. A total of 600 µl of the combined library is loaded into MiSeq Reagent V2 (500 cycles) kit cartridge.

A paired-end sequencing of 2 × 250 bp was performed using Illumina MiSeq® sequencer (Illumina Inc, San Diego, CA), at which the flow cell and reagents were loaded. After that, a custom sample sheet was prepared according to the manufacturer's guidelines using the Illumina Experiment Manager (IEM) software. For the purpose of this customized panel, the HaloPlex HS indexes used for every sample were manually modified. Read files (FASTQ) were only requested in the application option of IEM, according to manufacturer's patented software. The progress and quality of the run were examined throughout the run from the MiSeq sequencing screen, including cluster density, clusters passing filters, quality score measurement and estimated yield (Mb).

2.4.5 Bioinformatics and variants filtration

The FASTQ files containing raw sequencing data generated by Illumina MiSeq platform were utilized for alignment, mapping and data analysis using a commercially available software package "DNAStar Lasergene 15 Genomics Suite" (DNAstar, Madison, WI). This suite consists of four main applications: (1) SeqMan NGen, which is used to assemble and align patients genomic data to the human reference genome (hg19/ GRCh37), (2) SeqMan Pro, which is used to evaluate coverage, obtain enrichment report and downstream analysis to identify SNPs, small indels, large insertions and deletions, (3) Genomic visualization application, which is used to visualize genomic sequencing results and visually compare coverage across exons and genes, (4) ArrayStar, which is used for variant analysis using multiple variant annotation databases and algorithms such as dbSNP, ExAC, the Exome sequencing project (ESP), gnomAD, 1000 genomes project, ClinVar, SIFT, PolyPhen-2, MutationTaster and LR predictions.

In the first step following completion of data preprocessing using SeqMan NGen (Figure 2.5), a target coverage and enrichment report of each sample was checked for quality assessment using SeqMan Pro. The quality threshold for all variants included in the analysis was as following: read depth (RD) ≥ 20, genome quality (GQ) ≥ 20, mapping quality (MQ) ≥ 20 and base quality (BQ) ≥ 20 (Figure 2.5). The VCF data of each sample was annotated in ArrayStar and variants were filtered and prioritized using the following steps: (1) variants with allele frequency greater than expected for disease (>2% for autosomal dominant and >1% for autosomal recessive) were filtered out as likely does not affect function, (2) synonymous variants predicted not to effect splicing and not in conserved region and reported as likely neutral in databases were filtered out as likely does not affect function, (3) known variants reported in databases as likely neutral and multiple in silico analysis predict no effect on gene function were filtered out as likely does not affect function, (4) nonsense, frameshift, canonical +/- splice site, initiation-codon, single or multiple exon deletion that identified in patients with classical disease related phenotypes are maintained and classified as variants effecting function, (5) missense variants that are absent from allele frequency databases, predicted pathogenic by multiple in silico tools, segregate within family and found in patients with classical disease phenotype are maintained and classified as likely effect function, and finally (6) in-frame deletions/insertions that are not reported in any allele frequency databases, with multiple in silico analysis predicted pathogenic, protein length changes as a consequence of in-frame deletion/insertion and present in patients with classic disease phenotypes are maintained and classified as likely effect function.

For the assessment of potential splicing effects of the missense variants, the Human Splicing Finder software was used. For evaluating the evolutionary conservation of substituted residue, ConSurf web server along with GERP score and the phyloP conservation score were used. I- Mutant 3.0 server was used for assessing the effect of genetic alteration in the stability of the protein structure.

A series of databases associated with cystic kidney disease were interrogated for evaluating the obtained variants and identifying novel ones, including PKDB Mutation Database (http://pkdb.mayo.edu/), ARPKD/*PKHD1* Mutation Database, Leiden Open Variation Database (LOVD), HGMD and the previously published articles reporting *PKD1, PKHD1,*

HNF1B, WDR19, SDCCAG8 and NPHPs, mutations. The pathogenicity of novel variants was ascertained according to the revised criteria of the American College of Medical Genetics (Richards et al., 2015).

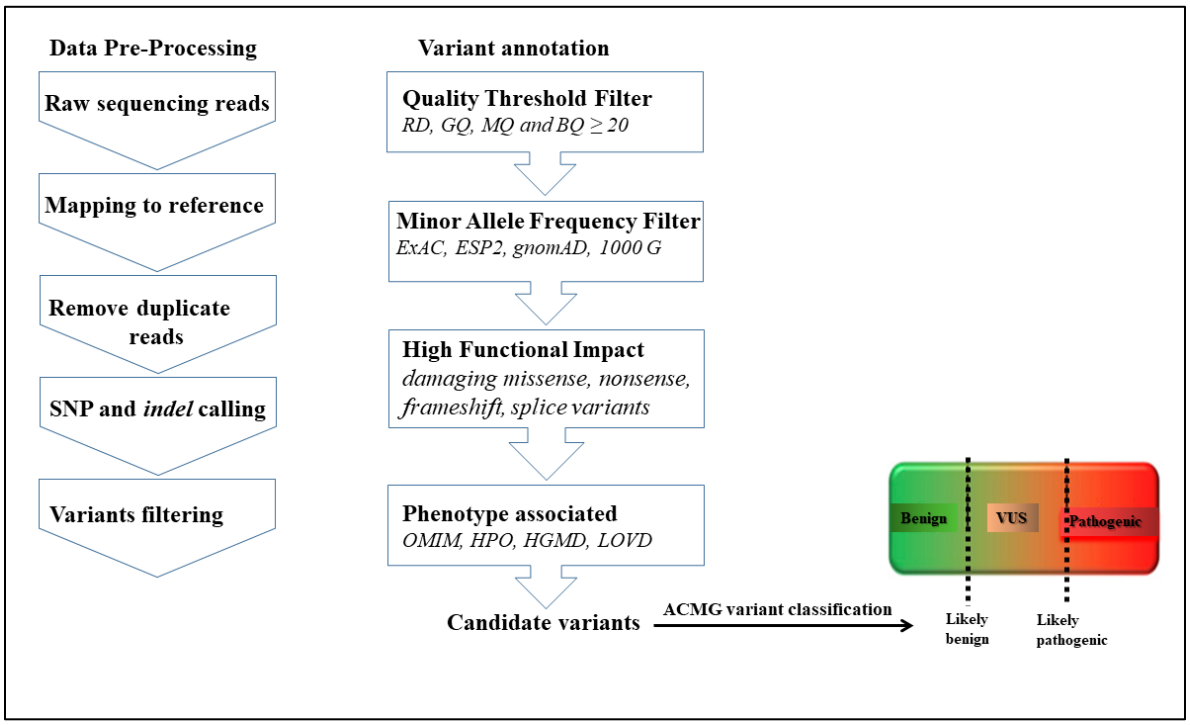

Figure 2.5 Workflow of raw data pre-processing and variants filtering strategy. Data pre-processing strategy involves multiple steps; including aligning the raw sequence to the reference genome (mapping), removing of PCR duplicate reads, variant calling and finally variant filtering. Different parameters are used in variant filtering and prioritizing; including quality threshold, allele frequency, potential impact on protein functioning, and disease-related phenotypes. 1000G, 1000 Genomes project; ACMG, American College of Medical Genetics and Genomics; BQ, base quality; ESP, exome sequencing project; ExAC, Exome Aggregation Consortium; GQ, genotype quality; HGMD, Human Genome Mutation Database; HPO, Human Phenotype Ontology project; LOVD, Leiden Open Variation Database; MQ, mapping quality, OMIM, Online Mendelian Inheritance in Men; RD, read depth; VUS, variant of uncertain significance.

2.4.6 Validation by Sanger Sequencing

All putative disease causing variants detected by NGS were validated by bi-directional fluorescent Sanger sequencing of PCR amplicons and their segregation with the disease was confirmed. Target regions were amplified using AmpliTaq Gold 360 Master Mix kit (Applied Biosystems) and Oligonucleotide PCR primers, which were designed using Primer3 program (http://primer3.ut.ee/). Primers were designed to cover the whole exon sequence at which variant is located, but for large exons, a set of overlapping fragments were amplified using multiple primers. The primer sequences are reported in Table 2.4. The annealing temperature for all primers was close to 60°C to confirm that similar PCR conditions can be applied for the amplification of all fragments.

The PCR was carried out using reagents and conditions stated in Table 2.5 and 2.6, respectively. The PCR products were verified on 1.5% agarose gel electrophoresis (120 V/cm, 30 min) and compared with appropriated marker of molecular weight. Subsequently, the PCR products were purified using ExoSAP-IT reagent (Applied Biosystems) and sequenced using BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems) using reagents and condition reported in Table 2.5 and 2.7, respectively. The PCR products were purified using ethanol and afterward loaded on ABI 3130 sequencer (4 or 16 capillaries). All sequences were assembled, aligned and analyzed by comparison against a reference sequence using the SequencePilot 4.2.2 software (JSI Medical Systems GmbH), at which a diagnostic report for each sample was generated.

The previously described protocol was applied to all genes, except the *PKD1* gene. In particular, the suspected *PKD1* SNVs, deletions and insertions were confirmed by long-rang PCR (LR-PCR) using the GeneAmp High Fidelity PCR System (Applied Biosystems), the sets of primers and PCR protocol published previously by (Tan et al., 2012). The LR-PCR products were then verified, purified and sequenced using the same protocol described above.

Primer Name	Gene - Exon	Sequence 5'--> 3'
PKHD1_3F	*PKHD1* Exon 3	CTGAGGCAGGTTAAATATTGCTT
PKHD1_3R	*PKHD1* Exon 3	GTCTGTTCGTCTCCCTTCAGG
PKHD1_6F	*PKHD1* Exon 6	GTGCCTCCTGTGTTTGTGAA
PKHD1_6R	*PKHD1* Exon 6	TCCAGTCTCCAACATCAACTCA
PKHD1_32AF	*PKHD1* Exon 32	AACACATGCCCTACCTTCCA
PKHD1_32AR	*PKHD1* Exon 32	AACATCACAGTTCAGGTTCCC
PKHD1_32BF	*PKHD1* Exon 32	GAAGTAACCTCTCCAACTCAGTC
PKHD1_32BR	*PKHD1* Exon 32	CCACAAATACCATCGGCTCAT
PKHD1_32CF	*PKHD1* Exon 32	TCTCTGACCACTGTGCTGAT
PKHD1_32CR	*PKHD1* Exon 32	TGAAACACTTGGGGCATAATGT
PKHD1_32DF	*PKHD1* Exon 32	TGATTAGGGGTCAGAGGTTAGC
PKHD1_32DR	*PKHD1* Exon 32	CCACTGCAAAGGTTAAGATGTCA
PKHD1_32EF	*PKHD1* Exon 32	AGGTAGATGGACTTTGGTATCACA
PKHD1_32ER	*PKHD1* Exon 32	TTTCCAGAAGTGAAAGGAGCTAC
PKHD1_58AF	*PKHD1* Exon 58	TCAGCCTTTTGTGGGGAAGA
PKHD1_58AR	*PKHD1* Exon 58	TGAAAGCCAAGAAGCCAGAG
PKHD1_58BF	*PKHD1* Exon 58	GCCTTCATCTCTATAAGGAAAGTGG
PKHD1_58BR	*PKHD1* Exon 58	TGCATGGATGTATGAAATGGCA
NPHP3_18F	*NPHP3* Exon 18	TGTCCTGGACTTTCTTGACCT
NPHP3_18R	*NPHP3* Exon 18	AAAGCCACGGGAGTTCATC
NPHP3_24F	*NPHP3* Exon 24	TAGGGGTCAGGATTCCAAACTC
NPHP3_24R	*NPHP3* Exon 24	ACCTGTCCCTCATAAAGACAAATT
NPHP4_6F	*NPHP4* Exon 6	AAGGTTGCGGCTGTACCAT
NPHP4_6R	*NPHP4* Exon 6	CAGGGTGAAGAATAAGTCATCCA
NPHP4_27F	*NPHP4* Exon 27	CCCTTGTTGGCCTCTCGT
NPHP4_27R	*NPHP4* Exon 27	CTGGAGGCGCTGGAAAAG
SDCCAG8_12F	*SDCCAG8* Exon 12	ATCCCTGGTGTTGCTTCTGA
SDCCAG8_12R	*SDCCAG8* Exon 12	TGCTGTTGTATTCTCACCATTCA
WDR19_22&23F	*WDR19* Exon 22&23	TTAGTGTTTGCCTTGTGATTGCA
WDR19_22&23R	*WDR19* Exon 22&23	CCAGAGGCACATTCATTTCCAAT
WDR19_32F	*WDR19* Exon 32	GGCCATCATCAAGGAGTTGT
WDR19_32F	*WDR19* Exon 32	GGGTGAGAGCTCTGGTCAGT
HNF1B_2F	*HNF1B* Exon 2	CCTAACCATCTGCTTGTCTGTCT
HNF1B_2F	*HNF1B* Exon 2	AGAGGGCAAAGGTCACTTCA

Table 2.4 Oligonucleotide primer sequences and amplified exons.

	Reagent	Volume (µl)
PCR amplification	AmpliTaq Gold 360 Master Mix	6.25
	primer F (10µM)	1
	primer R (10µM)	1
	DNA sample (50-100ng/µl)	1
	Dnase free water	up to 13.5
	Total	**13.5**
Sequencing	HPLC- water	5.5
	5X sequencing Buffer	2
	BigDye Terminator v3.1 Ready Reaction Mix	0.5
	volume	**8**
	Primer F or R (3.3 µM)	1
	PCR Product	1
	Total	**10**

Table 2.5 PCR and sequencing mixes for each sample. HPLC, High-performance liquid chromatography.

Steps	No. Cycles	Temperature (°C)	Time
1. Initialisation	1	95	15 min
2. Denaturation	13	94	30 sec
		62	30 sec
		72	30 sec
3. Annealing	8	94	30 sec
		46.5	30 sec
		72	30 sec
4. Elongation	16	94	30 sec
		54.5	30 sec
		72	30 sec
5. Final elongation	1	72	5 min
6. Termination	Hold	4	∞

Table 2.6 Thermal cycler program used for PCR reactions. hrs, hours; min, minutes; sec, seconds; Tem, temperature.

Steps	Number of Cycles	Temperature (°C)	Time
1	1	96	1 min
2	25	96	10 sec
		50	5 sec
		60	4 min
3	Hold	4	∞

Table 2.7 Thermal cycler condition used for sequencing samples. hrs, hours; min, minutes; sec, seconds; Tem, temperature.

2.4.7 Copy Number Variants (CNVs) Detection and Validation

CNVs, mainly deletions, were detected by depth analysis of NGS data using Lasergene software, where the read depth per gene / exon was compared with that of the same capture targets on the same NGS run. Later, array comparative genomic hybridization (array-CGH) was used to confirm CNVs, at which Affymetrix CytoScan HD array kit (Agilent Technologies, USA), which composed of approximately 2.6 million markers distributed throughout all chromosomes, was used. The protocol was performed by the array-CGH department in National Genetic Center in Oman as instructed by the manufacturer. Chromosome Analysis Suite (ChAS) v3.1 Software (Affymetrix, USA) was utilized for genomic annotation.

2.5 Sanger Screening of Hot-spot *PKHD1* mutations

Sanger sequencing screening of the *PKHD1* exons 3, 6, 32 and 58 was performed for another 23 patients from 15 different families with clinically suspected ARPKD, as well as the available samples of parents (n=10) and unaffected siblings (n=3). This was performed in the molecular laboratories in the National Genetic Centre in Oman using the same primers and procedure described in previous section (2.4).

2.6 Whole Exome Sequencing (WES)

2.6.1 Patients Inclusion and Clinical Evaluation

The first batch of WES samples includes five patients who were unsolved by the target NGS panel, while the second batch includes six genetically untested patients. All patients have clinical features strongly suggestive of ciliopathies. A consent form was attained from each patient and any family member involved in this study, as per the declaration of the MOH. Family pedigrees were drawn using invitae online tool (https://familyhistory.invitae.com).

2.6.2 DNA Isolation, Library Preparation and Exome Sequencing

gDNA was isolated from whole blood of patients and the available family members using a DNAeasy Blood & Tissue kit (Qiagen), as described in section 2.3. DNA extraction was performed in the National Genetic Centre in Oman.

DNA library construction and WES of the first batch samples were outsourced to EuroFins GATC Biotech (Germany), at which SureSelect Human All Exon V6 Enrichment Kit (Agilent Technologies, CA, USA) and Illumina HiSeq platform (Illumina, San Diego, CA, USA) were used. Analysis of raw data (FASTQ format) were performed by the outsourcer, including sequence reads mapping to the human reference genome hg19 using BWA (Li and Durbin, 2009), removal of PCR duplicates using Picard (http://picard.sourceforge.net), alignment refinement using GATK, coverage analysis and SNP and *indel* calling using GATK's Haplotype Caller (McKenna et al., 2010) (Appendix A2).

For the second batch WES samples, DNA library preparation and WES were outsourced to Novogene Co., Ltd (China), where SureSelect Human All Exon V6 Enrichment Kit (Agilent Technologies, CA, USA) and Illumina platform (Illumina, San Diego, CA, USA) were used. Similarly, analysis of raw sequencing reads was carried out by the outsourcer, where mapping, alignment, refinement and variant calling were performed. Statistics of mapping, coverage and depth of this batch samples are given in Appendix A2.

2.6.3 Variants Detection and Annotation

SNPs and INDELs VCF files were obtained from the outsourcer and were loaded into Qiagen Variant Ingenuity tool for variants filtration and annotation. The following filtering algorithm was applied to verify putative disease-causing variants:

(1) Confidence filter is used to filter low quality variants on the basis of variant call quality, read depth and allele fraction. (2) Common variant filter is used to conveniently exclude common variants observed in normal populations. Algorithm was set to exclude variants that are present with MAF of at least 3%of any of population databases (1000 Genomes project, ExAC, gnomAD, NHLBI ESP), unless this is a well-known pathogenic common variant. (3) Predicted Deleterious filter is used to rapidly categorize variants on the basis of (a) variants experimentally evidenced in the literature to be associated with disease-phenotype, (b) predicted or observed evidence to disturb gene function or expression.

2.6.4 Variants Validation by Sanger Sequencing

Sanger sequencing was utilized to confirm suspected disease causing variants and their segregation with the disease. Primer3 was utilized to design primer sequences (http://primer3.ut.ee/), which are shown in Table 2.8. PCR amplification was performed in the Institute of Genetic Medicine in Newcastle University using *Taq* PCR master mix (Qiagen) kit, as per the manufacturer instructions. Briefly, 30 µl PCR reaction was prepared containing 1 µl of gDNA (50-100 ng), 1 µl of forward and reverse primers (10 pmol), 15 µl Taq PCR Master Mix and 12 µl water. A touch-down PCR with the cycling conditions summarized in Table 2.9 were followed.

The amplified amplicons were verified on 1% gel and purified using ExoSAP-IT PCR cleanup reagent (Applied Biosystems). Sanger sequencing was outsourced to EuroFins GATC Biotech (Germany), at which bi-directional fluorescent sequencing on an ABI 3730 XL sequencer using BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems) was carried out. The obtained sequences were assembled and aligned compared to a reference sequence using the SequencePilot 4.2.2 software (JSI Medical Systems GmbH).

Gene / Exon	Primer Name	Primer Sequence, 5'->3'
TMEM138 / exon 5	TMEM138_EX5F	CCTGAGGCTTCTCTTCTGCT
	TMEM138_EX5R	CCTGCAAAGCAGCAAATGT
TMEM231 / exon 5	TMEM231_EX5F	TCAAACAGCCATCGTGGTTA
	TMEM231_EX5R	TTATAGGCAGGAGCCACCAC
COL4A5 / Exon 39	COL4A5_Ex39F	GTTGGAAATTGGAAAACTGGGTG
	COL4A5_Ex39R	AGGGGAAAGTGTGTGGTAGC
BBS9 / Exon 17	BBS9_Ex17F	TGTGTTCATTTTGCCTTCTGG
	BBS9_Ex17R	AGTATCCTCTGTGATGTGGTATC
WDR19 / Exon 32	WDR19_Ex32F	GAACAAAGCATGAATTGGGGC
	WDR19_Ex32R	CATGGGTGAGAGCTCTGGTC
NUP93 / Exon 12	NUP93_Ex12F	GTGGCTCAGGGTGTCATTTG
	NUP93_Ex12R	GAAGGGAAAGGTGGTTATGTCC

Table 2.8 Forward and reverse primer sequences used for WES variants verification.

Steps	**No. Cycles**	**Temperature (°C)**	**Time**	**Comments**
1	1	95	10 min	
2	13	94	30 sec	decrease by 0.70°C each time
		72	30 sec	
		72	1 min	
3	20	94	30 sec	
		50	30 sec	
		72	1 min	
4	1	72	5 min	
5	Hold	4	∞	

Table 2.9 Touch-down PCR conditions used for WES variants verification.

2.7 Statistical Analyses

Statistical analysis was performed using IBM SPSS Statistics 20 with the results expressed as frequencies and percentages for categorical variables and as median ±SD for contentious variables, as appropriate.

Chapter 3. Retrospective Analysis of Renal Replacement Therapy (RRT) Register in Oman[1]

3.1 Introduction and aims

Chronic kidney disease (CKD) is a common condition characterized by permanent kidney damage and reduced glomerular filtration rates leading to end stage kidney disease (ESKD), where renal replacement therapy (RRT) is necessary for long term survival. Globally, ESKD is a huge burden on health care systems. The number of patients receiving RRT worldwide in 2010 was estimated to be 2.6 million, whereas the estimated number of actual patients demanding RRT was 4.9 million (Liyanage et al., 2015). This RRT gap is one of the global challenges presented with the growing rate of ESKD.

Inherited kidney diseases are important causes of morbidity and may lead to both progressive CKD and ESKD. Inherited kidney disease accounts for approximately 20% of all CKD cases and is an important cause of ESKD (Hildebrandt, 2010). Renal registry studies suggest that at least 10% of ESKD in adults is related to inherited renal disease, with autosomal dominant polycystic kidney disease (ADPKD) making a large proportion of these cases (Australian and New Zealand Dialysis and Transplant Registry (ANZDATA), 2012). In the United States (US) and Europe, congenital anomalies of the kidney and urinary tract (CAKUT) and inherited nephropathies are the major causes of CKD among youngest ESKD groups (Harambat et al., 2012). The situation in the Middle East countries was revealed to be the same, but the prevalence of inherited kidney disease is reported to be much higher (up to 30%) compared to Europe due to high rates of consanguinity (Harambat et al., 2012).

In Oman, there has been a progressive increase in the ESKD incidence and prevalence over the last three decades (Al Ismaili et al., 2017). The incidence rate of ESKD patients receiving RRT in Oman at the end of 1998 was 21 per million population (PMP), whereas the calculated incidence in 2013 was 120 PMP (Al Ismaili et al., 2017). Along with the increasing rate of ESKD, a gradual increase in morbidity caused by CKD had been observed (Al Alawi et al., 2017b).

[1]adapted from Al Alawi et al. (2017a)

Hereditary disorders are massive burden on healthcare system in Oman, which are considered as the major causes of congenital malformations and perinatal deaths in hospitals (Rajab et al., 2013). The prevalence of some rare inherited kidney disease was reported to be higher compared to the worldwide prevalence due to high consanguinity (Rajab et al., 2005). However, there is no comprehensive data estimating the magnitude of inherited kidney disease in patients in Oman.

The main objectives of this study were to perform a comprehensive epidemiological and etiological report of ESKD patients commencing RRT in Oman with an emphasis on genetic causes and inherited kidney disease and compare our results with other countries. All newly registered Omani patients with ESKD commencing RRT over a fifteen-year period (2001- 2015) (n = 2,922) were analysed using the RRT register in Oman. All potentially genetic or inherited causes of ESKD were reviewed and the proportion and clinical characteristics of this cohort were reported. For more details of the RRT register, collected data and retrospective analyses performed please see Chapter 2 (Methods). The results of this study were published in Al Alawi et al. (2017a) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5480059/#B17).

3.2 Characteristics of ESKD Patients Commencing RRT

From 2001 to 2015, a total of 2,922 new patients commenced RRT due to different causes. Males contributed 57.1% (n = 1668) of the patients and females contributed 42.9% (n = 1254). The mean age of RRT commencement was 50.14 ± SD 17.5 years, while the median age was 53 years. Overall, 1321 (47.1%) cases of ESKD occurred among patients who were 45–64 years, whereas 884 (31.5%) occurred among patients who were ≤44 years and 599 (21.2%) in patients who were 65 years and over (Figure 3.1B).

Diabetic nephropathy was the most prevalent cause of ESKD (46%), followed by hypertensive nephropathy (HTN) (19%) and chronic glomerulonephritis (15%) (Figure 3.2). Inherited kidney disease contributed just 5% of the total RRT population. Other aetiologies, such as urological, tubulointerstitial kidney disease and vascular causes, comprised 11% of RRT population. However, a dramatically different picture was revealed when the primary diagnosis is given by age groups (Tables 3.1 and 3.2). In patients less than 20 years of age, inherited kidney disease was the most common primary cause of kidney disease (Table 3.1) accounting for 42% of ESKD in 0–12 years' age

group and 27% of ESKD in the 13–19 years' age group (Table 3.2). Therefore, in patients less than 20 years of age inherited kidney disease accounted for 65 of 200 cases (32.5%) of ESKD.

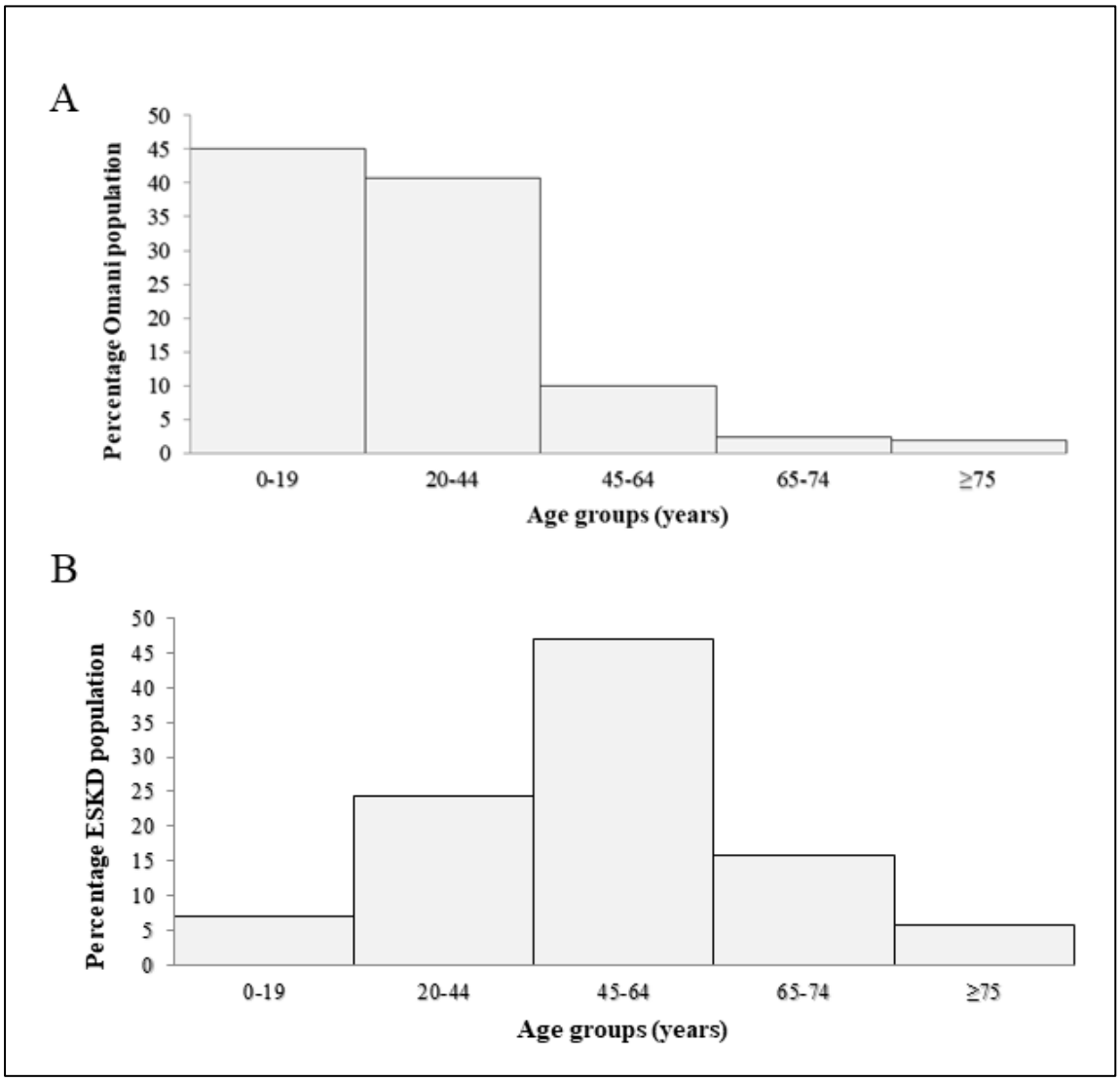

Figure 3.1 Age distribution of the ESKD population compared to the total Omani population. (A) Percentage age distribution of Omani population (National Center for Statistics and Information, 2015). (B) Percentage age distribution of ESKD population at initiation of renal replacement therapy (RRT). ESKD: end stage kidney disease.

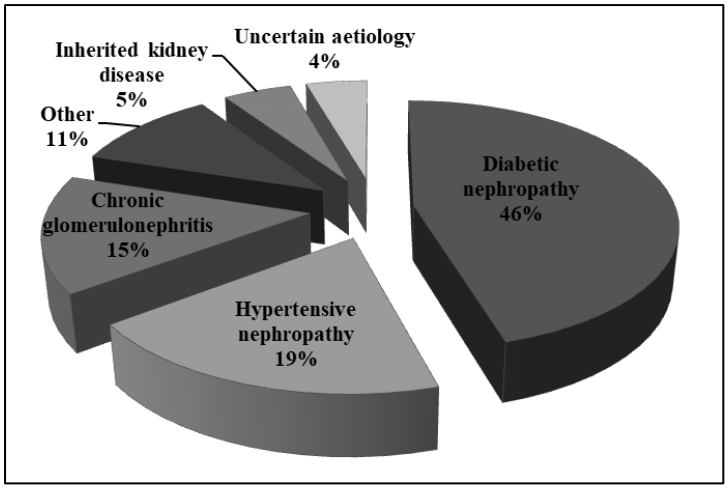

Figure 3.2 Aetiology of end stage kidney disease (ESKD) in Oman.

Primary causes of end stage kidney disease														
Age Group (years)	Diabetic nephropathy		Hypertensive nephropathy		Glomerulo-nephritis		Inherited kidney disease		Other		Uncertain aetiology		Total	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
0-12	2	0.071	0	0	16	0.571	30	1.07	15	0.535	8	0.285	71	2.5
13-19	0	0	4	0.143	45	1.605	35	1.248	33	1.177	12	0.428	129	4.6
20-44	173	6.17	125	4.458	187	6.669	33	1.177	119	4.244	47	1.676	684	24.4
45-64	769	27.43	253	9.023	139	4.957	36	1.284	77	2.746	47	1.676	1321	47.1
65-74	259	9.237	110	3.923	27	0.963	1	0.036	36	1.284	7	0.25	440	15.7
≥75	73	2.603	49	1.748	10	0.357	4	0.143	19	0.678	4	0.143	159	5.7
Total	1276	45.51	541	19.29	424	15.12	139	4.957	299	10.66	125	4.458	2804	100

Table 3.1 Distribution of primary kidney diagnosis by age in end stage kidney disease (ESKD) patients (2001-2015).
Percentages calculated after excluding patients without primary diagnosis data. *N*: number. %: percentage.

Age Group (years)	Inherited kidney disease	
	N	%
0-12	30	42.3
13-19	35	27.1
20-44	33	4.8
45-64	36	2.7
65-74	1	0.2
≥75	4	2.5
Total	139	5

Table 3.2 Distribution of inherited kidney disease by age in ESKD patients (2001-2015). *N*: number. %: percentage.

3.3 Characteristics of Inherited Kidney Disease Patients on ESKD

The distribution of males with inherited kidney disease was found to be higher (*n* = 79; 56.8%) than females (*n* = 60; 43.2%). Patients with inherited kidney disease started RRT at a younger age with a mean of 29.4 ± SD 20.1 and median age of 21 years (Figure 3.3).

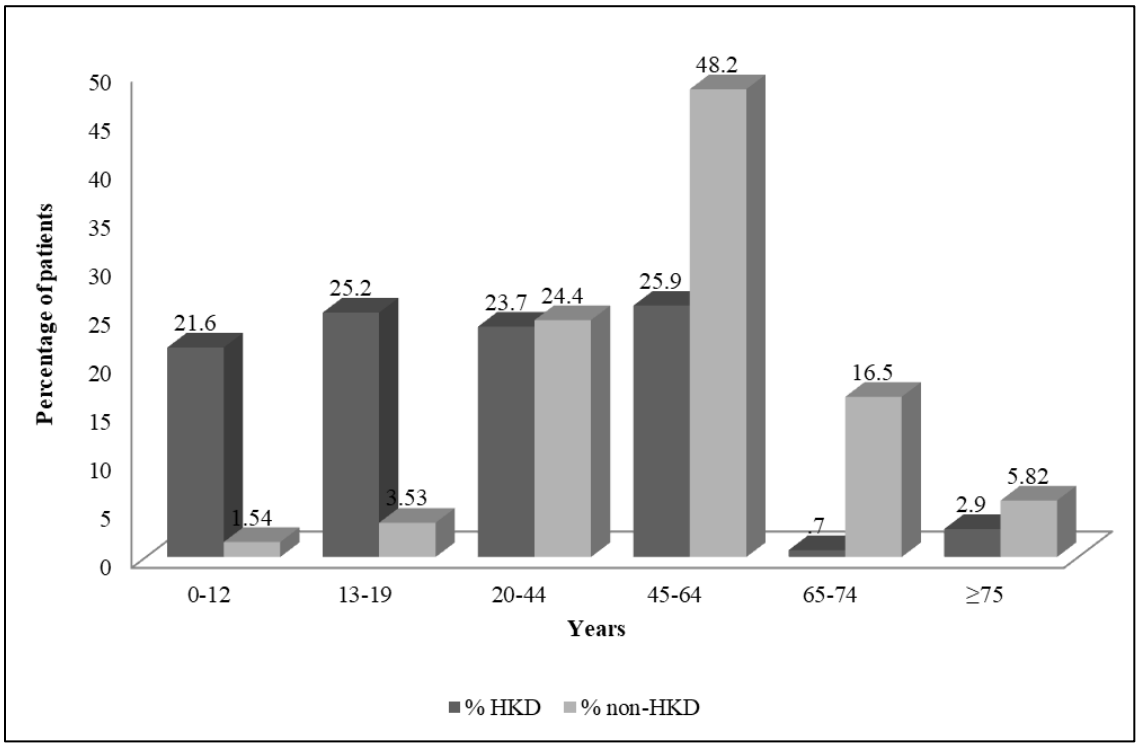

Figure 3.3 Comparison of patients with hereditary kidney disease (HKD) and non-hereditary kidney disease (non-HKD) across age groups.

A positive family history of disease was present in 36.7% of inherited kidney disease cases. Dialysis was the initial RRT modality in all inherited kidney disease patients (n = 115) except 24 patients, who received preemptive transplant in the form of living-related donor (n = 6) and living nonrelated donor (n = 18) (Table 3.3). Currently, in the cohort of inherited kidney disease patients, 37% have a functioning transplant, 38% are receiving haemodialysis, and 3.6% are receiving peritoneal dialysis (Table 3.3). A total of 25 patients (18%) have died during the study period with cardiac disease being the leading cause of death. Hypertension was the most common comorbidity in inherited kidney disease patients at initiation of RRT (54.7%), compared to diabetes (2.2%), ischemic heart disease (3.6%), cerebrovascular diseases (1.4%), and respiratory diseases (1.4%) (Table 3.3).

ADPKD was the most common inherited kidney disease diagnosis, accounting for 40.3% of inherited cases, followed by CAKUT (11.5%), Alport syndrome (9.4%), and ARPKD (7.2%) (Table 3.4).

	ADPKD		Other inherited kidney disease		Total inherited kidney disease population	
	N	%	N	%	N	%
Positive family history of disease	23	41.1	28	33.7	51	36.7
Comorbidity						
Diabetes mellitus	2	3.6	1	1.2	3	2.2
Hypertension	34	60.7	42	50.6	76	54.7
Ischemic heart disease	5	8.9	0	0.0	5	3.6
Cerebrovascular disease	1	1.8	1	1.2	2	1.4
Respiratory disease	1	1.8	1	1.2	2	1.4
Other	6	10.7	17	20.5	23	16.5
First RRT modality						
Haemodialysis	42	75.0	63	75.9	105	75.5
Peritoneal dialysis	1	1.8	9	10.8	10	7.2
Preemptive transplant, living related donor	1	1.8	5	6.0	6	4.3
Preemptive transplant, living nonrelated donor	12	21.4	6	7.2	18	12.9
Current status						
Haemodialysis	18	32.1	35	40.7	53	38.1
Peritoneal dialysis	1	1.8	4	4.7	5	3.6
Transplant	22	39.3	30	34.9	52	37.4
Lost to follow-up	2	3.6	1	1.2	3	2.2
Deceased	13	23.2	12	14.0	25	18.0
Cause of death						
Cardiac disease	5	38.5	3	25.0	8	32
Cerebrovascular disease	2	15.4	1	8.3	3	12
Infection	3	23.1	2	16.7	5	20
Other	1	7.7	4	33.3	5	20
Uncertain	2	15.4	2	16.7	4	16

Table 3.3 Comparison between Autosomal dominant polycystic kidney disease (ADPKD) and other causes of inherited kidney disease. *N*: number. %: percentage.

Inherited kidney disease	N	Proportion of inherited kidney disease (%)	Proportion of ESKD in this cohort (%)	Age group (*N*)			
				0-12	13-19	20-44	≥45
Autosomal dominant polycystic kidney disease (ADPKD)	56	40.3	2	1	1	17	37
Congenital anomalies of Kidney and Urinary Tract (CAKUT)	16	11.5	0.6	8	6	2	0
Alport syndrome	13	9.4	0.5	0	7	6	0
Autosomal recessive polycystic kidney disease (ARPKD)	10	7.2	0.4	3	6	0	1
Dysplastic cystic kidney	7	5	0.2	1	3	2	1
Steroid resistant nephrotic syndrome (congenital & childhood)	5	3.6	0.2	4	1	0	0
Primary hyperoxaluria	5	3.6	0.2	2	2	1	0
Prune-belly syndrome	5	3.6	0.2	2	2	1	0
Familial focal segmental glomerulosclerosis	5	3.6	0.2	1	2	2	0
Medullary cystic kidney	4	2.9	0.1	3	0	1	0
Familial interstitial nephropathy	2	1.4	0.1	1	1	0	0
Haemolytic uremic syndrome	2	1.4	0.1	2	0	0	0
Mesangioproliferative glomerulosclerosis	2	1.4	0.1	0	1	1	0
Membranoproliferative glomerulosclerosis	2	1.4	0.1	0	0	0	2
Nephronophthisis	1	0.7	0.0	1	0	0	0
Barter syndrome	1	0.7	0.0	0	1	0	0
Lowe's syndrome	1	0.7	0.0	0	1	0	0
Renal tubular acidosis (Type 1)	1	0.7	0.0	0	1	0	0
Undetermined familial disease	1	0.7	0.0	1	0	0	0
Total	139	100	5	30	35	33	41

Table 3.4 Inherited kidney diseases in end stage kidney disease (ESKD) population (2001-2015). *N*: number. %: percentage.

3.4 Discussion

This study represents a comprehensive, up-to-date population-based epidemiological and etiological report of Omani patients reaching ESKD and commencing RRT. It reveals that ESKD is more prevalent in males, with a ratio of 1.3, which is consistent with data reported from other countries (Hecking et al., 2014). The median age of incident ESKD patients starting RRT was 53 years and is in agreement with that reported in other Middle East countries with almost similar demographics and socioeconomic features, including Saudi Arabia (Al-Sayyari and Shaheen, 2011) and Jordan (Batieha et al., 2007), but substantially lower than that reported in European countries, including the United Kingdom (UK) (UK Renal Registry, 2013) , Croatia, Georgia and Cyprus (European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Registry, 2015) and the USA (USRDS, 2013). The data we present here from Oman shows that there is a sharp increase in the prevalence of ESKD with increasing age. We anticipate that as the population ages in Oman, the ESKD prevalence will increase. This trend is consistent with that reported from Saudi Arabia, which is comparable to the situation in the developed countries where the rate of elderly is recently decreased or stabilized (Al-Sayyari and Shaheen, 2011).

Diabetic nephropathy was the commonest underlying cause of ESKD and was more prevalent among older patients of 45 years and over. Eighteen years ago, the prevalence of diabetic nephropathy among RRT patients in Oman was reported to be 14.5% (Al-Marhuby, 1998), whereas in our study it accounted for 45.5% of ESKD population. The observed incidence of diabetic nephropathy leading to ESKD places Oman among the highest countries in the world. Table 3.5 allows a comparison in the percentage of incident ESKD patients due to diabetes mellitus in Oman and other countries.

In this study, we described for the first time the prevalence of inherited kidney disease causing ESKD in Oman. Inherited kidney disease comprises 5% of all causes of ESKD but was highly prevalent in paediatric ESKD patients. The detected prevalence of inherited kidney disease in our study is considerably lower than that reported in other countries including Libya (12%) (Alashek et al., 2013) and Australia (10%) (Mallett et al., 2014); however, it is consistent with the summarized estimate of the countries of the Gulf Cooperation Council (GCC) of 4.43% (Hassanien et al., 2012). Nonetheless, inherited and

congenital kidney disease is thought to be an important aetiology of ESKD in Omani population in which the consanguinity rate is relatively high (56.3%) (Tadmouri et al., 2009). The detected low frequencies of inherited kidney disease are expected to be due to high mortality rate among newborns with recessively inherited kidney disorders. CKD in paediatric patients is a devastating illness and the mortality rate for those with ESKD receiving RRT is expected to be 30–150 times higher when compared to a general paediatric population (McDonald and Craig, 2004). Moreover, since what is seen from ESKD is only the "tip of the iceberg" of CKD we expect inherited kidney disease patients with earlier stages of CKD are probably exceeding those reaching ESKD. Recently, Al Riyami et al. (2019) in their study comprising Omani children with different CKD stages (I-V) revealed that the overall incidence of CKD in Oman is higher than that of other countries and inherited kidney disease account for 32% of CKD aetiology. At diagnosis only 41.3% of patients are on CKD stage 5, while the remaining (58.6%) are at CKD stage 2-4.

Country	Diabetic	HTN	GN	UA	ADPKD	Reference
Jordan	29.2	14.2	12.3	12.4	-	(Batieha et al., 2007)
United Kingdom	15.9	6.1	19	16	9.9	(UK Renal Registry, 2013)
Libya	26.5	14.6	21.2	10.2	6.3	(Alashek et al., 2013)
India	20.5	4.5	34.5	19	5	(Sakhuja and Sud, 2003)
Pakistan	10	12	37	19	3	(Sakhuja and Sud, 2003)
Turkey	29.9	25.9	7.9	15.7	3.8	(Connor et al., 2013)
Oman	*45.5*	*19.3*	*15.1*	*4.5*	*2*	*This study*

Table 3.5 Primary causes of end stage kidney disease (ESKD) in Oman and other countries. HTN: Hypertensive nephropathy; GN: Glomerulonephritis; UA: uncertain aetiology; ADPKD: autosomal dominant polycystic kidney disease.

A comparison of Omani registry data from comparator countries shows a much smaller burden of ADPKD than Western countries and lowers than the summarized estimate of ADPKD in GCC countries (4.8%) (Hassanien et al., 2012) (Table 3.3). The reasons for this probably reflect the relative young population in Oman. An age comparison of the total

population of Omani versus age of ESKD population confirms this, where the population can be divided into youth group (0-14 years) that account for 21.6%, working age group (15-64 years) accounting for 57.3% and an elderly group (≥65 years) accounting for 4.3% (National Center for Statistics and Information, 2015) (Figures 3.1 (A) and (B)). Published data from Australia and New Zealand describe an increase in the contribution of ADPKD to ESKD over the last 5 decades (Fernando et al., 2017) and reflect an overall increase in age of the ESKD population. Similar observations have been made in other countries including Denmark (Orskov et al., 2010) and the USA (USRDS, 2013). Thus, over the next few decades the relative contribution of ADPKD to ESKD in Oman will likely increase towards 5–10%.

Estimating the prevalence and incidence of ESKD accurately is very important to evolve strategies to decrease the total burden of ESKD and support the preparation of a comprehensive health service delivery. The approach of using RRT registries may underestimate the accurate burden of ESKD, because of the uncertainty in the total of undiagnosed cases and missing of ESKD individuals who are not commencing RRT (Li et al., 2018). Data capture-recapture approach is now used in epidemiology to determine the accurate estimation and to essentially notify the gap in RRT facilities, at which various administrative data sources are utilized to reduce the effect of misclassification (Li et al., 2018).

In summary, this study represents a population-based etiological report of Omani ESKD commencing RRT from 2001 to 2015. It clearly shows that Oman is facing major factors that globally are fundamentally responsible for the growing incidence of ESKD in adults, namely, an aging population and a high burden of diabetes mellitus. Therefore, health care providers must concentrate on strategic actions that highlight primary prevention, early detection, and dynamic management of CKD population. For the first time, the prevalence of inherited kidney disease causing ESKD in Oman has been accurately described and this data emphasizes need to measure the frequencies of inherited kidney disease patients in earlier stages of CKD and assess their rate of progression to ESKD.

Chapter 4. Targeted Next Generation Sequencing of Omani Patients with Inherited Cystic Kidney Disease[2]

4.1 Introduction and aims

Chronic kidney disease (CKD) is defined as anomalies in the structure or function of the kidney that are present for more than 3 months and have implications for health. Inherited kidney diseases are a major cause of CKD and often lead to progressive CKD resulting in end-stage kidney disease (ESKD). Cystic kidney diseases are common inherited causes of ESKD in both children and adults, accounting for 6%–12% of cases (Harambat et al., 2012, Torres and Harris, 2019).

Inherited forms of cystic kidney have been associated with dysfunction of the primary cilia (Braun and Hildebrandt, 2017). These diseases are frequently called renal ciliopathies and are part of an increasing number of inherited diseases that include autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD)(Bergmann, 2019), tuberous sclerosis complex (TSC) (Bissler and Christopher Kingswood, 2018), autosomal dominant tubulointerstitial kidney disease (ADTKD) (Zaucke et al., 2010), nephronophthisis-related ciliopathies (NPHP-RC) (Srivastava et al., 2017), Bardet-Biedl syndrome, Senior-Löken syndrome, Meckel Gruber syndrome, Joubert syndrome, and others (Mitchison and Valente, 2017).

Molecular genetic analysis of individuals and families with cystic kidney disease is crucial in order to determine accurate diagnosis, prognosis, genetic counselling, and medical and educational management (Mallett et al., 2016, Alkanderi et al., 2017, Mallett et al., 2017). However, genetic testing requires time and cost-effective approaches that will not overburden healthcare systems. Next generation sequencing (NGS, also called massively parallel sequencing) technologies are dramatically increasing sequencing capacity in routine clinical diagnosis, and speeding up genetic mutation identification. Gene panel approaches through parallel sequencing of targeted subsets of disease-associated genes as well as whole-exome and whole-genome sequencing are increasingly becoming part of routine clinical service for the investigation of inherited kidney disease (Al-Hamed et al., 2016, Mallawaarachchi et al., 2016, Groopman et al., 2019). Specific to cystic kidney disease, various studies have applied NGS to deliver a disease-specific molecular diagnosis. Targeted NGS panels have been used extensively for analysis of ADPKD (Rossetti et al., 2012, Lanktree et al., 2019), ARPKD (Bergmann, 2017), and NPHP-RC (Chaki et al., 2012).

[2]Adapted from Al Alawi et al. (2019) (URL: https://www.kireports.org/article/S2468-0249(19)31468-8/fulltext)

In Oman, inherited kidney diseases are relatively common, leading to a significant healthcare burden (Rajab et al., 2013). Congenital malformations and genetic disorders are associated with 39% of perinatal deaths in hospitals (Rajab et al., 2013). However, to date, access to molecular genetic diagnostics is limited in Oman. We sought to establish an NGS platform for the molecular genetic diagnosis of cystic kidney disease in Oman that would allow for the first time a picture of the underlying molecular genetic causes of cystic kidney disease. In this pilot study of 53 patients with cystic kidney disease, we applied a diagnostic panel targeting 49 genes and achieved an overall molecular genetic diagnosis rate of 75%. The results were published in Al Alawi et al (2019) (URL:https://www.kireports.org/article/S2468-0249(19)31468-8/fulltext)

4.2 Panel Performance

The NGS panel (Tables 2.1 and A1.1) showed a good capture yield and high sequencing quality with mean coverage depth of 875.3 ± 541 SD (Table 4.1). An average of 2.6 million filtered reads were generated per sample, with 2.0 million of these aligned uniquely to the target region, leading to 76.5% average reads enrichment (65% to 83%) and hence indicating high sensitivity of the capture method used. On average, this panel provided 98.6%, 97.8%, 95.7%, and 89.3% base reads on target, with base coverage of 20×, 30×, 50×, and 100×, respectively (Table 4.1). Sufficient coverage of all *PKD1* exons was obtained through designing capture primers and enrichment technique (Table 4.2).

4.3 Patients Characteristics

The 53 patients included in this study were from different regions throughout Oman (Figure 4.1) and included one prenatal (2%), 30 pediatric (from birth to 13 years; 57%), and 22 teenage/adult (>13 years; 42%) cases. The median age was 10 years (range: 0–63 years), and 28 (53%) were female. At the time of referral, 39 (74%) had a known family history of kidney disease.

Performance Metrics	Mean (Average)	Standard Error of the Mean ($SE_{\bar{x}}$)	Standard Deviation (SD)
Total Length of targeted reference	244088	0	0
Total aligned reads	2607397.1	231596.2	1553594.3
Targeted aligned reads	2006310.1	182732.0	1225803.4
Read enrichment %	76.5	0.6	4.1
Total aligned bases	391703116	35675192.3	239316465.0
Targeted aligned bases	311088772.2	29061958.1	194953541.5
Base enrichment %	79.0	0.6	3.8
Mean region coverage depth	875.3	80.7	541.3
Uniformity of coverage (Pct > 0.2*mean)	95.464	0.2	1.4
Target coverage at 1X	99.5	0.03	0.2
Target coverage at 10X	99.1	0.1	0.7
Target coverage at 20X	98.6	0.2	1.5
Target coverage at 30X	97.8	0.4	3.0
Target coverage at 50X	95.7	1.1	7.3
Target coverage at 100X	89.3	2.9	19.7
Insert size median	302.4	6.9	46.2
Insert size 25th percentile	270.7	6.2	41.3
Insert size 75th percentile	350.6	8.0	53.7
Captured Variants	807.8	29.4	197.3
Variants (Percent found in dbSNP)	44.4	1.1	7.6
Variant Ts/Tv ratio	1.0	0.03	0.2
Variant Het/Hom ratio	7.7	0.4	2.5

Table 4.1 NGS panel performance metrics. Read enrichment: Number of targeted reads / total number of aligned reads (expressed as a percentage). Base enrichment: Number of targeted aligned bases / total aligned bases (expressed as a percentage). Uniformity of coverage (Pct > 0.2*mean): Percentage of targeted regions with average coverage > 20% of the mean region coverage depth. Variant Ts/Tv ratio: Number of nucleotide substitutions that are transitions / number of substitutions that are transversions.

Genomic Coordinate		Length	Exons Captured	Mean Coverage Depth	Mean% Covered	Mean Read Count
Start	End					
2185425	2185740	316	1	109	53%	685
2169257	2169429	173	2	1074	100%	1932
2169064	2169236	173	3	523	100%	1833
2168626	2168896	271	4	873	100%	2039
2167741	2168513	773	5	363	100%	2337
2167439	2167723	285	6	335	100%	1152
2166783	2167104	322	7	506	100%	1800
2166479	2166695	217	8	738	100%	2230
2165942	2166169	228	9	587	100%	1640
2165328	2165676	349	10	491	100%	1395
2164120	2164976	857	11	637	100%	6984
2163111	2163343	233	12	269	90%	627
2162738	2163014	277	13	1078	100%	3121
2162290	2162524	235	14	323	100%	1243
2158202	2161922	3721	15	601	100%	16207
2157833	2158083	251	16	538	100%	2531
2156755	2156999	245	17	654	100%	1798
2155815	2156728	914	18-20	450	100%	3051
2155272	2155525	254	21	502	100%	908
2154448	2154693	246	22	512	100%	1343
2153216	2153946	731	23	835	100%	4347
2152764	2153021	258	24	946	100%	2882
2152331	2152684	354	25	595	100%	2024
2152011	2152307	297	26	569	100%	1811
2149594	2150617	1024	27-30	583	100%	4227
2147678	2148035	358	31-32	1040	100%	3880
2147098	2147554	457	33-34	548	100%	3010
2143494	2144261	768	35-37	457	100%	3947
2142904	2143144	241	38	1032	100%	2832
2142430	2142643	214	39	1047	100%	1890
2141997	2142239	243	40	310	100%	863
2141731	2141957	227	41	946	100%	2271
2141373	2141648	276	42	145	59%	578
2139677	2141225	1549	43-46	388	100%	4337

Table 4.2 Depth of coverage of the captured regions of *PKD1* gene using NGS panel. Eleven multiplexed samples were sequenced using the designed NGS panel and run on the MiSeq System. PCR duplicates and highest and lowest performers were removed from analysis. Performance was calculated from average coverage for each exon by depth and percent and average of the number of reads in each exon.

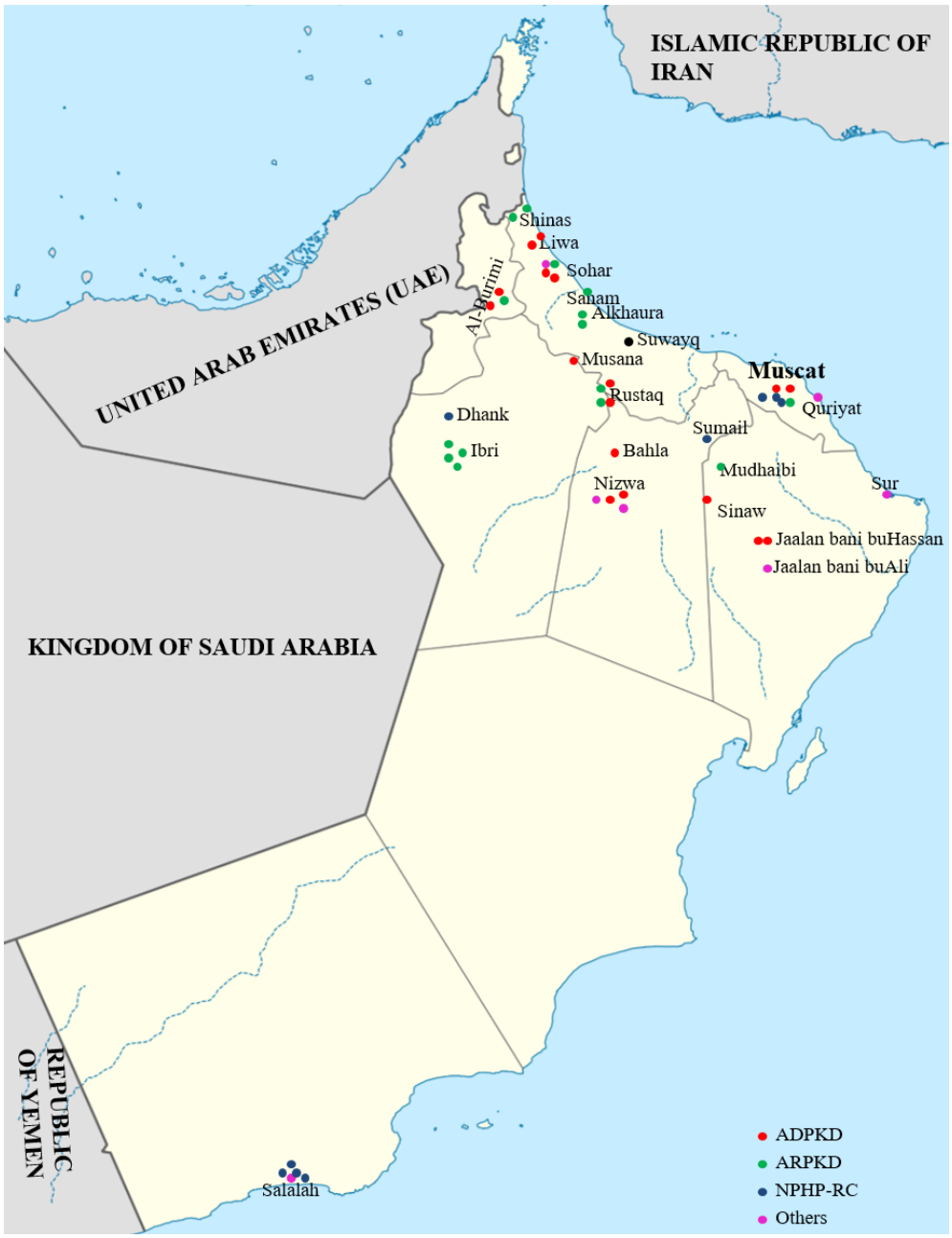

Figure 4.1 Geographical distribution of patients with cystic kidney disease patients included in this study. ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; NPHP-RC, nephronophthisis-related ciliopathy.

4.4 Molecular Genetic Diagnosis and Correlation with Clinical Phenotype

The clinical diagnosis of the patients included ADPKD (*n* = 16; 30%), ARPKD (*n* = 16; 30%), NPHP-RC (*n* = 12; 23%), ciliopathy syndromes (*n* = 5; 10%), and unspecified cystic kidney disease (*n* = 4; 7.5%). Clinical features and key family history details are shown in Table 4.3. Molecular genetic investigations identified disease-causing variants in 40 of 53 (75%) patients (Figure 4.2A). Disease-causing variants were detected in *PKD1*, *PKHD1*, *NPHP1*, *NPHP3*, *NPHP4*, *SDCCAG8*, *HNF1B,* and *WDR19* (Figure 4 .2B).

Upon evaluation, molecular genetic testing confirmed the clinical diagnosis of 33 (62%), changed the diagnosis in 3 (6%), and revealed a diagnosis in 3 patients (6%) with unspecified cystic kidney disease (Figure 4.3). Overall, 12 (55%) variants were previously reported as disease causing, and 10 (46%) were novel (Table 4.4). Causative variants include 13 different single nucleotide variants (SNVs; 10 missense and 3 nonsense), 7 small insertions/deletions (INDELs; 5 deletions [≤20 base pairs] and 2 insertions), one large INDEL, and one whole-gene deletion (Table 4.4). According to the American College of Medical Genetics and Genomics (ACMG), 17 variants were classified as pathogenic, 4 as likely pathogenic, and one as a variant of uncertain significance (Table 4.4) (Richards et al., 2015).

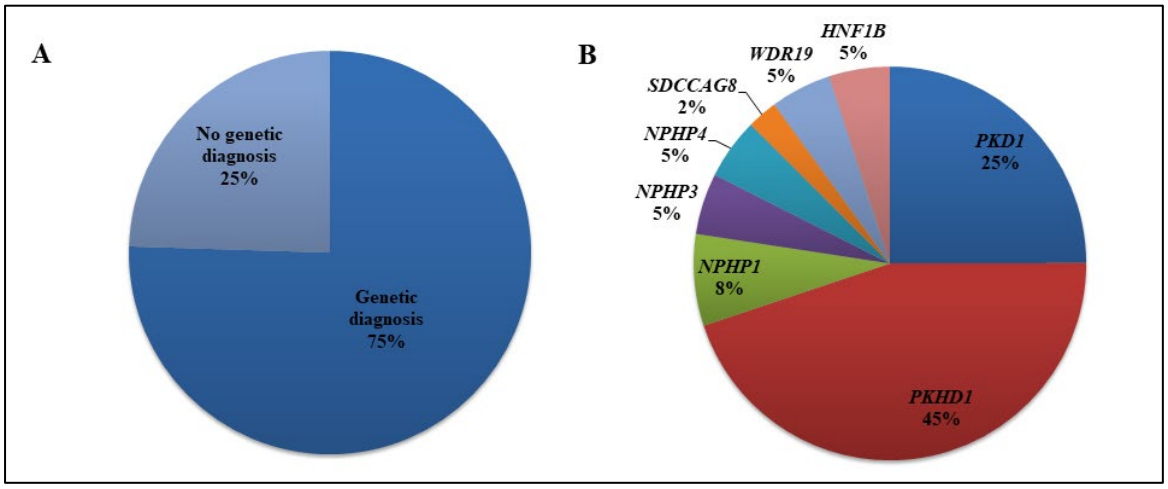

Figure 4.2 Molecular genetic diagnosis rate and genotype of studied patients. (A) Percentage of patients with an identified molecular genetic etiology of underlying cystic kidney disease. (B) Distribution of causative cystic kidney disease genes.

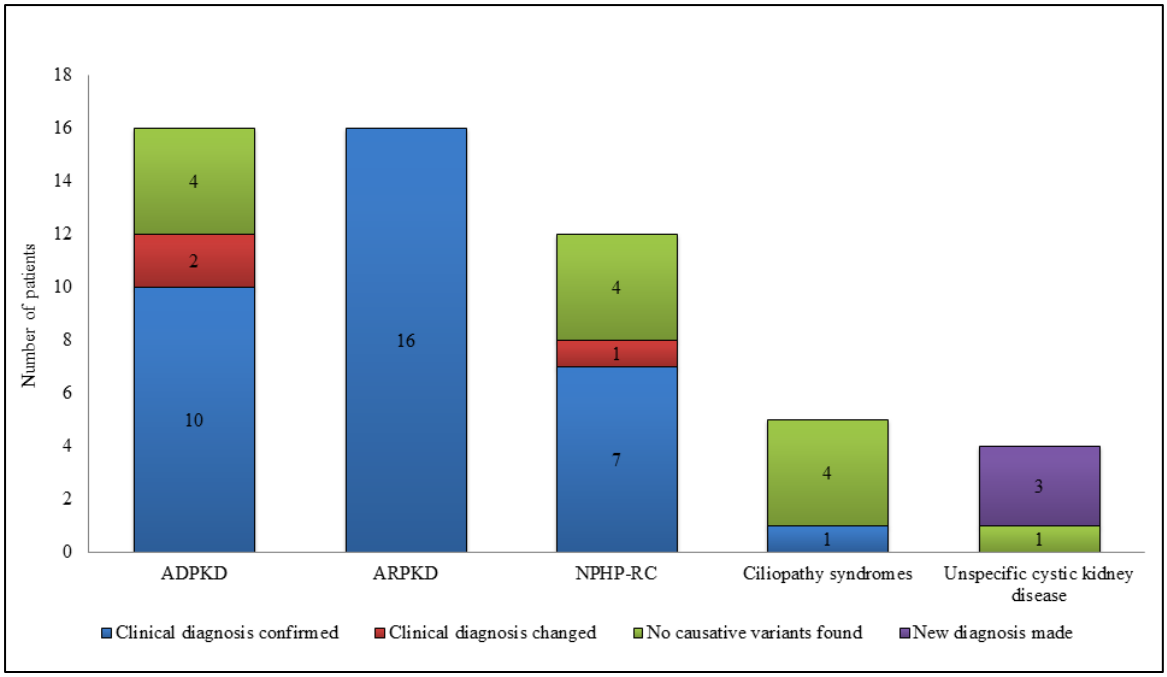

Figure 4.3 Comparison between suspected clinical diagnosis and molecular genetic diagnosis. ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; NPHP-RC, nephronophthisis-related ciliopathies.

The majority of patients referred for ADPKD were teenagers/adults (88%; 14 of 16). Genetic testing identified 8 different *PKD1* pathogenic variants, including 1 novel missense, 1 novel nonsense alteration, 2 small deletions, 2 novel small insertions, and 1 large INDEL (Table 4.4 and Figure 4.4).

A molecular genetic etiology of ARPKD was obtained in a total of 18 unrelated patients, in which 4 *PKHD1* different missense variants were detected. These variants were c.107C>T, p.(Thr36Met); c.406A>G, p.(Thr136Ala); c.4870C>T, p.(Arg1624Trp), and c.9370C>T, p.(His3124Tyr) (Table 4.4). The c.107C>T, p.(Thr36Met) *PKHD1* variant was identified homozygously in 10 patients and compound heterozygously in 4 other patients (Table 4.4). The p.(Thr136Ala) allele (Figure 4.5) has not been reported previously or described in any databases. However, mutation evaluation algorithms considered this variation pathogenic.

Patient ID	Age at referral	Gender	Clinical diagnosis	Additional clinical features	Family history	Specific tests findings	Region in Oman
P1	47 Y	F	ADPKD	Hypertension. ESKD 47 Y.	Brother: ADPKD (CKD stage 4). Sister: ADPKD (ESKD).	Renal USS: bilateral polycystic kidneys with several hepatic cysts.	Buraimi
P2	26 Y	F	ARPKD	CKD stage 4.	Brother: ARPKD	Renal USS: small kidneys with loss of CMD and multiple tiny cysts	Saham
P3	4 M	M	ARPKD	Hypertension, respiratory distress and ESKD from birth. Early death.		Antenatal USS showed bilateral enlarged kidneys.	Shinas
P4	37 Y	F	ADPKD	Hypertension. ESKD 33 Y.	ADPKD in multiple family members.	Abdo USS: multiple kidney cysts, simple hepatic cysts and two pancreatic cysts.	Sinaw
P5	12 Y	F	ARPKD	Portal hypertension. CKD stage 1.	History of sibling death (aged 4 Y).	Renal USS: large echogenic kidneys, hepatosplenomegaly.	Ibri
P6	2 Y	M	ARPKD	Hypertension and iron deficiency anaemia. CKD stage 3A.	Sibling died of ESKD. 3 paternal cousins with ARPKD. Parents are consanguineous.	Abdo USS: cystic kidneys and hepatomegaly.	Sohar
P7	3 D	M	MKS	Dysmorphic features, occipital encephalocele and polydactyl. CKD stage 1.	2 sibling deaths: encephalocoele (aged 1 Y); hydrocephalus (aged 7 Y). Parents are consanguineous.	Antenatal USS: occipital encephalocele and bilateral ventriculomegaly.	Nizwa
P8	5 M	F	ARPKD	Hypertension and recurrent respiratory	Sibling death: respiratory distress.	Antenatal USS: oligohydramnios. Postnatal USS: hepatomegaly and	Ibri

				infection. CKD stage 2.		enlarged cystic kidneys.	
P9	16 Y	M	ARPKD	Chronic bronchial asthma, hyperparathyroidism, short stature, ESKD from birth.	Parents are consanguineous.	Abdo USS: both kidneys enlarged with multiple cysts. Liver biopsy: congenital hepatic fibrosis.	Khabura
P10	3 Y	F	NPHP	Recurrent UTI, infantile cystic kidney disease, CKD stage 1.	2 affected siblings; one died with ESKD.	Abdo USS: small kidneys with cysts and congenital liver fibrosis.	Salalah
P11	19 Y	F	ARPKD	Portal hypertension and oedema, ESKD 14 Y.	Sibling with ARPKD. Parents are consanguineous.	Abdo USS: cystic kidneys with hepatic fibrosis and hypersplenisim.	Buraimi
P12	3 M	F	MKS	Dysmorphic features, occipital encephalocele, polydactyl, diaphragmatic hernia. ESKD 1 Y.	Sibling died perinataly with encephalocele. Parents are consanguineous.	Abdo USS: large cystic kidneys.	Quriyat
P13	2 Y	M	JBTS	Global developmental delay, hypotonia, polycystic kidneys, poor visual fixation.		Brain MRI: molar tooth sign, small vermis. Renal USS: polycystic kidneys.	Sur
P14	1 Y	F	NPHP	Failure to thrive, chronic anaemia, ESKD 1 Y.		Renal biopsy: cystic dilation of tubules, glomerulosclerosis and advanced interstitial scarring. Liver biopsy: bile duct proliferation, and hepatic fibrosis. Renal USS: increased echogenicity with loss of CMD and cortical cysts noted in left kidney.	Liwa

P15	2 Y	F	NPHP	Hypertension, ESKD 1 Y.	2 affected siblings; one died due to ESKD.	Abdo USS: small kidneys with cysts and congenital liver fibrosis.	Salalah
P16	2 M	F	ARPKD	CKD stage 4, hypertension.	4 siblings with perinatal deaths with ARPKD. Parents are consanguineous.	Renal USS: large kidneys with multiple cysts.	Ibri
P17	2 D	F	Antenatal cystic kidney disease	Antenatal severe enlarged kidneys and lung hypoplasia. Perinatal death.		Antenatal scan showed enlarged kidneys and lungs hypoplasia.	Sohar
P18	41 Y	M	ADPKD	Hypertension and diabetes, ESKD 39 Y.	Mother and brother with ADPKD.	Imaging (CT, US): bilateral polycystic kidney, few calcification in the wall of cysts. Several hepatic and pancreatic cysts.	Sohar
P19	6 Y	M	BBS	Polydactyly hands and feet, obesity, developmental delay, retinitis pigmentosa, CKD stage 1.		Imaging (US) showed no renal cyst yet.	Nizwa
P20	43 Y	F	ADPKD	Hypertension and recurrent UTI, CKD stage3.	Mother, 2 brothers and 3 sisters with ADPKD.	Abdo MRI: kidneys enlarged with several cysts. Some of cysts are haemorrhagic. Multiple liver cysts.	Muscat
P21	fetus	U/A	Antenatal cystic kidney disease		Family history of 3 fetal deaths.	Antenatal USS: kidneys enlarged with no CMD. Lung hypoplasia.	Suwaiq
P22	3 Y	M	ARPKD	Hypertension, CKD stage 3A.	1 affected sibling. Parents are consanguineous.	Abdo USS: kidneys enlarged, echogenic parenchymal mild hydronephrosis. Hepatic fibrosis.	Rustaq

P23	12 Y	M	NPHP	Short stature and delayed bone age. Dyslipidaemia. CKD stage 3A.	Parents are consanguineous.	Renal USS: echogenic kidneys.	Liwa
P24	8 Y	F	NPHP	Chronic, recurrent respiratory infections, retinal degeneration, ESKD 8 Y.	Sister: ESKD.	Renal USS: increased echogenicity of both kidneys with poor CMD with cortical scars.	Sumail
P25	2 M	F	ARPKD	Hypertension and hypoaldosteronism, lung hypoplasia, perinatal death.	History of older sibling death (2 days) with similar features.	US imaging showed bilateral enlarged kidneys with multiple cysts and liver haemangioma.	Khabura
P26	17 Y	F	ARPKD	Hypertension, anaemia, ESKD 13 Y.	3 siblings with ARPKD: 2 died.	Renal USS: enlarged kidneys with multiple small cysts and loss of CMD. Liver fibrosis.	Mudhaibi
P27	20 Y	M	ARPKD	Abdomen pain and urinary tract infection. CKD stage 3.	3 brothers with ARPKD.	Abdo USS: liver fibrosis, splenomegaly and both kidneys increased echogenicity, loss of CMD and small cysts.	Rustaq
P28	28 Y	F	ARPKD	ESKD 11 Y.	Father and sister with ARPKD.	Abdo USS: hepatomegaly and echogenicity due to hepatic fibrosis. Bilateral enlarged kidneys.	Shinas
P29	1 Y	F	ARPKD			Renal USS: enlarged kidneys with increased echogenicity and nephrocalcinosis.	Muscat
P30	3 Y	F	NPHP	Developmental delay, hyperparathyroidism, right hip dysplasia and failure to thrive, ESKD 4 Y.	Parents are consanguineous.	Renal USS: echogenic kidneys and poor CMD, no focal lesion or hydronephrosis.	Dhank

P31	5 Y	F	NPHP	Retinitis pigmentosa, bilateral conductive hearing loss, ESKD 6 Y.		Renal USS: both kidneys small in size, with bilateral cysts.	Muscat
P32	40 Y	M	ADPKD	Hypertension, hyperuricaemia and hyperlipidaemia, ESKD 39 Y.	Maternal uncle: ADPKD.	Abdo CT: multiple cystic lesions on both kidneys, multiple small renal stones in left kidney.	Nizwa
P33	31 Y	M	ADPKD	Renal calculi.	Father and brother with ADPKD.	Renal USS: bilateral enlarged kidneys with multiple cysts and stones.	Rustaq
P34	55 Y	F	ADPKD	Hypertension and diabetes, ESKD 37 Y.	Brother and 2 sons with ADPKD.	Renal USS: bilateral cysts.	Sohar
P35	48 Y	M	ADPKD	Hypertension, ESKD 50 Y.	Sister with ADPKD.	Abdo USS: fatty liver, bilateral increased renal cortical echogenicity, bilateral renal cortical cysts.	Jaalan bani buHassan
P36	29 Y	F	ADPKD	Hypertension, ESKD 31 Y.	Father and paternal uncle: ADPKD.	Renal USS: bilateral cysts.	Jaalan bani buHassan
P37	53 Y	M	ADPKD	Hypertension, ESKD 48 Y.	Mother and maternal uncle: ADPKD.	Renal USS: bilateral enlarged kidneys with multiple cysts.	Buraimi
P38	2 Y	F	ARPKD	Hypertension, CKD stage 3A.		Abdo USS: enlarged kidneys with multiple small cysts and loss of CMD and liver fibrosis.	Suwaiq
P39	13 Y	F	NPHP	CKD stage 2.		Abdo USS: kidneys increased cortical echogenicity with loss of CMD and cortical cysts.	Muscat
P40	12 Y	M	NPHP	ESKD age 10.	2 siblings with ESKD and 1 with CKD stage 4.	Abdo USS: kidneys increased echogenicity and loss of CMD.	Sumail

P41	10 Y	M	MKS	Occipital encephalocele, dysmorphism, failure to thrive, and visual loss. CKD stage 3B.		Renal USS: large kidneys with loss of CMD and presence of multiple cysts.	Jaalan bani buAli
P42	7 Y	F	ADPKD	Abdominal pain.		Renal USS: large kidneys with multiple cysts.	Muscat
P43	47 Y	M	ADPKD	Hypertension, abdominal pain. CKD stage 4.	Father and paternal uncle: ADPKD.	Renal USS: large kidneys with multiple cysts.	Musana
P44	5 M	M	ARPKD	Hypertension, oligohydramnios, ESKD.		Renal USS: enlarged kidneys with increased echogenicity with loss of CMD and small cysts.	Ibri
P45	44 Y	M	ADPKD	Hypertension and haematuria. ESKD 45 Y.	2 paternal aunts and 2 paternal uncles with ADPKD. 3 Brothers and 1 daughter with ADPKD.	Renal USS: enlarged cystic kidneys.	Bahla
P46	10 Y	F	NPHP	Abdominal pain, developmental delay, delayed speech. CKD stage 4.	Brother with ESKD and developmental delay.	Renal USS: hypoplastic dysplastic kidneys.	Salalah
P47	63 Y	M	ADPKD	Hypertension, CKD stage 3B.		Renal USS: cortical renal cysts.	Rustaq
P48	13 Y	M	Unexplained kidney failure	ESKD 12 Y.	Sister with CKD stage 3.	Renal USS: echogenic kidneys with loss of CMD and multiple cysts.	Salalah
P49	2 M	F	NPHP	Hypertension, liver fibrosis, nystagmus, developmental delay, ESKD from birth.	Consanguineous parents. One sibling with liver fibrosis, died aged 3 Y.	Abdo USS: hepatic fibrosis, mild splenomegaly, kidneys with increased echogenicity and loss of CMD.	Muscat

P50	11 Y	F	NPHP	CKD stage 3. Mild hearing loss.		Renal USS: large kidneys with increased in echogenicity.	Salalah
P51	8 Y	M	ADPKD	CKD stage 2.		Renal USS: echogenic kidneys, loss of CMD.	Nizwa
P52	2 D	M	UCKD	Dysmorphic with severe oligohydramnios.	Parents are consanguineous.	Antenatal USS: enlarged cystic kidneys with severe oligohydramnios.	Sohar
P53	23 Y	M	ADPKD	ESKD 13 Y.			Rustaq

Table 4.3 Clinical phenotype and evidence of family history of renal disease. Abdo, Abdominal; ADPKD, autosomal dominant kidney disease; ARPKD, autosomal recessive kidney disease; BBS, Bardet Biedl syndrome; CKD, chronic kidney disease; CMD, corticomedullary differentiation; CT, computed tomography; D, day; ESKD, end stage kidney disease; F, female; JBTS, Joubert syndrome; M, male; M, month; MKS, Meckel Gruber syndrome; NPHP, nephronophthisis; U/A, unavailable; UCKD, unspecific cystic kidney disease; USS, ultrasound scan; UTI, urinary tract infection; Y, year.

Patient	Initial diagnosis	Gene	Transcript	c. change	aa. change	Type	ACMG Classifi.	dbSNP ID	F/ seg.	Reference
P1	ADPKD	*PKD1*	NM_001009944.2	c.7428C>G	p.Cys2476Trp	SNV	likely pathogenic	NA	Yes	**Novel**
P2	ARPKD	*PKHD1*	NM_138694.3	c.9370C>T	p.His3124Tyr	SNV	pathogenic		Yes	Furu et al. (2003)
				c.4870C>T	p.Arg1624Trp	SNV	pathogenic	rs2003 91019		Onuchic et al. (2002)
P3	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944	Yes	Ward et al. (2002)
P5	ARPKD	*PKHD1*	NM_138694.3	c.4870C>T	p.Arg1624Trp	SNV	pathogenic	rs2003 91019	No	Onuchic et al. (2002)
				c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944		Ward et al. (2002)
P6	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944	No	Ward et al. (2002)
P7	MKS	*WDR19*	NM_025132.3	c.2608G>A	p.Asp870Asn	SNV	uncertain significane	rs2019 63605	Yes	No citation
P8	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944	Yes	Ward et al. (2002)
				c.406A>G	p.Thr136Ala	SNV	pathogenic			**Novel**
P9	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944	No	Ward et al. (2002)
P11	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944	No	Ward et al. (2002)
P14	NPHP	*NPHP3*	NM_153240.4	c.3448C>T	p.Gln1150X	SNV	pathogenic		Yes	**Novel**
P16	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs1378 52944	No	Ward et al. (2002)
P18	ADPKD	*PKD1*	NM_001009944.2	c.12604_12631delGGCCGGCTGGGGACAAGGTGTGAGCCTG	p.Gly4202fs*146	Indel	pathogenic		Yes	Rossetti et al. (2012)
P20	ADPKD	*PKD1*	NM_001009944.2	c.5014_5015delAG	p.Arg1672fs*97	Indel	pathogenic		Yes	Rossetti et al. (2012)

P21	UCKD	*NPHP3*	NM_153240.4	c.2529delA	p.Tyr844Thrfs*5	Indel	pathogenic		Yes	**Novel**
P22	ARPKD	*PKHD1*	NM_138694.3	c.406A>G	p.Thr136Ala	SNV	pathogenic		Yes	**Novel**
P23	NPHP	*NPHP4*	NM_015102.3	c.3784A>T	p.Arg1262X	SNV	pathogenic		No	**Novel**
P24	NPHP	*SDCCAG8*	NM_006642.3	c.1420delG	p.Glu474fs	Indel	pathogenic	rs397515335	Yes	Otto et al. (2010)
P25	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
P26	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
P27	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
				c.406A>G	p.Thr136Ala	SNV	pathogenic			**Novel**
P28	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
				c.4870C>T	p.Arg1624Trp	SNV	pathogenic	rs200391019		Onuchic et al. (2002)
P29	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
				c.4870C>T	p.Arg1624Trp	SNV	pathogenic	rs200391019		Onuchic et al. (2002)
P32	ADPKD	*PKD1*	NM_001009944.2	c.6264dupG	p.Arg2089Alafs*19	Indel	pathogenic		Yes	**Novel**
P33	ADPKD	*PKD1*	NM_001009944.	c.12301delC	p.Leu4101Trpfs*97	Indel	pathogenic		Yes	**Novel**
P34	ADPKD	*PKD1*	NM_001009944.2	c.12604_12631delGGCCGGCTGGGGACAAGGTGTGAGCCTG	p.Gly4202fs*146	Indel	pathogenic		Yes	Rossetti et al. (2012)
P37	ADPKD	*PKD1*	NM_001009944.2	c.9340C>T	p.Gln3114X	SNV	pathogenic		Yes	**Novel**
P38	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	No	Ward et al. (2002)
P39	NPHP	*HNF1B*	NM_000458.2	c.443C>T	p.Ser148Leu	SNV	likely pathogenic	rs121918674	No	Edghill et al. (2006)
P40	NPHP	*NPHP4*	NM_015102.3	c.673G>T	p.Gly225Cys	SNV	likely pathogenic	rs540402276	No	No citation
P42	ADPKD	*PKD1*	NM_001009944.2	c.7421dupG	p.Ser2475Leufs*26	Indel	pathogenic		No	**Novel**

P43	ADPKD	*PKD1*	NM_001009944.2	c.2711_2712delAG	p.Glu904Glyfs*196	Indel	pathogenic		Yes	**Novel**
P44	ARPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
				c.406A>G	p.Thr136Ala	SNV	pathogenic			**Novel**
P45	ADPKD	*PKD1*	NM_138694.3	c.6264dupG	p.Arg2089Alafs*19	Indel	pathogenic		Yes	**Novel**
P46	NPHP	*NPHP1*	NM_001009944.2	NPHP1 deletion		WGD	pathogenic		No	Hildebrandt et al. (1997)
P48	UCKD	*NPHP1*	NM_025132.3	*NPHP1* deletion		WGD	pathogenic		No	Hildebrandt et al. (1997)
P49	NPHP	*WDR19*	NM_000458.2	c.3533G>A	p.Arg1178Gln	SNV	likely pathogenic	rs79436363	No	Halbritter et al. (2013)
P50	NPHP	*NPHP1*	NM_138694.3	*NPHP1* deletion		WGD	pathogenic		No	Hildebrandt et al. (1997)
P51	ADPKD	*HNF1B*	NM_138694.3	c.494G>A	p.Arg165His	SNV	pathogenic	rs121918675	Yes	Bellanne-Chantelot et al. (2004)
P52	UCKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	Yes	Ward et al. (2002)
P53	ADPKD	*PKHD1*	NM_138694.3	c.107C>T	p.Thr36Met	SNV	pathogenic	rs137852944	No	Ward et al. (2002)
			NM_138694.3	c.406A>G	p.Thr136Ala	SNV	pathogenic			**Novel**

Table 4.4 Molecular genetic findings of target kidney gene panel. aa. Change, amino acid change; ACMG Classifi., American College of Medical Genetics and Genomics classification; ADPKD, autosomal dominant kidney disease; ARPKD, autosomal recessive kidney disease; BBS, Bardet Biedl syndrome; c. change, nucleotide change; dbSNP, single-nucleotide polymorphism database; F/seg, family segregation; Indel, insertion/deletion; JBTS, Joubert syndrome; MKS, Meckel Gruber syndrome; MOI, mode of inheritance; NA, not applicable; NPHP, nephronophthisis; SNV, single-nucleotide variant; U/A, unavailable; UCKD, unspecific cystic kidney disease; WGD, whole-gene deletion.

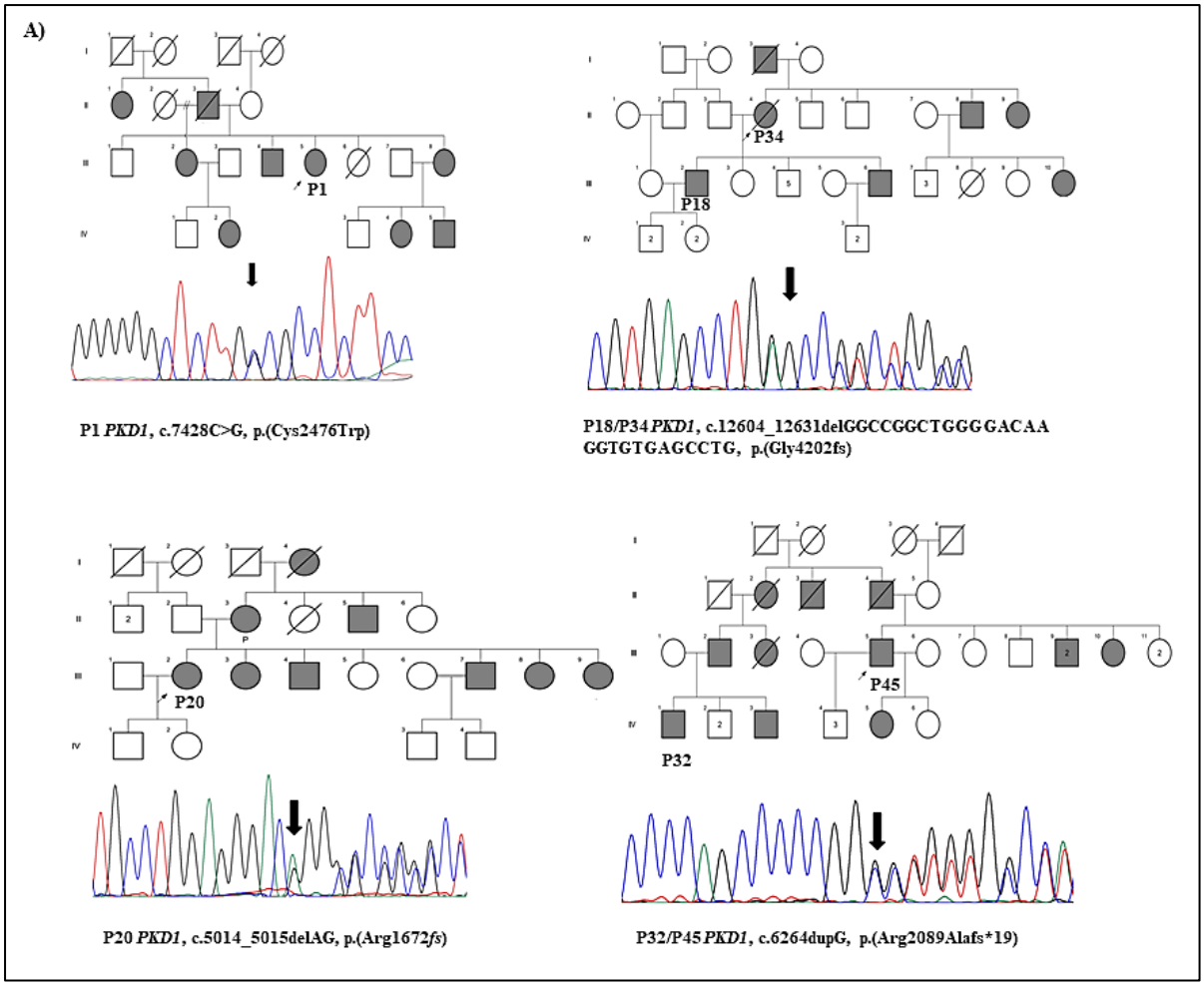

Figure 4.4 Pathogenic *PKD1* variants detected in Omani autosomal dominant polycystic kidney disease (ADPKD) patients. A) Pedigree diagrams showing family structure of patients (P) P1, P18/P34, P20 and P32/P45. Chromatograms showing *PKD1* pathogenic variants that were detected and Sanger sequence confirmed in those patients. Squares specify males; circles specify females. Arrow points to proband; filled squares and circles specify affected individuals in the family.

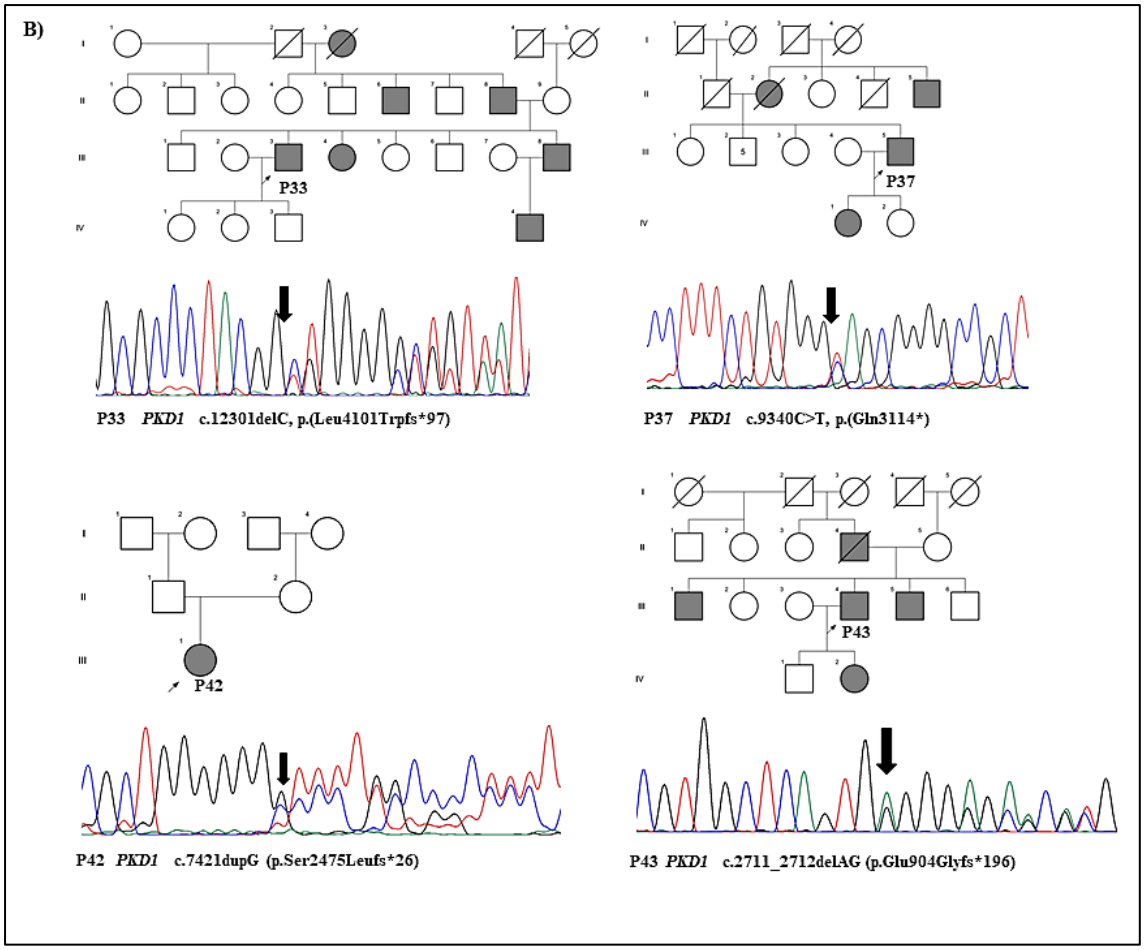

Figure 4.4 (Cont.). B) Pedigree diagrams showing family structure of patients (P) P33, P37, P42 and P43. The chromatograms present *PKD1* pathogenic variants that were detected and validated. Squares specify males; circles specify females. Arrow points to proband; filled squares and circles specify affected individuals in the family.

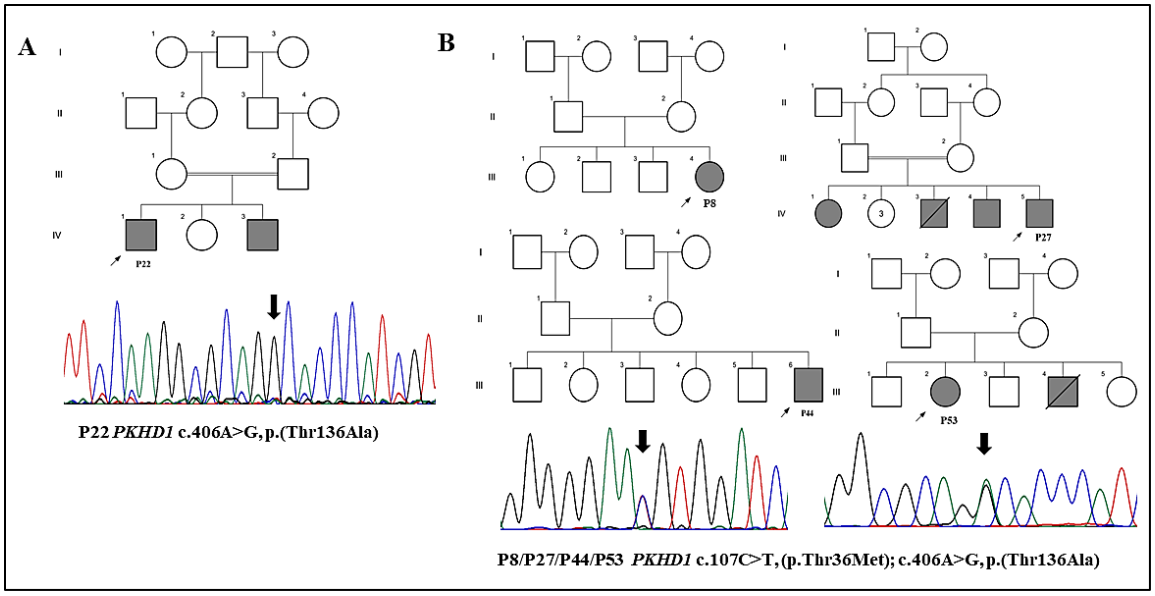

Figure 4.5 Pathogenic *PKHD1* variants detected in Omani autosomal recessive polycystic kidney disease patients. (A) Family pedigree of patients (P) P22 and Sanger sequencing chromatogram showing novel *PKHD1* pathogenic homozygous missense variant c.406A>G p.(T136A). (B) Family pedigree of patients P8, P27, P44 and P53 and Sanger sequencing in which variant c.406A>G p.(T136A) was detected in compound heterozygous with c.107C>T, p.(T36M). Squares indicate males; circles indicate females. Arrows point to the proband; filled squares and circles indicate affected individuals in the family.

A total of 9 patients were found to carry causative variants associated with NPHP-RC in *NPHP1*, *NPHP3*, *NPHP4*, *SDCCAG8,* and *WDR19* (Table 4.4). Copy number variations were detected in 3 of the patients (P46, P48, and P50), in which a homozygous deletion of ~862 kb in size that contains 16 genes, including 3 OMIM genes: *NPHP1* (OMIM: 607100), *RGPD6* (OMIM: 612709), and *MALL* (OMIM: 602022), was validated by comparative genomic hybridization array (Figure 4.6).

Two novel pathogenic variants were detected in the *NPHP3* gene. A homozygous nonsense variant p.(Gln1150*) was identified in a 1-year-old female (P14) who presented with hypertension, cystic kidney disease, liver fibrosis, splenomegaly, and ESKD (Table 4.4; Figure 4.7). In a second consanguineous family (P21), we identified a homozygous 1-bp deletion in c.2529delA in exon 18 of the *NPHP3* gene in a fetal sample, resulting in frameshift and premature termination of p.(Tyr844Thrfs*5) (Figure 4.7). This family has a history of 2 fetal deaths with features of oligohydramnios, and antenatal scan showed

bilateral enlarged kidneys with loss of corticomedullary differentiation and lung hypoplasia (Table 4.3; Figure 4.7).

A homozygous missense p.(Gly225Cys) and nonsense p.(Arg1262*) variant were detected in *NPHP4* in probands P40 and P23, respectively, both with a clinical diagnosis of NPHP (Table 4.3; Figure 4.8). The p.(Gly225Cys) variant (rs540402276) is reported in the ExAC database only once in an African population in its heterozygous state with a very low minor allele frequency, whereas p.(Arg1262*) is a novel nonsense variant.

A homozygous frameshift deletion p.(Glu474fs*20) in the *SDCCAG8* gene was identified in P24 with a clinical diagnosis of NPHP, reaching ESKD at the age of 8 years and extra renal features of retinitis pigmentosa (Table 4.3; Figure 4.8). A 2-month-old patient (P49) with renal impairment, dilated bile ducts, and bilateral echogenic kidneys was found to carry a known homozygous missense variant p.(Arg1178Gln) located in exon 31 of the *WDR19* gene (rs79436363; Table 4.4; Figure 4.8) that had previously been reported in a patient with Senior-Loken syndrome-8 (Halbritter et al., 2013).

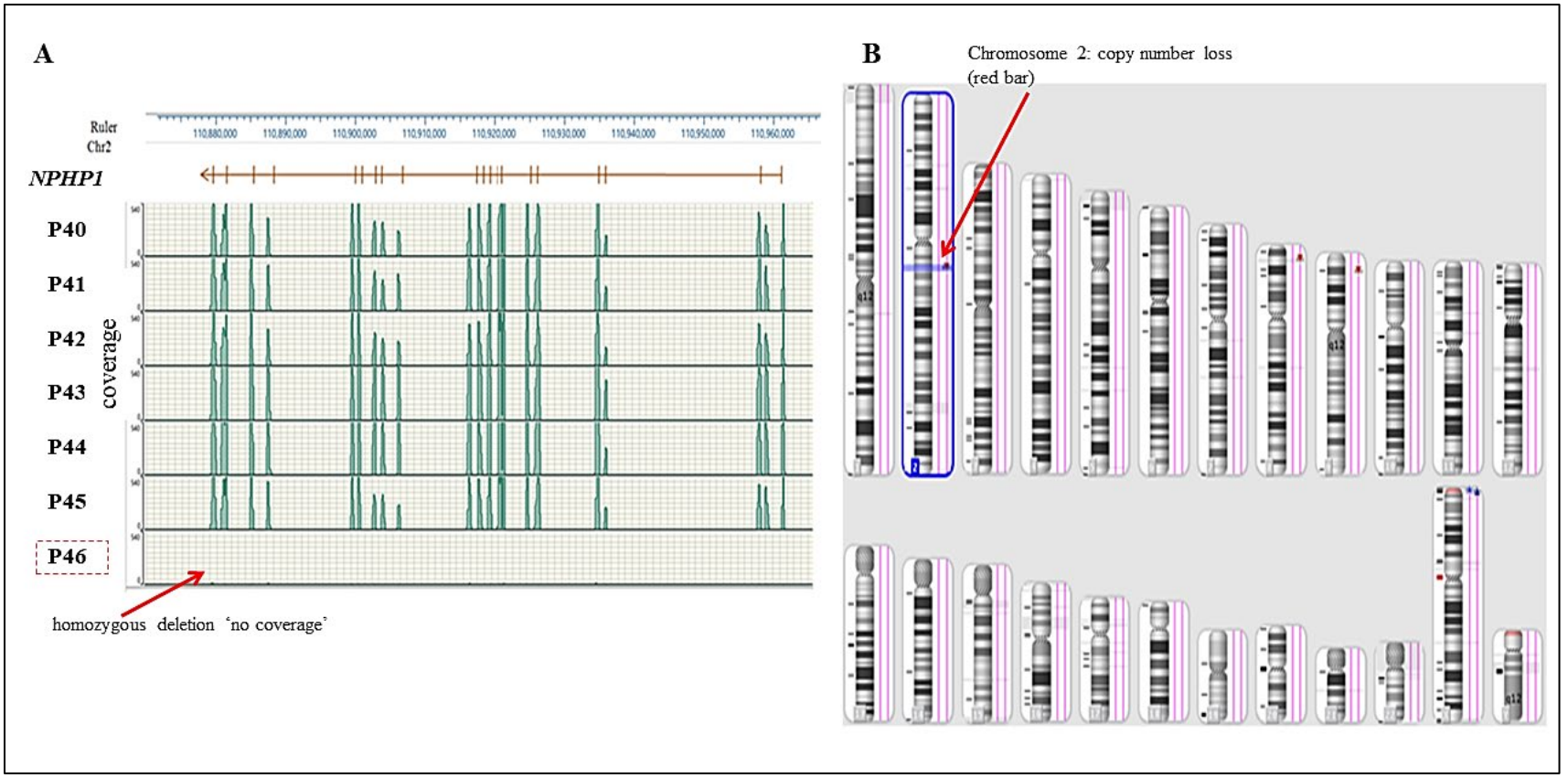

Figure 4.6 Detection of *NPHP1* deletion using target NGS panel and confirmation by CGH-array. A. Representation of the depth of coverage of *NPHP1* gene in 7 samples of the same run. In P46, there is no coverage of any of the *NPHP1* exons (demonstrated by the lack of green peaks) indicating a homozygous deletion. Green peaks indicate the depth of coverage of the sequenced exons. B. Representative results of the array-CGH data of patient P46. The red bar in the left side of chromosome 2 (outlined in blue) indicates copy number loss where a homozygous deletion of 862 kb in size was detected that contains 16 genes, including *NPHP1* (OMIM: 607100), *RGPD6* (OMIM: 612709) and *MALL* (OMIM: 602022).

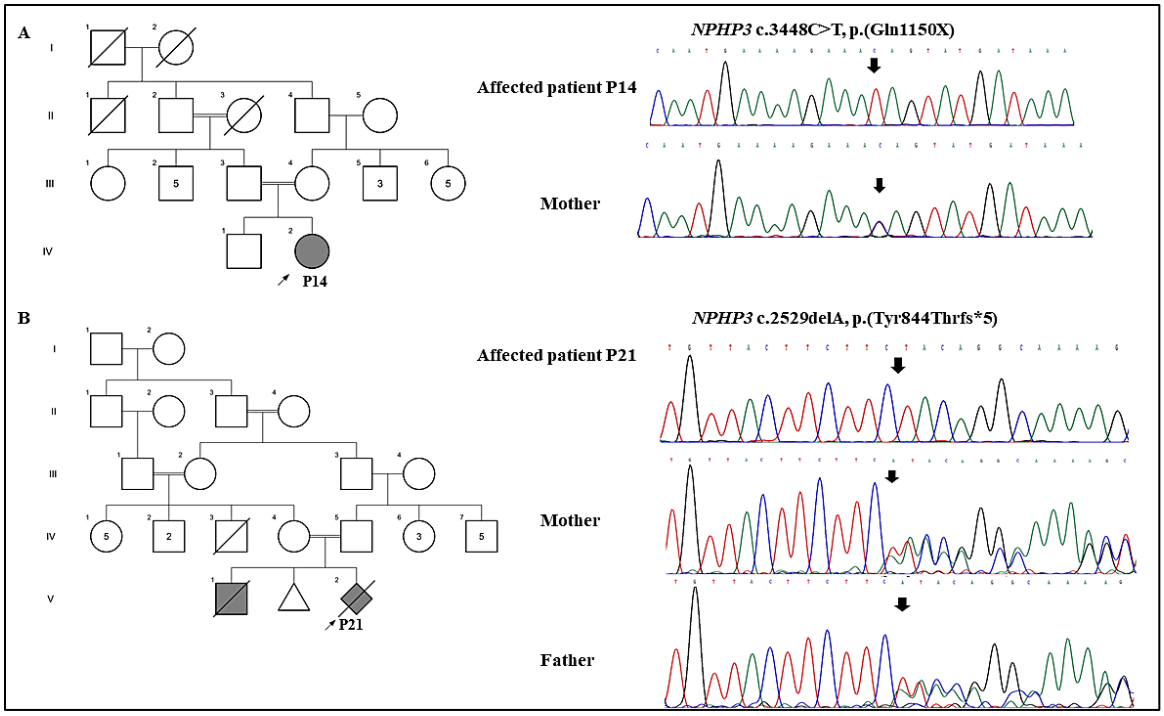

Figure 4.7 Next-generation sequencing identifies two novel truncating variants in the Nephrocystin 3 (*NPHP3*). Pedigrees and Sanger sequence chromatograms showing familial segregation of the detected *NPHP3* pathogenic variants. (A) Segregation analysis of a homozygous nonsense variant Gln1150X identified exon 24 of the NPHP3 in patient P14. (B) Pedigree and chromatograms showing the inheritance pattern of a lethal homozygous 1-bp deletion (2529delA) in exon 18 of the *NPHP3* gene in fetus sample (P21) leading to frameshift and premature termination (Tyr844Thr*fs**5). Squares indicate males; circles indicate females. Arrows point to the proband; filled squares and circles indicate affected individuals in the family.

The NGS panel was able to provide a new molecular diagnosis for some patients with a *de novo* mode of inheritance. For example, in a 10-year-old male (P51) with CKD secondary to bilateral echogenic kidneys mimicking ADPKD but without a family history of disease, a heterozygous pathogenic SNV p.(Arg165His) was identified in *HNF1B* (Table 4.4; Figure 4.8). Segregation analysis of parents and unaffected siblings revealed this to be a likely *de novo* mutation. Another assumed *de novo* *HNF1B* missense variant p.(Ser148Leu) was identified in a 13-year-old female (P39) who had antenatal polycystic kidneys and no family history of disease (Table 4.4; Figure 4.8). A molecular genetic diagnosis was found in one patient (P7) with a multisystem ciliopathy suggestive of Meckel Gruber syndrome, including dysmorphic features, encephalocele, and polydactyl. In this patient, a homozygous missense

variant p.(Asp870Asn) (rs201963605) in the *WDR19* gene was detected that co-segregated with the other affected sibling with a similar phenotype (Figure 4.8).

The gene panel failed to identify a molecular genetic diagnosis in 13 patients, including 4 patients with an ADPKD-like phenotype (P4, P35, P36, and P47), 5 patients with an NPHP-like phenotype (P10, P15, P17, P30, and P31), and 4 with a more complex multisystem ciliopathy phenotype including Meckel Gruber syndrome (P12 and P41), Joubert syndrome (P13), and Bardet-Biedl syndrome (P19). Bearing in mind that P12, P13, P15, P30 and P31 were further genetically evaluated using WES later, as described in chapter 6.

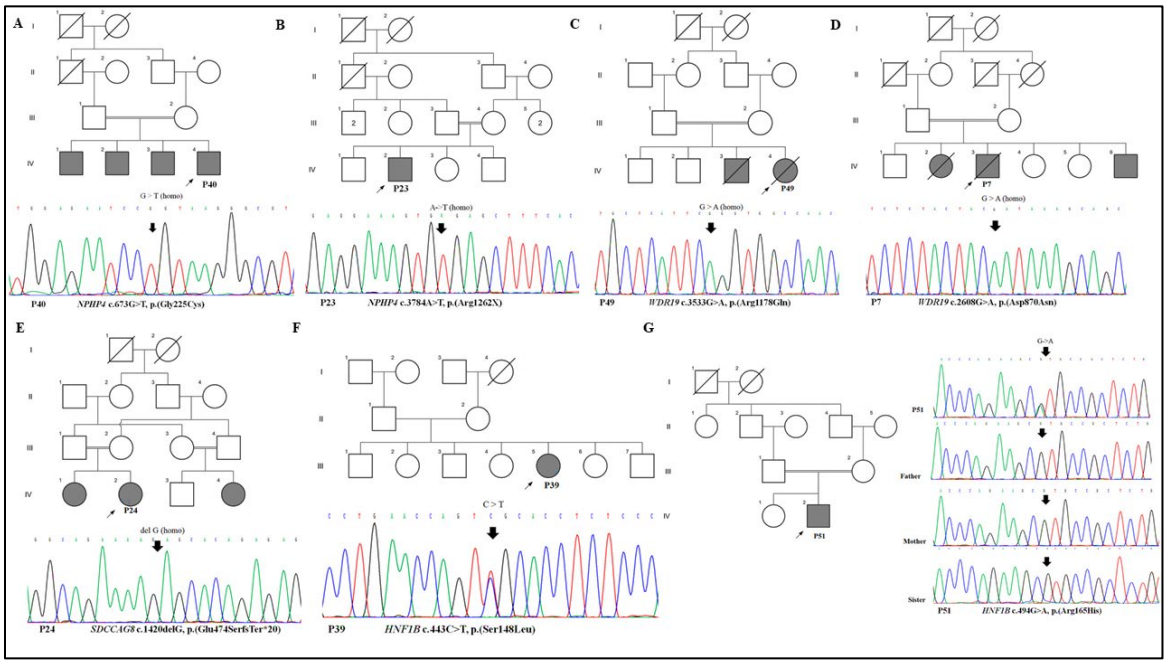

Figure 4.8. Seven different unrelated pedigrees involved in this study and disease-causing variants identified by Next-generation sequencing. (A) and (B) A homozygous missense and nonsense variants (Gly225Cys and Arg1262X) identified and confirmed by Sanger traces in *NPHP4* in patients P40 and P23, respectively. (C) A deceased two month old patient (P49) confirmed to carry a known homozygous missense variant Arg1178Gln located in exon 31 of the *WDR19* gene associated with Senior-Loken syndrome-8. (D) (E) In NPHP patient (P24) with ESKD and retinal degeneration, a homozygous frameshift deletion (Glu474fs*20) in *SDCCAG8* gene was detected and familial segregation was confirmed in other affected sibling (IV1) and cousin (IV4). (F) and (G) Pedigrees showing *de novo* occurrence of the known *HNF1B* Ser148Leu *and* Arg165His in P39 and P51, respectively. In P51, *de novo* inheritance was confirmed by Sanger sequencing of all family members. Squares indicate males; circles indicate females. Arrows point to the proband; filled squares and circles indicate affected individuals in the family.

4.5 Discussion

Employing NGS diagnostic panels for the high-throughput detection of disease-causative variants through interrogation of multiple genes simultaneously has become a common approach in the genetic testing of inherited kidney disease. The present study represents the first comprehensive genetic analysis of inherited cystic kidney diseases and renal ciliopathies in patients from Oman using a customized NGS panel. Our results demonstrate the efficiency of the design and application of this targeted panel for genetic diagnosis of patients with different phenotypes.

Generally speaking, the diagnostic yield of NGS panels greatly depends on the patient population selected and the variant calling threshold. We have shown that an NGS panel consisting of 49 cystic kidney disease–associated genes applied to 53 proband patients was capable of resolving 75%, consistent with the reported diagnostic yield of other targeted NGS studies (Bullich et al., 2018). Our high rate of detection may be explained by clear phenotypic characterization of patients and detailed family history. As a result, the clinical diagnosis was confirmed by molecular testing in 33 (62%) patients. The molecular diagnostic results enabled a change in clinical diagnosis in 3 patients (resolving the overlapping clinical phenotypes of NPHP/ARPKD and ADPKD/renal cysts and diabetes syndrome) and a precise diagnosis in 3 patients who had an unclear or atypical cystic kidney disease phenotype (Table 4.4).

Defining the genetic etiology of disease is fundamental in terms of medical intervention, disease management, and future family planning. Overall, a genetic etiology was obtained in 73% of pediatric patients, in which ARPKD (37%) and NPHP-RC (27%) were the most prevalent disease. In contrast, genetic diagnosis was achieved in 77% of adults, most of whom had either ADPKD (41%) or ARPKD (32%). Similar to other studies, no significant difference was evident among pediatric and adult patients, in terms of genetic diagnostic rate (Mallett et al., 2017).

Among the 49 panel genes, only 8 contributed to our diagnostic yield. These were *PKHD1* (45%), *PKD1* (25%), *NPHP1* (8%), *NPHP3* (5%), *NPHP4* (5%), *WDR19* (5%), *HNF1B* (5%), and *SDCCAG8* (2%). In total, 22 different disease-causative variants were

identified in 40 patients, which include 10 missense, 3 nonsense, 5 small deletion, 3 small insertions, one large INDEL, and a whole-gene deletion affecting *NPHP1* (Figure 4.6). Of those, 10 (46%) variants were novel findings in the genes *PKD1* (n = 6), *PKHD1* (n = 1), *NPHP3* (n = 2), *and NPHP4* (n = 1). Homozygous variants/deletions were detected in more than half our patients (23 of 40), in keeping with the known consanguinity. The custom of consanguineous marriages is strongly adhered to in the Omani community, with the rate estimated to be 56% (Tadmouri et al., 2009), owing to social, cultural, geographic, and economic factors(Al Alawi et al., 2017a).

In terms of molecular testing, the *PKD1* gene is considered to be complex due to its large size (46 exons), the presence of 6 *PKD1* pseudogenes, and its high allelic heterogeneity. Nonetheless, recent studies using capture-based methodology had successively covered all exonic regions of this gene (Trujillano et al., 2014). Our results confirm that we were capable of sequencing, using long-range polymerase chain reaction, all exons of the *PKD1* gene and detecting different types of genomic variants, thus providing accurate genotyping data of ADPKD patients. Eight pathogenic variants of *PKD1* were identified in 10 suspected ADPKD patients (from 8 different families). We failed to solve 4 cases in which the clinical diagnosis of ADPKD was suspected. The coverage of *PKD1* exons 1, 12, and 42 (Table 4.2) was lower than the remaining exons but acceptable. These cases now need to be examined for copy number and structural variants in *PKD1* as well as alternative genetic causes, including *DNAJB11* (Cornec-Le Gall et al., 2016) and *GANAB* (Porath et al., 2016), which were not included on our panel.

ARPKD is a severe, early-onset type of cystic kidney disease caused by biallelic mutations in the *PKHD1* gene, which consists of 67 exons that encode a 4074 amino acid type I transmembrane protein called fibrocystin/polyductin(Braun and Hildebrandt, 2017). Almost 748 *PKHD1* variants have been reported to date in the ARPKD mutation database (www.humgen.rwth-aachen.de) scattered along the entire length of the gene with no mutational hot spots (Bergmann et al., 2004a). Missense variation is a common mechanism of disease in the *PKHD1* gene, where half of the reported variants are missense. Most of the *PKHD1* mutations are private, and most *PKHD1* patients may have compound heterozygous mutations. ARPKD is one of the most common genetic disorders in Oman,

with an estimated birth incidence of 1 in 12,000 births (Rajab et al., 2013). In this study, only 4 different pathogenic missense variants were identified in *PKHD1*. The four variants, p.(Thr36Met), p.(Thr136Ala), p.(Arg1624Trp), and p.(His3124Tyr), involve substitutions of highly conserved amino acids and were all reported previously, except p.(Thr136Ala). The high frequency of these missense mutations suggests founder mutations in *PKHD1* in Omani patients.

NPHP disease-causative variants were detected in 10 of 15 patients suspected clinically to have NPHP. Among Omani NPHP patients, *NPHP1* gene deletion was identified in 3 unrelated patients from the same geographic region. *NPHP3* mutations were responsible for infantile and juvenile NPHP in consanguineous families, where a novel deletion c.2529delA was associated with neonatal death and novel nonsense associated with cystic kidneys, hepatic fibrosis, and splenomegaly leading to ESKD before 1 year of age.

The lack of identification of causative variants in 13 (25%) patients, including 5 with suspected NPHP and 4 with multisystem ciliopathies, supports the genetic heterogeneity of renal ciliopathies, for which the spectrum of associated genes is continually expanding and the gene panel failed to include the latest known causes. For such patients, NGS panels containing additional known NPHP and renal ciliopathy genes, or whole-exome sequencing/whole-genome sequencing approaches, need to be applied. In addition, deletion-duplication analysis using multiplex ligation-dependent probe amplification in candidate genes and chromosomal microarray analysis are other future approaches for our unsolved cases. There is a growing argument to move to whole-exome sequencing and whole-genome sequencing approaches for molecular genetic diagnostics, given the constant need to update gene panels, the ability of whole-genome sequencing to provide information on structural variant and gene copy number, and the falling costs of these unselected methods. However, data analysis and interpretation continue to be challenges for such approaches and have slowed their implementation into molecular genetic diagnostic services.

4.6 Conclusion

In conclusion, we have demonstrated that for inherited cystic kidney disease, a targeted NGS panel is a comprehensive, noninvasive, and efficient tool for genetic diagnosis of patients. With this approach, a diagnostic yield of 75% was obtained in Omani patients with inherited cystic kidney disease. In addition, NGS panel sequencing allows large disease genes, such as *PKD1*, to be sequenced. This study represents a comprehensive molecular genetic overview of Omani patients with inherited cystic kidney disease and their associated clinical phenotypes and contributes to the knowledge of causative mutations in renal ciliopathy genes.

Chapter 5. Clinical and Genetic Characteristics of Autosomal Recessive Polycystic Kidney Disease in Oman

5.1 Introduction

Autosomal recessive polycystic kidney disease (ARPKD) is one of the most frequent cystic kidney diseases in infants and children primarily causing kidney and liver associated mortality and morbidity. Classically, ARPKD manifests prenatally as enlarged echogenic kidneys with Potter's syndrome or postnatally during childhood or adolescence. It is characterized by bilateral echogenic cystic kidneys caused by dilatation of the renal tubular collecting ducts and congenital hepatic fibrosis secondary to malformation of the liver biliary ducts. ARPKD is also associated with systemic and portal hypertension. Current therapies focus on treating ARPKD symptoms (Guay-Woodford et al., 2014).

ARPKD is caused by mutations in the *PKHD1* gene, which is located on chromosome 6 (p12.3-p12.2). Consistent with disease phenotype, high expression of *PKHD1* is found in foetal and adult kidney, with low levels detected in the liver, pancreas and arterial walls (Bergmann, 2017). The longest open reading frame transcript (NM_138694.4) consists of 67 exons encoding the integral membrane protein fibrocystin / polyductin (*FC/*PD). It was shown that the severity of ARPKD is determined by the type of mutations rather than the location on the *PKHD1* gene (Denamur et al., 2010). There are over 700 different mutations associated with an ARPKD phenotype (Ebner et al., 2017). The size of *PKHD1* and its heterogeneous mutational spectrum has previously been an obstacle to molecular diagnosis. However, recent advances in massive parallel sequencing / next generation sequencing (NGS) facilitate large scale screening for pathogenic mutations in *PKHD1*.

The importance of early diagnosis and management of ARPKD through genetic testing is widely recognized. Precise diagnosis can improve the clinical management of patients, avoiding the exposure to unnecessary and invasive assays and enhance early detection of kidney and extra-kidney complications.

Inherited kidney disease is a leading cause of end stage kidney disease (ESKD) in children in Oman (Al Alawi et al., 2017a). In a hospital-based study, the observed birth incidence of

ARPKD was evaluated to be 1 in 12,000 births (Rajab et al., 2005). However, there are no population-based studies evaluating the incidence of ARPKD in the Omani population as a whole. A recent study showed that hereditary kidney disease accounts for 32% of etiologies causing chronic kidney disease (CKD) in children and ARPKD is the leading cause, accounting for of 12% of CKD (Al Riyami et al., 2019). The objective of this study was to demonstrate the utility of molecular genetic testing in patients suspected with ARPKD in an Omani population. In addition, we describe the clinical and genetic profile of this cohort of ARPKD patients.

5.2 Patients Selection

A group of 75 samples, from 33 unrelated families were referred to the National Genetic Centre in Oman between January 2015 and December 2018 for the genetic study of inherited kidney disease. The kidney pathology results as well as clinical information, including age at clinical diagnosis, neonatal ventilation, CKD, liver disease, splenomegaly, hypertension, urinary concentration defect, urinary tract infection (UTI), pulmonary hypoplasia, oesophageal varices and renal replacement modalities were available for analysis. Additional demographic information including familial history of ARPKD, parental consanguinity and geographical distribution were also obtained. Family pedigrees described in this study are illustrated in Figure 5.1 and 5.2. Initially, for 18 unrelated patients, molecular study was performed through next generation sequencing (NGS) using targeted gene panel as previously described in chapter 4 (Figure 5.1). Based on the NGS results, Sanger sequencing of the *PKHD1* exons 3, 6, 32 and 58 was performed for another 15 different families with suspected ARPKD (Figure 5.2).

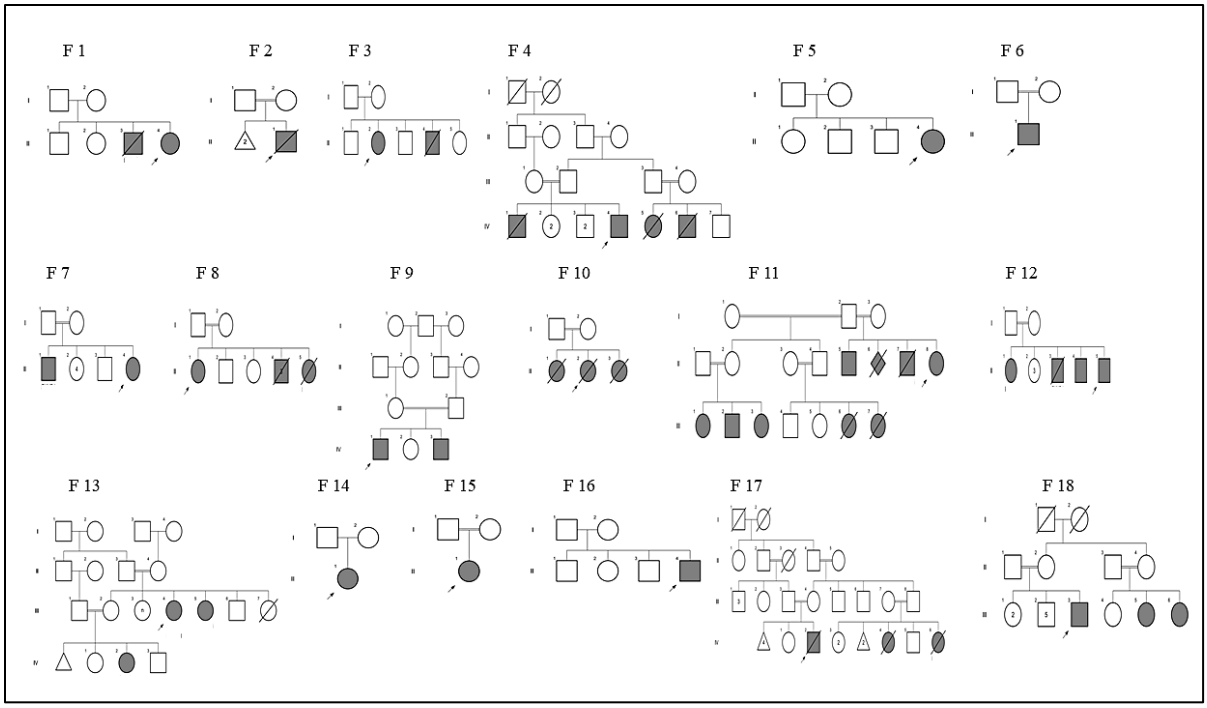

Figure 5.1 Pedigrees of the ARPKD families analysed by targeted NGS approach. Squares represent males, circles represent females. Filled symbols indicate the affected status. Double-horizontal bars illustrate parental consanguinity. F: Family. All families (F1-F18) were genetically solved by this approach.

5.3 Clinical Characteristics of Patients

In total, 75 samples (40 (53.3%) males and 35 (46.7%) females) from apparently 33 unrelated families were enrolled. The cohort includes clinically suspected ARPKD patients (*n*=41; n=20 males and n=21 females), their parents (*n*=24) and siblings or relatives (*n*=10) (Table 1).

Molecular genetic diagnosis of biallelic *PKHD1* mutations was confirmed in 30 families, giving a detection rate of 91%, through targeted gene panel (*n*=18 families) and Sanger sequence (*n*=12 families). Twenty-four families (72.7%) reported a family history of kidney disease with an autosomal recessive inheritance pattern, while none of the molecularly unsolved families reported a family history of kidney or liver disease. Parental consanguinity was known in 22 families (66.7%).

The initial clinical diagnosis was made prenatally in eight patients (19.5 %), during infancy (0-1 year) in 21 patients (51.2%), during early childhood (2-8 years) in 9 patients (22.0%), and in later years (9-13 years) in 3 patients (7.3%) (Table 5.1). The main clinical features included hypertension (73.2%), congenital hepatic fibrosis (CHF) (73.2%), CKD (61%) with median age of onset 3 years, splenomegaly (46.3%), pulmonary hypoplasia (17%) and perinatal death (14.6%) (Table 5.1). Twelve patients (26.8%) developed ESKD at mean age of 13 years.

	N	**%**
ARPKD patients	41	100
Males	20	49
Females	21	51
Age at diagnosis:		
Prenatal	8	19.5
Birth-1st Month	6	14.6
2-12 Months	15	36.6
1-8 Years	9	22.0
> 8 Years	3	7.3
Clinical Features:		
Hypertension	30	73.2
Congenital Hepatic fibrosis (CHF)	30	73.2
Splenomegaly	19	46.3
Pulmonary hypoplasia	7	17.1
Perinatal deaths (< 28 days)	4	9.8
Postneonatal deaths (28 days - 1 Year)	2	4.9
Chronic Kidney Disease (CKD)	25	61.0
End Stage Kidney Disease (ESKD)	12	30.0

Table 5.1 Clinical characteristics of suspected ARPKD patients from Oman. ARPKD: autosomal recessive polycystic kidney disease; N: number; %: percentage.

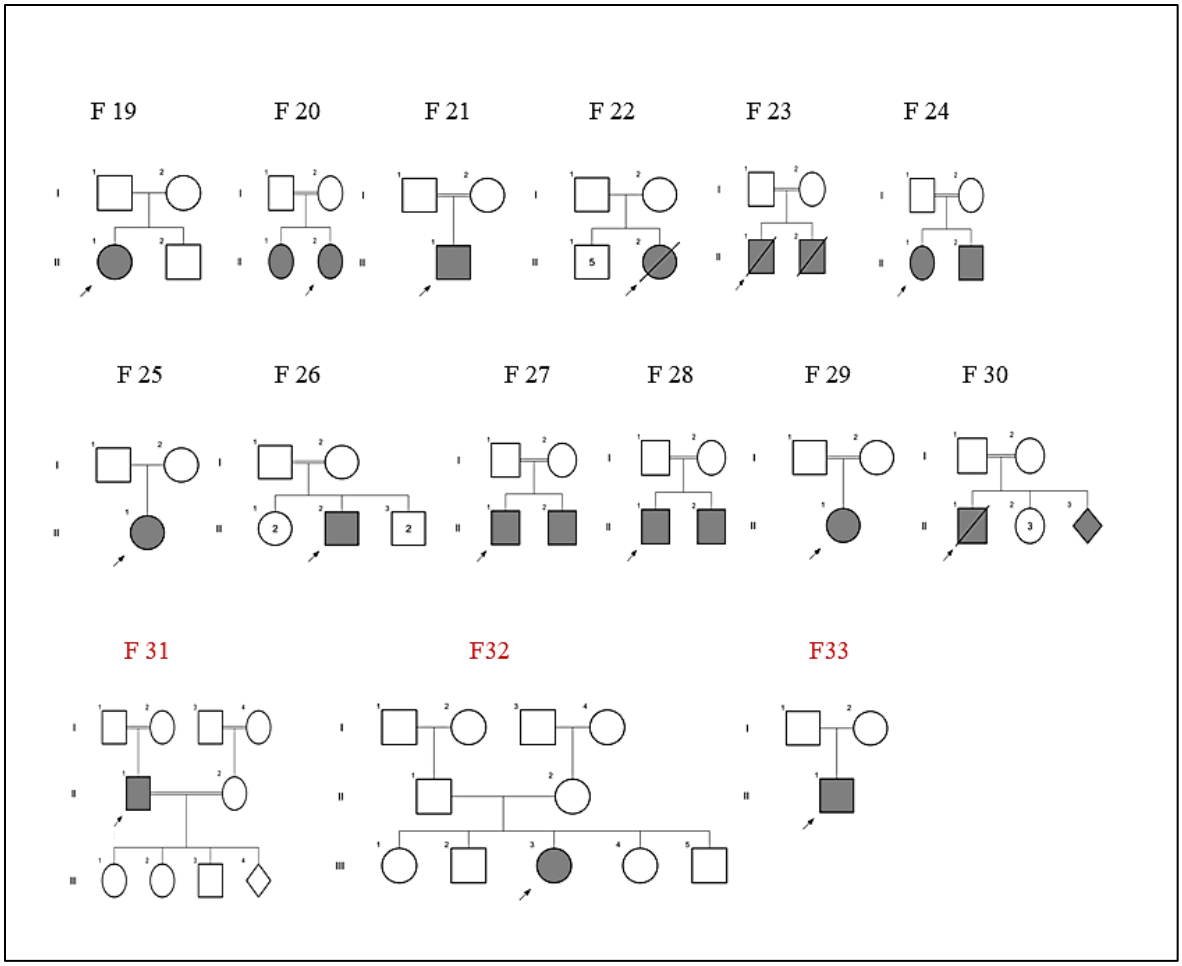

Figure 5.2 Pedigrees of the ARPKD families analysed by Sanger sequencing screening of target *PKHD1* alleles. Squares represent males, circles represent females. Filled symbols indicate the affected status. Double-horizontal bars illustrate parental consanguinity. F: Family. Families F19-F30 were genetically solved, while F31-F33 (red) were unsolved by this technique.

5.4 Genetics of Omani ARPKD Patients

In total only 4 *PKHD1* missense pathogenic mutations were identified: c.107C>T, p.(T36M); c.406A>G, p.(T136A); c.4870C>T, p.(R1624W) and c.9370C>T, p.(H3124Y) located in exons 3, 6, 32 and 58, respectively. Twenty-one families (63.6%) carried homozygous alleles, while only 9 (27.3%) carried compound heterozygous alleles. The most commonly detected mutation was p.(T36M), where 16 families (48.5%) carried it in homozygous state, while 8 families (24.2%) were compound heterozygous for this allele and the p.(T136A) allele (5 families) or the p.(R1624W) allele (3 families). Four families carried the homozygous mutation p.(R1624W), whilst one family carried the homozygous mutation p.(T136A). One family had compound heterozygous mutations p.(R1624W) and p.(H3124Y). The clinical presentations and genotypes are summarized in Table 5.2. Sanger sequencing chromatograms of the four different mutations in the *PKHD1* gene are shown in Figure 5.3A.

The p.(T136A) missense mutation is located in a highly conserved nucleotide (phyloP, 3.199; PhastCons, 1) and predicted to be pathogenic (SIFT, Polyphen2 and MutationTaster). To date, it has not been reported in the Exome Aggregation Consortium (ExAc), the Genome Aggregation Database (gnomAD) or the 1000 Genome Browser (Table 5.3).

Family No.	Sex	Age	Peri-/ neonatal death	PP	PH	HTN	CKD	ESKD with RTX	ESKD with CAPD / HD	Congenital hepatic fibrosis	Splenomegaly	EV	UTI	CHF	Genotype (aa change)
1	F	4 y				●	●			●	●	●			R1624W; H3124Y
2	M	p	●	●		●	●							●	T36M; T36M
3	F	3 y				●				●	●	●			T36M; R1624W
4	M	1 y				●	●			●	●				T36M; T36M
5	F	5 m				●				●					T36M; T136A
6	M	8 y				●	●		●	●	●		●		T36M; T36M
7	F					●	●		●	●	●				T36M; T36M
8	F	2 m				●	●		●	●	●	●			T36M; T36M
9	M	1 m				●				●					T136A; T136A
	M	p				●				●					
10	F	p	●	●	●	●	●			●					T36M; T36M
11	F	2 y				●	●	●		●					T36M; T36M
	F	2 y					●	●		●					
12	M	7 m				●	●		●	●	●			●	T36M; T136A
	M	At birth				●				●	●			●	
13	F	12 m					●	●							T36M; R1624W
	F	5 m				●				●	●				
	F	5 m					●	●		●	●		●		
14	F	6 m			●					●					T36M; R1624W
15	F	2 y				●				●					T36M; T36M
16	M	p				●	●			●			●		T36M; T136A
17	M	p	●	●	●		●					●			T36M; T36M

Table 5.2 Summary of genotype-phenotype outcomes of ARPKD patients with *PKHD1* mutations. Age refers to the age at initial clinical diagnosis. F, females; M, males; PP, Potter's phenotype; PH, pulmonary hypoplasia; HTN, hypertension; CKD, chronic kidney disease; ESKD, end stage kidney disease; RTX, renal transplantation; CAPD, continuous ambulatory peritoneal dialysis; HD, hemodialysis; EV, esophageal varices; UTI, urinary tract infections, CHF, congestive heart failure; aa, amino acid; m: month, y: years; p, prenatal.

Family No.	Gender	Age	Peri-/ neonatal death	PP	PH	HTN	CKD	ESKD with RTX	ESKD with CAPD / HD	Congenital hepatic fibrosis	Splenomegaly	EV	UTI	CHF	Genotype (aa change)
18	M	13 y				●	●		●						T36M; T136A
19	F	1 m				●				●	●		●		T36M; T36M
20	F	10 m				●	●		●	●	●				T36M; T36M
	F	At birth			●	●	●		●	●	●		●		
21	M	At birth				●	●			●	●				T36M; T36M
22	F	At birth	●	●		●	●							●	T36M; T36M
23	F	p	●	●	●	●	●								T36M; T36M
24	F	6 m					●		●	●	●				T36M; T36M
25	F	p			●	●	●			●	●		●		T36M; T136A
26	M	At birth				●				●					R1624W; R1624W
27	M	5 m				●	●			●	●				R1624W; R1624W
	M	9 m				●									
28	M	11 y								●	●				R1624W; R1624W
	M	1 y				●	●			●					
29	F	4 y								●					R1624W; R1624W
30	M	p	●		●										T36M; T36M

Table 5.2 (cont.).

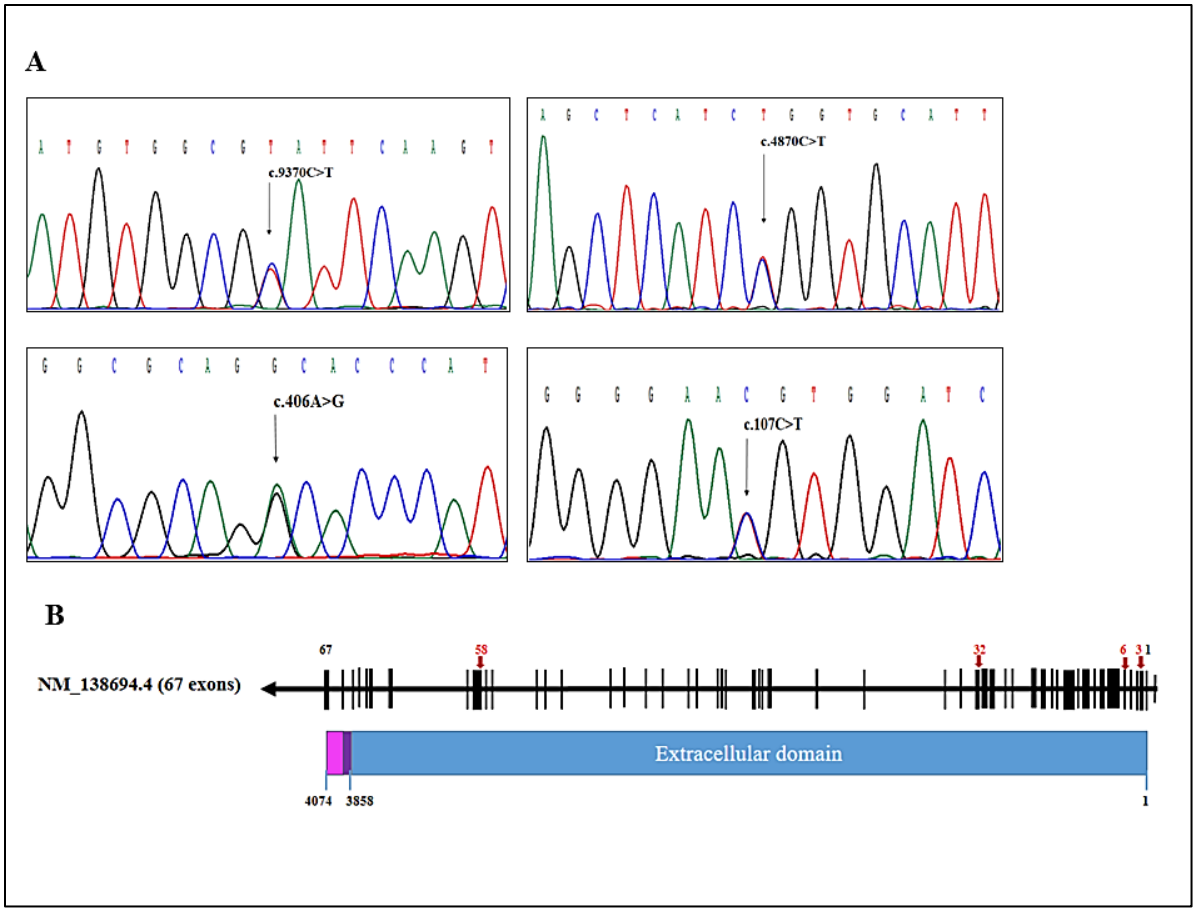

Figure 5.3 Representation of the missense variants of the *PKHD1* gene detected in ARPKD patients in relation to the gene exon structure and protein domains. A. Chromatograms of the identified mutations in the *PKHD1* gene. Heterozygous sequence variants are arrowed with the nucleotide changes indicated (reference sequence NM_138694.4) B. Simplified structure of the *PKHD1* transcript (longest open reading frame) (NM_138694.4) consisting of 67 exons that encode for a 4074 amino acid. Mutations are identified by red arrows.

Location		Exon 3	Exon 6	Exon 32	Exon 58
Nucleotide variation		c.107C>T	c.406A>G	c.4870C>T	c.9370C>T
Amino acid variation		p.T36M	p.T136A	p.R1624W	p.H3124Y
dbSNP ID		rs137852944	NA	rs200391019	rs1554218666
MAF	ExAC	0.0005193 (63/121312)	not found	0.0001812 (22/121394)	not found
	1000 Genomes Project	0.000199 (1/5008)	not found	not found	not found
	gnomAD (total)	0.0005094 (144/282706)	not found	0.0001379 (39/282816)	not found
	ESP (Exome Variant Server)	0.00031 (4/13006)	not found	0.00015 (2/13006)	not found
	ClinVar (global MAF)	0.0002	not found	NA	NA
	UK 10K	0.00054 (4/7428)	not found	0.00013 (1/7428)	not found
Genomics England, 100,000G project		16×	not found	6×	not found
PKHD1 mutation database		86×	not found	15×	4×
Origin		Germany, Caucasian-American, UK, Spain, Czech Republic, Finland, Netherlands, Australia		Saudi-Arabia, Caucasian-American, Israel, Netherlands, Czech Republic, Finland-Greece	Italy, Turkey
References		Ward et al (2002); Rossetti et al (2003); Bergmann et al (2004); Sharp et al (2005); Losekoot et al (2005); Gunay-Aygun et al (2009).		Onuchic et al (2002); Gunay-Aygun et al (2009).	Furu et al (2003); Bergmann et al (2004a); Bergmann et al (2005).

Table 5.3 Allele frequencies and worldwide distribution of the four *PKHD1* mutations detected in Omani ARPKD patients. dbSNP, single-nucleotide polymorphism database; ExAC, Exome Aggregation Consortium,; ESP, NHLBI Exome Sequencing Project (Exome Variant Server); gnomAD, The Genome Aggregation Database; MAF, minor allele frequency; NA, not available; UK 10K, The 10,000 genome project, United Kingdom.

5.5 Geographic Distribution of *PKHD1* Mutations in Oman

The p.(T36M) allele was detected in patients from all governorates of the country except, Musandam, Al Wusta and Dhofar (Figure 1). The missense p.(T136A) allele was detected in its homozygous state in one family from Al Batinah South and in heterozygous state in families from Ad Dakhiliyah (*n*=1), Al Dhahirah (*n*=2), Al Batinah south (*n*=2) (Figure 5.4). We postulate that this mutation may be a founder allele in this population.

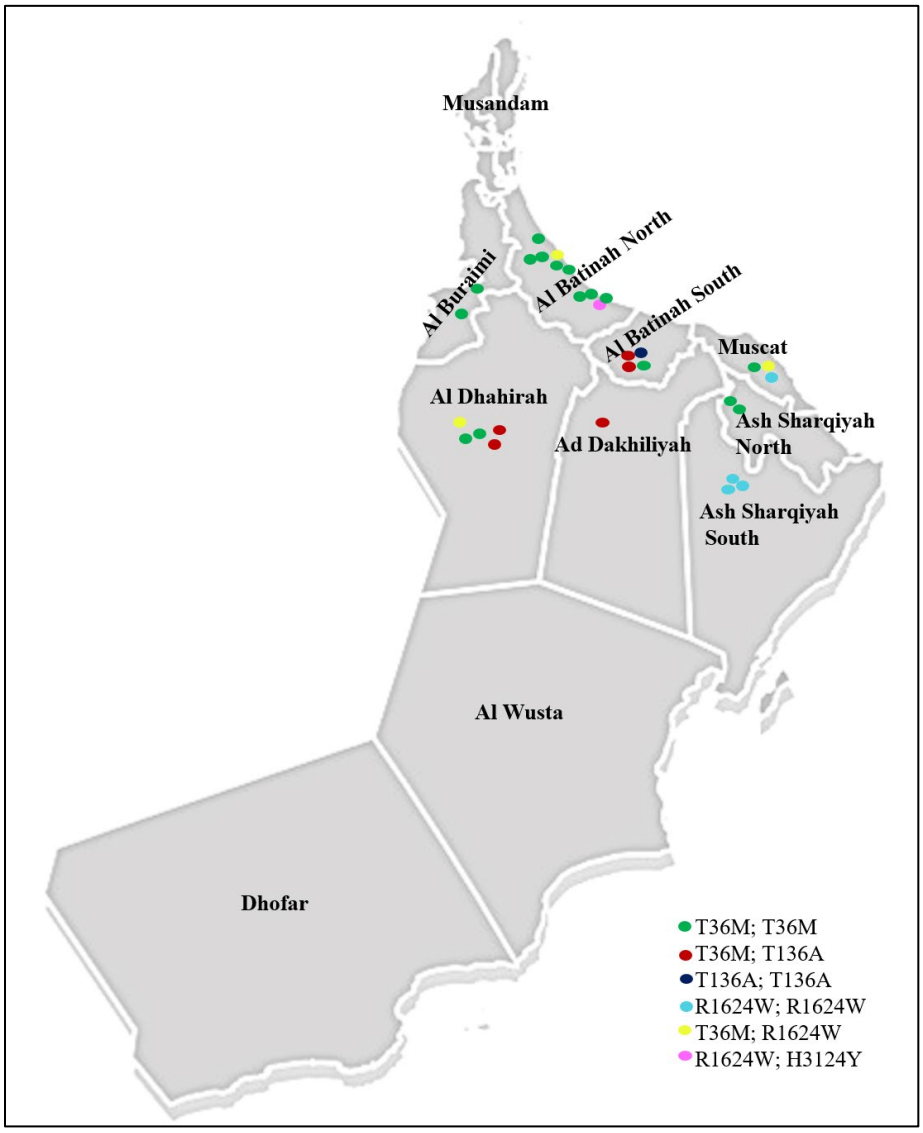

Figure 5.4 Geographical distribution of the 4 missense *PKHD1* mutations in ARPKD patients from Oman. Each family is presented by plot. Each different mutation is indicated by a different colour.

5.6 Discussion

Inherited kidney diseases, including ARPKD are leading causes of CKD and ESKD in children in Oman, leading to significant morbidity and mortality. Previous studies from Oman have provided ARPKD-associated morbidity data but lacked molecular genetic data (Al-Lawati, 2013, Al Riyami et al., 2019). In this study we have provided a clinical and molecular genetic analysis of *PKHD1* in a cohort of 41 patients.

Most study patients had early onset ARPKD disease reflected by age at initial diagnosis. Eight were diagnosed prenatally, 21 before their first year of life, 11 during childhood and only 1 patient during adolescence. These early-onset phenotypes are in agreement with that reported from other studies (Bergmann et al., 2005b). Clinical analysis of our ARPKD patients showed that the frequently associated morbidities were also common in our patients including systemic hypertension (79%), congenital hepatic fibrosis (78.4%), splenomegaly (48.6%), pulmonary hypoplasia (21.2%), CKD (7.6%) and neonatal death (15%). It is estimated that 30-50% of ARPKD patients die shortly after birth due to respiratory failure, whereas kidney failure is a rare cause of neonatal death (Bergmann et al., 2005b). With the advancement in renal replacement therapy modalities, the survival rate of neonates and children with ARPKD is improved. In our patients, twelve (24.4%) developed ESKD by mean age of 13 years and hence required either renal replacement therapy (*n*=8) or kidney transplantation (*n*=4). It has been reported that ARPKD patients with corticomedullary kidney pathology are expected to develop respiratory distress at birth and rapid deterioration of glomerular function, while the glomerular function is maintained for longer time in those with only medullary disease (Gunay-Aygun et al., 2010).

As the *PKHD1* is a large gene and in order to identify the causative mutations in our ARPKD patients, we initially applied targeted NGS gene panel for 18 unrelated patients. Four missense mutations were identified as genetic causes of ARPKD in this cohort, within exon 3, 6, 32 and 58. Therefore, we proceeded with Sanger sequencing of these exons alone for the molecular diagnosis of other ARPKD patients (*n*=23) from 15 different families. In total, 30 out of 33 families were solved, achieving a diagnostic rate of 91%, hence providing cost effective targeted PCR analysis of these specific alleles as a convenient diagnostic tool. Failure to detect mutations in three unrelated probands using the Sanger sequence approach

may be explained by the heterogeneity of ARPKD disease where mutations in other recessive cystogenes that phenocopy ARPKD might occur. It is also worth mentioning that none of these patients have family history of disease, which may be due to mutation in autosomal recessive genes that lead to ARPKD-like phenotypes such as the *DZIP1L* (Lu et al., 2017) or even in dominant cystic kidney disease genes such as *HNF1B*, *PKD1* and *PKD2* that often occur *de novo* or even in an autosomal recessive manner (Bergmann, 2019). Although all of these genes were included in the NGS target panel except *DZIP1L*, the 3 unsolved cases were merely processed by Sanger sequencing. Thus, target NGS panel or whole exome sequence are the suggested option for the 3 unsolved patients. With the current improvements of high throughput sequencing of different renal ciliopathy genes, many patients with cystic kidney disease phenotypes can receive a precise diagnosis.

To date, 748 unique *PKHD1* variants have been recorded in the Human *ARPKD/PKHD1* Mutation Database. Approximately 45% of these variants are missense alterations resulting in substitution of conserved amino acids, which usually leads to partial or complete dysfunction of FC/PD. All of the *PKHD1* variants detected in this study, as well, are missense alterations of highly conserved amino acids. Although three of these variants are reported in the *ARPKD/PKHD1* Mutation Database, p. (T36M) is the most persistent mutation found in *PKHD1* in ARPKD patients to date. Structurally, FC/PD is an integral membrane protein consisting of a large amino terminal extracellular domain (about 3,860 aa) containing various glycosylation sites, a single transmembrane (TM) segment and a short cytoplasmic C-terminal tail (about 195 aa) comprising four potential protein kinase A phosphorylation sites (Bergmann, 2017) (Figure 1.4, page 24). The localization of FC/PC to cilia and its integral structure predicted a sensory role at which FC/PD acts as receptor transducing the extracellular information into the cell through stimulation of signal cascades, thus controlling cell-cell adhesion and proliferation (Bergmann, 2017). The 4 missense variants identified in this study are located in exons encoding the extracellular domain (Figure 5.3B) and are either very rare or not observed in the reference databases (Table 5.3).

The *PKHD1* sequencing results of our patients did not find two previously described truncating mutations, corresponding with perinatal lethal phenotypes (Bergmann et al., 2004b, Bergmann et al., 2005b). The most frequent change identified in Omani families was

p.(T36M) located in exon 3, detected homozygously in 16 families (48.5%) and heterozygously in 8 families (24.2%), accounting for almost 73% of the families. Patients with homozygous change in *PKDH1* (n=18) had an earlier age of onset and an increased severity of disease (6 had a severe perinatal presentation and died at peri-/neonatal age, while the remaining had either infantile or early childhood presentation leading to ESKD) (Table 5.2). Consistent with observations made by Bergman et al. (2004), the p.(T36M) variant often leads to intra-familial and inter-familial phenotypic variability in the age of onset and severity. Additionally, p.(T36M) in combination with the missense changes p.(T136A) and p.(R1624W) in some cases caused a relatively severe form of ARPKD, which is in agreement with previous reported studies (Bergmann et al., 2003, Furu et al., 2003, Obeidova et al., 2015).

The pathogenic *PKHD1* allele p.(T36M) has been described in many populations and ethnicities. Whether this allele is a highly conserved ancestral change that is frequent in some populations such as the Central European population (Bergmann et al., 2003, Rossetti et al., 2003), or caused by recurrent mutational events is uncertain (Bergmann et al., 2005b). The p.(T36M) allele appears to be common in European genomes, with an expected carrier frequency of 1:412 (Ward et al., 2011). However, detections of this change in patients from different ethnicities and origins are suggestive that p.(T36M) is a *PKHD1* 'hotspot' mutation caused by the frequent methylation events of cytosine to thymine in the CpG sites (Bergmann et al., 2003, Sharp et al., 2005). It is also assumed that the substitution of the amino acid Threonine to Methionine creates potential alternative translation start codon that may be even stronger than the original start codon (Furu et al., 2003). The protein product initiating from position c.107 would be predicted to lead to complete loss of protein function due to improper protein folding (Furu et al., 2003).

The missense change, p.(R1624W), was previously reported in patients from different ethnicities, including Caucasian Americans (Sharp et al., 2005, Gunay-Aygun, 2009), Dutch (Losekoot et al., 2005), Czech Republicans (Obeidova et al., 2015), Slovenians (Smolović et al., 2018), Saudi Arabians (Sharp et al., 2005, Al-Hamed et al., 2016, Edrees et al., 2016) and Kuwaitis (Vivante et al., 2017). The p.(R1624W) mutation has been described with late onset or older ARPKD presentations when present homozygously (Sharp et al., 2005, Al-Hamed et

al., 2016) and heterozygously in *trans* with other truncating or missense change (Sharp et al., 2005, Smolović et al., 2018). In contrast, 6 of our patients with the p.(R1624W) mutation developed clinical features of ARPKD in infancy, 5 presented during childhood period and 1 at 11 years of age. The p.(H3124Y) combined with p.(R1624W) was found in a 26 year old patient with stage 4 CKD, who was initially diagnosed with polycystic kidney disease in early childhood (Table 5.2). These findings are in contrast to those made by Bergmann et al. (2004a) and Gunay-Aygun (2009) that correlated p.(H3124Y) with a severe perinatal-fatal phenotype.

These results therefore demonstrate that establishing genotype-phenotype correlations in ARPKD is challenging. Any correlation is complicated by the large number of missense variants distributed over the entire length of the coding exons of *PKDH1* and its complex splicing pattern (Bergmann, 2017). It was believed that two truncating mutations are associated with severe perinatal lethality and at least the presence of one missense is required for survival beyond the neonatal period. However, evidence is accumulating on the increased pathogenicity of some missense mutations that may cause complete loss of function effects (Bergmann et al., 2005b). Recently, a two year old child with two truncating mutations in *PKDH1* was reported to survive the neonatal period without ESKD, highlighting the significance of functional studies of germline mutations and interrogating the previously claimed genotype-phenotype correlations (Ebner et al., 2017). The wide variability in ARPKD severity among patients may be explained by differences in *PKHD1* mutations, influences of modifiers genes and environmental factors (Bergmann, 2019).

ARPKD is generally a severe form of paediatric ciliopathy with recognized phenotypic variability. While a significant number of ARPKD patients surviving the neonatal period reaches adulthood, some patients have an adulthood presentation and their kidney function differs between normal to moderate kidney insufficiency to ESKD (Buscher et al., 2014). Although bilateral kidney enlargement with multiple cysts is the major clinical characteristic, liver manifestations remain essential symptomatic disease complications in ARPKD patients. Liver disease seems to manifest later than kidney disease usually with progressive hepatic fibrosis and portal hypertension (Buscher et al., 2014). Hypersplenism, portal hypertension, and variceal bleeding are major liver involvements that may develop as a result of

progressive liver fibrosis. In rare cases, both kidney and liver disease may present in late adolescence or in adulthood (Fonck et al., 2001). The low prevalence, limited clinical information and atypical sonographic pattern of adult ARPKD patients can challenge the clinical diagnosis and management, hence genetic testing may be demanded for the establishment of definite diagnosis (Burgmaier et al., 2019). In this study, the absence of late presenting ARPKD in our cohort may be due to restricted patients recruiting criteria to mainly cystic kidney disease phenotypes.

The Omani population is characterized by a unique structure of tribal communities occupying definite geographical regions. This structure is conserved over many generations and has created genetic isolates (Rajab et al., 2013). The custom of consanguineous marriages as well as within-tribe (endogamous) marriages are extremely conserved in Oman, accounting for 56.3% (Tadmouri et al., 2009) and 20.4% of total marriages, respectively (Rajab and Patton, 2000). Over 300 genetic diseases have been identified in the Omani population (Rajab et al., 2015). The high frequency of recessive disorders in this population is probably related to a combination of genetic drift, consanguinity, and geographical isolation. The detection of only 4 pathogenic variants in different geographical regions of the country may be explained by the presence of *PKHD1* founder alleles and reveals a high degree of homogeneity in this population. Similarly, genetic studies of population isolates such as Finnish, French, Ashkenazi Jews and Africans represent a powerful method of finding founder mutations in *PKHD1*, which can be utilized for efficient diagnostic testing of at-risk individuals and pregnancies in these populations (Table 5.4) (Bergmann et al., 2003, Michel-Calemard et al., 2009, Quint et al., 2016, Lambie et al., 2015).

Currently there is no clinical cure for ARPKD other than managing the clinical complications (Buscher et al., 2014). Together translational research and clinical trials in patients may facilitate successful drug development in coming future. With the absence of clinical biomarkers and lack of comprehensive assessment of the available therapeutic options for ARPKD patients on one hand and great morbidity and mortality of disease on the other hand, there is a serious need for prospective and retrospective population studies and construction of an international clinical database. Such effort can elaborate the current understanding of ARPKD and deliver more information on extrarenal manifestations and treatment options.

Recently, the German Society for Pediatric Nephrology (GPN) and the European Study Consortium for Chronic Kidney Disorders Affecting Pediatric Patients (ESCAPE) collaborated to initiate an international multicenter registry of ARPKD (ARegPKD) (Ebner et al., 2015). The continued identification of *PKHD1* variants and their associated phenotypes is to be encouraged and inclusion of cohorts from different ethnicities is valuable and should be encouraged.

Origin	Nucleotide change	Amino acid change	Exon	Mutation Type	References
Finnish	c.1486C>T	p.R496*	16	Nonsense	Bergmann et al., 2003
	c.10412T>G	p.V3471G	61	Missense	
French	c.7350+653A>G	p.G2451fs*18	IVS46	Intronic / Pseudo exon activation	Michel-Calemard et al., 2009
Ashkenazi	c.3761_3762del CCinsG	p.A1254Gfs*49	32	Frameshift / Indel or Duplication	Quint et al., 2016
Afrikaner	c.1880 T>A	p.M627K	20	Missense	Lambie et al., 2015

Table 5.4 Different *PKHD1* founder mutations associated with different ethnicities.

5.7 Conclusions

In conclusion, this study shows that NGS identification of *PKHD1* mutations and subsequent screening of only 4 exons of the *PKHD1* gene was sufficient to identify the expected causative alleles in 91% of the studied patients and was suggestive of founder effects in this gene. There are many advantages for identifying high frequency limited disease associated mutations in a population, including simplifying the diagnostic testing, providing genetic counselling for individuals at risk and allowing rapid detection of mutations in other family members.

Chapter 6. Use of Whole Exome Sequencing in diagnosis of Omani Patients with Inherited Kidney Diseases

6.1 Introduction

Without doubt, the potential use of NGS technologies in basic research of inherited kidney diseases has contributed in the detection of novel causative genes and mutations, which provides accurate diagnosis and enhances the current knowledge of genotype-phenotype correlations in these disorders (Gee et al., 2014). Therefore, nowadays, with the advent of the ever-reducing costs, NGS technologies has gradually shifted to precision medicine to be implemented in clinics as a routine diagnostic tool (Mann et al., 2019). However, there is a debate on which format of NGS approaches to be used for diagnosis of patients. Although WGS provides genetic information on all genome level, including intronic, intergenic and regulatory regions, the sequencing cost and difficulties of massive data interpretation and storage remain critical issues keeping this approach not preferred as first diagnostic level. Focusing only on protein-coding regions through WES decreases the sequencing costs and produces manageable genetic data for interpretation, which enhances its extensive usage in diagnosis leading to the discovery of previously unrecognized renal disease genes and disorders (Braun and Hildebrandt, 2017, Groopman et al., 2019). However, in terms of costs and data interpretations and storage, targeted gene panels serve as the best first tier diagnostic format for many renal diseases, keeping in mind the advantages of enriching problematic genomic regions such as *PKD1* gene (Al-Hamed et al., 2016). Targeted gene panels, provide they are updated, are useful in the diagnosis of patients with clinical evidence of renal ciliopathies, whereas WES and WGS are best implemented in patients with a more non-specific clinical diagnosis or where gene panels have failed to provide a diagnosis.

Nowadays WES is becoming part of routine clinical and diagnostic practice (Groopman et al., 2019). In the case of heterogeneous renal ciliopathies, WES has been extensively applied in research studies as well as for diagnostic utility to detect various novel genes and variants (Otto et al., 2010, Mann et al., 2019). In this chapter, WES was used to determine the genetic causes of chronic kidney disease (CKD) in children suspected with recessively inherited kidney disease in a group of 11 unrelated patients. The first batch of WES experiments included five patients unsolved by the target NGS panel, which are P12 (re-identified as M46), P13 (re-identified as M48), P15 (re-identified as M44), P30 (re-

identified as M43) and P31 (re-identified as M47). Clinical features of these samples are highly suggestive of recessive pattern of renal ciliopathies and can be found in chapter 4 (Table 4.3). The second batch of WES experiments included six genetically untested patients, mostly with unspecific cystic kidney disease causing ESKD and their clinical presentations are described in Table 6.1.

Patient ID	Gender	Age	Suspected diagnosis	Clinical presentations	Family history	Parental consang.
P3	M	3 Y	UCKD	Enlarged cystic kidneys with unknown aetiology.	No	No
P9	F	4 Y	UCKD	Hypertension and iron deficiency anaemia. ESKD on PD.	Yes	No
P12	F	9 Y	UCKD	Hypertension and iron deficiency anaemia. CKD stage 4.	Yes	Yes
P15	M	12 Y	CKD of uknown aetiology	Hypertension, hearing loss on hearing aid. CKD stage 3.	Yes	No
P18	M	6 Y	UCKD	Hypertension, iron deficiency anaemia, DD, hyperparathyroidism, and retinal dystrophy. ESKD on PD.	Yes	Yes
N36	F	1 Y	BBS	Polydactyly present in all limbs.	Yes	U/A

Table 6.1 Characteristics of inherited kidney disease patients undergoing WES. BBS, Bardet Biedl syndrome; CKD, chronic kidney disease; consang., consanguinity; DD, Developmental delay; ESKD, end stage kidney disease; F, female; M, month; M, male; PD, peritoneal dialysis; U/A, unavailable; UCKD: unspecific cystic kidney disease; Y, year.

6.2 Patients Characteristics

WES was carried out for 11 apparently unrelated paediatric patients (Female: 7; Male: 4) with a clinical suspicion of different inherited kidney diseases, including unspecific cystic kidney disease (UCKD; *n* = 5), NPHP (*n* = 2), CKD of uknown aetiology (*n* = 1), BBS (*n* = 1), JBTS (*n* = 1) and MKS (*n*=1). Nine out of 11 (82%) had a positive family history of disease and seven had extrarenal manifestations (Table 6.1).

6.3 Exome Sequencing Data

Quality control of WES revealed that on average 95.2% of the reads were properly mapped to the reference genome and details of the depth, coverage and target covered of all samples can be found in the appendix A2. The average coverage depth was 187.9× for the first batch of WES samples (M43-M48) and 103.9× for the second batch of samples (P3-N36). Comparable coverage of target coding regions was achieved among the 11 cases with an average of 96.4% of the exome being covered at least 20-fold (Figure 6.1). In the analysis of these cases, the filter was set at ≥ 20-fold for both homozygous and heterozygous variants.

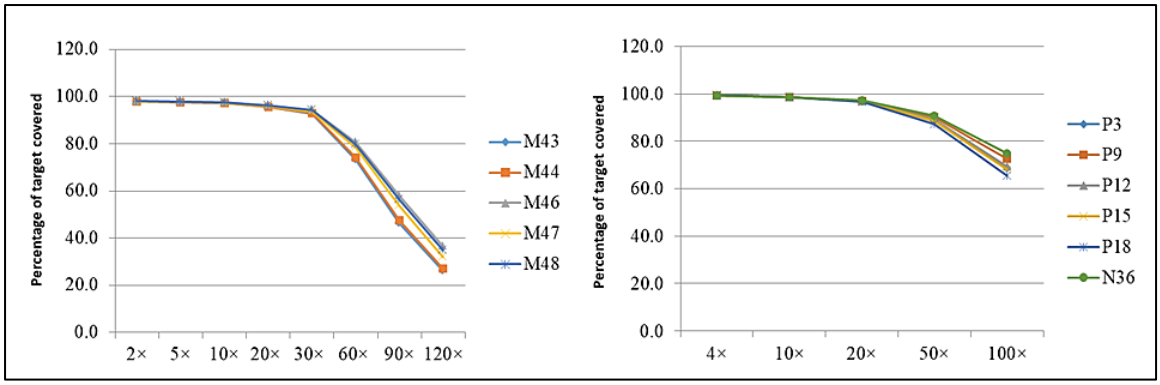

Figure 6.1 Coverage of coding regions across the studied patients.

6.4 Molecular Genetic Findings

Different filters were used for sorting detected variants, given primary interest to rare homozygous variants causing potential protein effects, keeping in mind high level of parental consanguinity (54.5%) in the studied patients. To detect homozygosity stretches of the genomes, WES genotype data were used to create homozygosity mapping using the online homozygosity mapper tool (http://www.homozygositymapper.org/). Family pedigrees the of children with positive results of WES are illustrated in Figure 6.2.

Using ACMG criteria, definite genetic diagnosis was obtained in 6 out of the 11 patients, leading to an overall diagnostic yield of 54.5% (Table 6.2). A total of six different single nucleotide variants (SNVs) were detected in six distinct known nephropathy genes (*TMEM231*, *TMEM138*, *NUP93*, *COL4A5*, *WDR19* and *BBS9*) and were confirmed by Sanger sequencing. Segregation of pathogenic causative allele with family members was confirmed for *NUP93*, *COL4A5* and *WDR19*.

Based on the distribution, missense variants were the most frequent (4/6), followed by nonsense (1/6) and splice site loss (1/6) (Table 6.2). Across the causative variants, the presumed pattern of inheritance was autosomal recessive in 83.3% (5/6), at which homozygous causative variants were detected, and X-linked in 16.7% (1/6) of cases (Table 6.2).

Three of the identified causative variants were not previously reported in any databases; including the missense c.710A>G; p.Y237C in *TMEM231* and c.1319T>C; p.F440S in *NUP93* as well as the nonsense c.3475C>T; p.Q1159* in *COL4A5* (Table 6.2). All tested samples were examined for mutations in ACMG actionable genes but none was found.

Patient ID	M46	M48	P9	P15	P18	N36
Molecular diagnosis	Meckel syndrome 11 (OMIM:615397)	Joubert syndrome 16 (OMIM:614465)	Nephrotic syndrome, type 12 (OMIM:616892)	Alport syndrome 1, X-linked (OMIM:301050)	Senior-Loken syndrome 8 (OMIM:616307)	Bardet-Biedl syndrome 9 (OMIM:615986)
Gene	*TMEM231*	*TMEM138*	*NUP93*	*COL4A5*	*WDR19*	*BBS9*
Sequence variant	NM_001077416: c.710A>G; p.Y237C	NM_016464: c.389A>G; p.Y130C	NM_014669.5: c.1319T>C; p.F440S	NM_033380: c.3475C>T; p.Q1159*	NM_025132: c.3533G>A; p.R1178Q	NM_198428.3: c.1789+1G>A
Zygosity	AR (hom)	AR (hom)	AR (hom)	X-linked (hemi)	AR (hom)	AR (hom)
Interpretation	Missense	Missense	Missense	Nonsense/Stop gain	Missense	Splice-site loss
ACMG Classification	Uncertain significance	Likely pathogenic	Uncertain significance	Pathogenic	Likely pathogenic	Pathogenic
dbSNP ID	NA	rs387907135	NA	NA	rs79436363	rs201938124
MAF	Variant was neither found in ExAC, gnomAD nor 1000G.	3.98×10^{-6} (gnomAD)	Variant was neither found in ExAC, gnomAD nor 1000G.	Variant was neither found in ExAC, gnomAD nor 1000G.	6.35×10^{-5} (gnomAD)	7.96×10^{-6} (T) (gnomAD)
SIFT Pred.	Damaging	Deleterious	Damaging		Tolerated	
PolyPhen-2 Pred.	Possibly Damaging	Probably damaging	Possibly Damaging		Probably Damaging	
Mutation Taster	Disease causing	Disease causing	Disease causing	disease causing	disease causing	
CADD Score	22.7	25.7	33	35	24.6	25
References	*Novel*	Lee et al (2012)	*Novel*	*Novel*	Halbritter et al. (2013)	Nishimura et al. (2005)

Table 6.2 Molecular genetic findings of Omani children detected by WES. ACMG, American College of Medical Genetics and Genomics; CADD, Combined Annotation Dependent Depletion; dbSNP, single-nucleotide polymorphism database; ExAC, Exome Aggregation Consortium database; gnomAD, The Genome Aggregation Database; NA, not applicable; Pred. prediction; 1000G, 1000 Genomes Project.

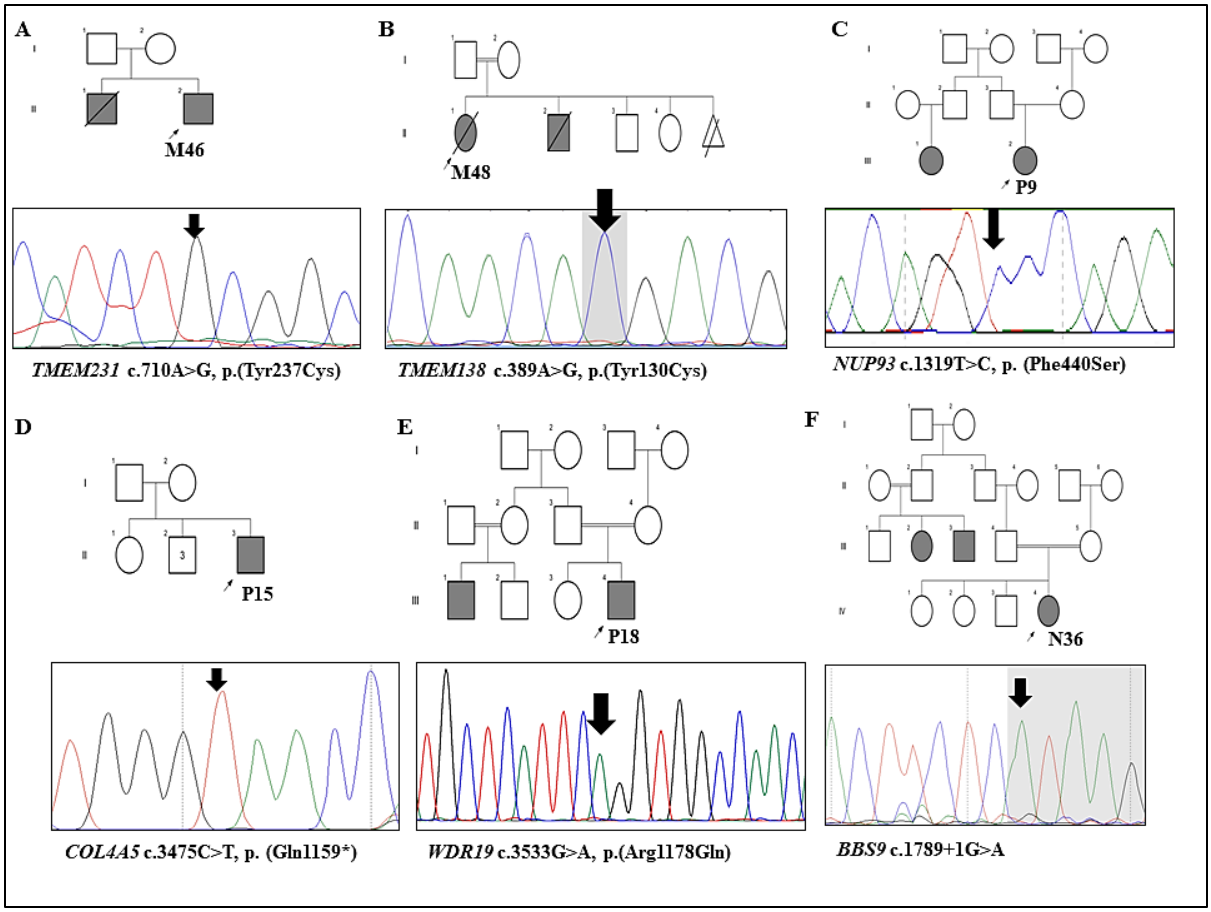

Figure 6.2 Pedigrees and electropherograms generated by Sanger sequencing confirming the disease causative variants detected by WES. A. Pedigree of patient M46 and the chromatogram showing the homozygous missense p.Y237C variant in *TMEM231* gene. B. Pedigree of patient M48 with clinical diagnosis of JBTS that was confirmed by the detection of pathogenic missense variant in *TMEM138* gene. C. Extended pedigree of P9, who has genotype confirming steroid resistance nephrotic syndrome caused by missense mutation (p.F440S) in *NUP93* gene, which was also confirmed on her affected cousin. D. Pedigree of patient (P15) with clinical presentation of Alport syndrome, confirming X-linked inheritance by the detection of the *COL4A5* nonsense (p.Q1159*) variant. E. Extended pedigree of P18 and the chromatopherogram confirming genotype of Senior-Loken syndrome by the detection of pathogenic missense variant (R1178Q) in *WDR19* gene. F. Pedigree of consanguineous family genetically confirmed to harbour a known splice site variant (c.1789+1G>A) in *BBS9* gene.

6.5 Clinical Significances of WES

WES findings have valuable impact on both early clinical diagnoses and management of studied children as well as genetic counselling. In four out of the 11 examined children (M46, M48, P15 and N36) the genetic findings confirmed the clinical diagnosis. This information is valuable since it clarifies the accurate mode of inheritance and facilitating proper counselling of family members as well as guide the setting kidney transplant and selection of living related kidney donors. In patient P15, for instance, WES results differentiate between X-linked and autosomal recessive form of Alport syndrome and allows proper (auditory and ophthalmology) screening and counselling of family members (Table 6.2; Figure 6.2). The detection of causative variants in *NUP93* gene in P9 can spare the patient and the affected cousin unnecessary exposure to immunosuppressive therapy and patients may refer for kidney transplant (Table 6.2; Figure 6.2). Management of diabetes, hypertension and weight in the case of patient N36 (with BBS) and other affected family members is critical to avoid the damage of important organs, such as eye and kidney (Table 6.2; Figure 6.2). The recognition of the accurate genetic diagnosis in the two families of patients M46 and M48 can potentially provide accurate genetic advice about their increased reproductive choice and the possibility of preimplantation genetic diagnosis (PGD).

6.6 Discussion

In paediatric population, CKD is a major contributor to health-care burden leading to severe morbidity and mortality. At least 17% of those with ESKD are considered as CKD with unknown aetiology, where the primary kidney disease is not clear (de Haan et al., 2019). In addition, the primary clinical diagnosis of CKD patients is most often inaccurate (de Haan et al., 2019). Thus, in the developing era of precision medicine, WES is used as an essential tool that provides novel diagnostic perspectives for the detection of the causes of CKD. Knowledge of genetic causes has valuable clinical implications in therapeutic intervention, improving prognosis, guide family counselling or managing settings of kidney transplantation (Ayme et al., 2017). Despite been rare, inherited kidney diseases represent one of the most common causes of CKD and ESKD, accounting for up to 10% of adults and almost all children commencing renal replacement therapy (Devuyst et al., 2014). The possibility of monogenic causes in those with unknown aetiology of CKD or with atypical

clinical presentation is assumed to be high (de Haan et al., 2019). At least 500 different genetic causes have been associated with childhood CKD (Connaughton et al., 2019).

In this pilot study, we examined the utility of WES in the diagnosis of 11 different Omani children with childhood onset CKD clinically suspected with inherited kidney disease. A conclusive genetic diagnosis was achieved in over half (6/11) of the cases, corresponding to an overall diagnostic rate of 54.5%. This result is within the reported diagnostic yield of WES. The high diagnostic yield achieved is probably a consequence of studying a cohort with high consanguineous and familial cases. A wide-range of genetic studies have been performed in childhood CKD population and different diagnostic yields were achieved due to differences in the inclusion criteria or patients and the study design. In his study of families with inherited kidney disease, Mallett et al. (2017) reported high diagnostic yield of 46%, reflecting the significant ability of WES in underlying the potential genetic causes of most renal phenotypes. In another recent study, Groopman et al. (2019) reported higher diagnostic yield in patients with congenital and cystic kidney disease (23.9%). Furthermore, regardless of the primary kidney diagnosis, higher diagnostic yield was associated with a positive family history of CKD, history of parental consanguinity and presentations of extrarenal features (Groopman et al., 2019, Mann et al., 2019). Thus using a combination of homozygosity mapping along with WES genotype data is always recommended as a powerful linkage approach for consanguineous families to identify rare genetic causes (Belkadi et al., 2016).

Although WES provides massive amount of data, 5 patients still remained unsolved in this study. Interpretation of a high prevalence of novel and extremely rare variants is still restricted by the incomplete knowledge of the total human protein-coding genes as well as the incorrect obligations of variants pathogenicity and incorrect association of genes with the disease in the literature. At present, up to 70% of protein-coding genes have no recognized human disease phenotype (Lek et al., 2016). False gene-disease associations are familiar in the literature and clinically valuable databases of variants pathogenicity, such as HGMD, comprise various errors causing benign variants being falsely selected out of the data and allocated as plausible diagnosis (Ghouse et al., 2017). This situation is predicted to improve as further genomes are sequenced, however the significance of large data collections

containing populations of both healthy individuals and patients with rare diseases cannot be ignored. In addition, studying more families with similar clinical phenotypes from the same population may facilitate linking novel undiscovered genes to the disease phenotype in those unsolved patients.

In fact, for certain inherited kidney disease, it is often a challenge to differentiate between them and misdiagnosis may happen, because the majority are genetically very heterogeneous and are associated with a broad range of clinical features. However, genetic testing using NGS approaches provides more accurate characterization of overlapping renal diseases, where most of kidney diseases with unknown primary aetiology were identified and some were reclassified (de Haan et al., 2019). In this study, WES confirmed the clinical diagnosis in 4 (66.7%) and recognized a first-time diagnosis in 2 children referred with ESKD of unknown aetiology (33.3%). In a similar study of large consanguineous or familial cohort (*n* = 79) of children clinically diagnosed with NPHP, genetic diagnostic yield of 63% was reported, at which the clinical diagnosis was confirmed in 64% and changed to different molecular diagnosis in the remaining 36% (Braun et al., 2016). Other studies have also documented the impact of WES in reclassification of the diagnosis of overlapping kidney disease such as mesangial proliferative glomerulonephritis (MPGN), focal segmental glomerulosclerosis (FSGS) and autosomal recessive and dominant Alport syndrome, where patients with Alport syndrome often misdiagnoses as MPGN or FSGS (Lata et al., 2018). In his study, Lata et al. (2018) also demonstrate the impact of WES in providing accurate diagnosis and prompting the medical management of CKD patients with unknown aetiology, although the study was limited by small, selected size of studied cohort.

This study has some limitation, including small sample size that does not give a generalized image of broader childhood renal ciliopathy population from Oman. However, an enhanced assessment of the utility of WES in the clinical diagnostic practice of these disorders may be given through systematic WES analysis of a larger, unselected cohort. Moreover, the diagnostic gap in this study may be caused by the common technical limitation of WES, including the detection of structural variant breakpoints, sequencing difficult regions with repetitive elements or GC-rich regions, discriminating highly homologous genomic regions with pseudogenes. These limitations are attributed to the short-read lengths that are utilized

to generate high genomic coverage and depth (Mantere et al., 2019). These limitations are assumed to be resolved through using long-reads sequencing platforms that compromise these technical challenges and improve the detection of genetic variants (Mantere et al., 2019). Thus the emerging future of long-read sequencing based WGS could enhance the diagnostic yield of patients with inherited kidney disease or unknown aetiology of CKD and provide more conclusive primary kidney disease diagnosis. This can be supported by recent reports of WGS obtaining higher molecular diagnostic yield compared with WES, where 20-40% of those unsolved by WES were genetically conclusive by WGS (Ellingford et al., 2016).

Recent advancements in medical genetics through the use of massively parallel sequencing have not only advents the discovery of novel causative variants, genes and phenotypes, but also revolutionized our vision about genetic kidney diseases where novel disorders were identified as well as previous disease were redefined (Stokman et al., 2016). However, all types of NGS-based testing (Target panel, WES and WGS) have some shared limitations, including the inability to obtain enough coverage of genomic regions with highly repetitive GC-content sequence, such as that in *MUC1* gene. In his study of six unrelated families with medullary cystic kidney disease type 1 (MCKD1), Kirby et al. (2013) highlighted the challenges of these technologies in detecting the causative monogenic causes of some Mendelian disorders, such as MCKD1, where only long-range polymerase chain reaction and molecular cloning successfully performed the task. Moreover, in many patients with acquired diseases, NGS- testing is of limited importance and transformation of genetic results into clinical setup may be challenging (Stokman et al., 2016). In the field of kidney disease, the majority of genetic testing studies are narrowed to a research setting, thus until now the knowledge of its diagnostic efficacy in clinical practice is still limited (de Haan et al., 2019). In addition, managing the medical ethics raised by these technologies, including uncertain variants and incidental findings, in addition to the social concerns is still challenging (Clarke, 2014).

6.7 Conclusion

WES of patients with different inherited kidney disease show promising consequences as diagnostic tool for children in the CKD population. It has the potential to resolve those cases with clear suspicion of renal ciliopathies, as well as those with uncertain aetiology causing CKD. The clinical impacts of positive WES results in therapeutic choice, genetic counselling and guidance of kidney transplant are critical. However, genetic counselling on the prospective effects of a positive test result is crucial, bearing in mind the possibility of incidental findings. Although further studies from the Omani population are required, we predict an expanding impact of NGS-based diagnosis, both gene panels and WES in clinical practice in the very near future.

6.8 Future Scope of This Study

The future scope of this study is to construct the foundation for a comprehensive nephrongenetic services in Oman, thus certain agenda are to be followed up after this research in order to continue progress in genomics of kidney disease. These include the followings: (1) **CKD statistical visions:** by remain make-up of systematic data of CKD epidemiology and related statistics with precise disease diagnosis. (2) **Biobank and biosamples:** to create a sort of first kidney disease specific patients' registry and biorepository with DNA/RNA samples to be archived at the National Genetic Centre in Muscat. (3) **Local genetic database:** to continue generating kidney gene mutation and DNA variants database to be shared with the international scientific community. (4) **Genotype-phenotype correlation:** to enhance assessment of patients' diagnosis and prognosis of a disease. (5) **Founder phenomena**: to continue detection of founder mutations that may segregate in large families from different regions of Oman and that will simplify molecular diagnosis. (6) **Segregation assessment** of variants pathogenicity with uncertain clinical significance that are thought to be potential cause of a disease.

This study has generally been very successful and has brought potential genetic diagnostics for over two-third of studied patients, however, there are different aspects that would be taken into consideration if this study is to be repeated. With respect to tackling the burden of inherited kidney disease throughout the country, multi-resources are to be used for collecting defined data of patients with kidney disease including different health-system surveillance or registries, such as the intensive care units, out-patients clinics and private-hospitals data. For molecular genetics and as post-genome sequencing step, interpretation and functional validation of genomic variants would be given enough space of the research by using model systems in more specific functional assays. For examples, to evaluate the impact of intronic, missense and synonymous variants of uncertain clinical significance (VUS) in a physiologically relevant manner patient-specific cellular models, such as human urine-derived renal epithelial cells (hUREC) would be used, which has been shown as an extremely powerful *in vitro* model of kidney disease (Molinari et al., 2018). Furthermore, to verify variants predicted to induce potential transcription or splicing impairment, such as splice-site

or even missense variants, functional studies would be done to proof pathogenicity using either whole-blood RNA or patient-specific hUREC to isolate tissue-specific RNA and then cDNA is made by using reverse transcriptase (RT-PCR). Finally, for the WES unsolved cases, I would use recently developed tools and pipelines designed specifically for the detection and reporting of structural variants using short read NGS data.

Chapter 7 Concluding Discussion

There are over 750 million people worldwide affected with kidney disease, thus the burden is much higher than those liveing with diabetes, cancer or even AIDS/HIV (Crews et al., 2019). Inherited kidney diseases are one of the major contributors to CKD burden, where up to 10% of adults and over 70% of children reaching ESKD are expected to harbour genetic causes (Groopman et al., 2019). However, studying such rare diseases has considerable challenges, where the patient populations are limited and the progress of treatments is delayed by small patient cohorts and lack of commercial feasibility. Collaborative research and progress of new technologies and methodologies are strategic to overcoming these challenges.

Currently, the focus of nephrogenetics in Oman is primarily made on establishing accurate genetic diagnoses to explain clinical phenotypes using the significantly improved diagnostic power of genomic technologies. In this pilot study of Omani patients suspected with renal ciliopathies, the diagnostic yield achieved by genomic sequencing is 73.4%; including a targeted kidney gene panel, mutation specific screening and WES experiments. On the other hand, the remaining ~25% of unsolved families are potentially valuable cohort for the detection of novel genes causing renal ciliopathies, where much remains to be discovered. Furthermore, for the common *PKHD1* mutations detected in the study patients, further investigations are required to verify founder affects in Omani population as well as other Arabs populations.

This study establishes a solid background to understand the genotype-phenotype of some inherited kidney diseases in Omani population. Thus, our results are anticipated to have wider applications across different types of renal ciliopathies, which are usually severe, lifelong and sometimes life-limiting conditions. However, appropriate investment in professional clinical assessment is crucial to ensure that the diagnosis proposed by genomic analysis is clinically comprehensive. The biological complexity of these diseases in term of clinical manifestations and inheritance highlights the importance of accurate documentation of clinical genotype-phenotype associations. It is also important to evaluate the clinical utility and factors impacting genomic diagnosis consistency and defining the amount and type of phenotypic information demanded to provide safe practice of genomic medicine. Success of

this is often attained through multidisciplinary settings of clinicians, scientists and bioinformaticians.

Accurate genetic diagnosis that correlates the clinical features of the patients with the molecular cause is the foundation for safe medical practice of these conditions. The rapid emergence of genomics and the better understanding of the molecular background of renal diseases have already started to change the quality of care that can be provided nowadays. The future is anticipated to bring improved strategies that will deliver comprehensive clinical applications and lead to new therapeutic options. However, optimizing the application of genome diagnostics is challenging since every single parameter of the analytical pipeline currently used is still developing. To be precise, clinicians are still getting to know when to request genetic testing; sequencing companies are still emerging long-read technologies; bioinformaticians are progressing algorithms to identify and prioritize distinct types of variants; population databases are emerging in terms of depth and illustration of different ethnicities; rapid gene discoveries continue in gene-disease associations and in identifying pleiotropy; variant databases and the literature are developing though intensely polluted with false-pathogenicity contents. Thus, to improve accurate diagnosis and reduce the risk of misdiagnosis or overdiagnosis, the fundamental objective will be to advance variant detection and filtration that can be achieved by concentrating attention on patient ascertainment, phenotyping, comprehensive discovery of disease-associated genes and variants and proper expertise. Therefore, a multidisciplinary team consisting of bioinformaticians, clinical scientists and specialist clinicians has critical role in maintaining safe and efficient practice of genome diagnostics of different inherited kidney diseases.

The advent of NGS technologies have made the major impact in revolutionizing the fields of transcriptomics, epigenomics, peptidomics, proteomics and metabolomics leading to a rapid growth in the experimental data of disease-altered molecules, therapeutic targets and biomarkers (Rhee, 2018). Moreover, in the practice of nephrology, omics technologies have been utilized to improve the diagnostic and prognostic value of urinalysis, including the extensive use of proteomics in the discovery of urinary biomarkers in CKD, acquired kidney injuries and ADPKD that may facilitate clinical therapeutic benefits (Rhee, 2018). The future of effective therapeutic development require a comprehensive understanding of the molecular

background of disease, that can be accelerated through integrating genomics with multi-omics data along with deep phenotyping or standard imaging and biochemical assays. Indeed, the use of genomics, which are the only omics implemented into routine clinical diagnostic practice, with multi-omics data is anticipated to improve the clinical diagnostic ability by investigating the functional significance of genetic variation on particular tissues. Furthermore, stratifying each patient according to their molecular genetic basis in NURTuRE, the National Unified Renal Translational Research Enterprise (UK) is a promising project that is intended to enable more demanding clinical trials and evaluate new therapies for patients with CKD and nephrotic syndrome (Ding et al., 2019).

Appendix

A1. Target-capture Panel Design

Design	Information
Design Name	Genotypic_EA977_PolyCystic_Capture_Array
Design ID	03787-1459331610
Design Category	HaloPlex High Sensitivity
Species	*Homo. sapiens* (hg19, GRCh37, February 2009)
Platform	Illumina
Read Length	250 bp
Target Summary	
Target Region Size	243.165 kbp
Amplicon Summary	
Total Amplicons:	11712
Total Target Bases Analyzable	242.446 kbp
Total Sequenceable Design Size	557.073 kbp
Target Coverage	99.70%
Recommended Minimum Sequencing per Sample	111.414 Mbp

Table A1.1 Summary of design from cystic kidney disease Targeted NGS panel. bp, base pair; GRCh37, Genome Reference Consortium Human Build 37; hg19, human reference genome version; kbp, kilo base pair; Mbp, mega base pair.

Target ID	Interval	Regions	Size (bp)	Coverage	High Cov.	Low Cov.
AHI1	chr6:135606714-135813425	30	6849	99.88	30	0
ANKS6	chr9:101494736-101558823	16	4269	100	16	0
ARL6	chr3:97486902-97516943	7	1261	100	7	0
BBIP1	chr10:112660068-112677965	5	962	100	5	0
BBS1	chr11:66278081-66299558	18	3620	100	18	0
BBS10	chr12:76739543-76742188	2	2372	100	2	0
BBS12	chr4:123662998-123665230	1	2233	100	1	0
BBS2	chr16:56518623-56553824	17	3955	100	17	0
BBS4	chr15:72978519-73029978	17	3796	100	17	0
BBS5	chr2:170336014-170382256	17	3437	100	17	0
BBS7	chr4:122746965-122791518	19	4053	100	19	0
BBS9	chr7:33185815-33644888	24	5303	100	24	0
BICC1	chr10:60272854-60588701	21	5135	100	21	0
CEP164	chr11:117209253-117282934	30	7482	100	30	0
CEP290	chr12:88442911-88535134	52	12925	99.69	51	1
CEP83	chr12:94702539-94806316	15	3606	100	15	0
DCDC2	chr6:24174908-24358028	11	2727	99.6	11	0
GLIS2	chr16:4382232-4387575	4	2138	100	4	0
HNF1B	chr17:36047233-36104925	9	2666	100	9	0
IFT27	chr22:37154305-37171801	9	1581	100	9	0
INVS	chr9:102866754-103063006	17	5095	100	17	0
IQCB1	chr3:121489142-121547857	13	3097	100	13	0
LZTFL1	chr3:45867756-45957160	12	2527	100	12	0
MKKS	chr20:10385845-10401435	5	2405	100	5	0
MKS1	chr17:56282862-56296922	19	4196	100	19	0
MUC1	chr1:155158561-155162684	8	2523	100	8	0
NEK8	chr17:27055782-27069055	11	3607	100	11	0
NPHP1	chr2:110881318-110962595	22	4643	100	22	0
NPHP3	chr3:132400704-132441249	29	7003	99.13	28	1

NPHP4	chr1:5923275-6046399	31	7542	100	31	0
PKD1	chr16:2139678-2185740	34	17303	100	34	0
PKD2	chr4:88928836-88996896	15	4407	100	15	0
PKHD1	chr6:51483829-51949781	67	18960	100	67	0
PRKCSH	chr19:11546889-11560277	11	3246	100	11	0
REN	chr1:204124094-204135471	10	2221	100	10	0
RPGRIP1L	chr16:53635938-53734685	27	6868	99.58	27	0
SDCCAG8	chr1:243419426-243663137	21	4486	99.87	21	0
SEC63	chr6:108192858-108279263	22	4602	100	22	0
TMEM67	chr8:94767093-94828730	29	6527	99.02	28	1
TRIM32	chr9:119459972-119462033	1	2062	100	1	0
TSC1	chr9:135771572-135804309	20	5644	100	20	0
TSC2	chr16:2098221-2138661	41	9998	98.06	40	1
TTC21B	chr2:166731215-166810265	28	6842	99.99	28	0
TTC8	chr14:89291002-89343804	18	3640	98.13	17	1
UMOD	chr16:20344586-20364105	12	3432	100	12	0
WDPCP	chr2:63349091-63815455	21	4474	99.26	20	0
WDR19	chr4:39184128-39280320	34	7597	100	34	0
XPNPEP3	chr22:41253136-41322489	14	3167	98.26	13	1
ZNF423	chr16:49525136-49856646	8	4681	100	8	0

Table A1.2 Coverage and size (bp) of targeted genes included in the NGS panel and the number of target regions in each gene. Target ID: gene entered in the Targets list. Interval: the genomic interval of the target. Regions: the number of regions within this target. Size: the total size (in base pairs) of the regions. Cov: Coverage. High Coverage: Number of regions where analyzable amplicon overlap >= 90%. Low Coverage: Number of regions where analyzable amplicon overlap < 90%.

A2. Whole Exome Sequencing

A. First WES batch (unsolved samples by targeted-NGS)[3]

1. Sequence Quality Metrics:

Sample	Total Reads	LQ Reads	Single Reads	HQ Reads
M43	80,971,872	1,454,930 (1.8%)	1,359,802 (1.7%)	78,157,140 (96.5%)
M44	77,972,988	1,404,860 (1.8%)	1,303,958 (1.7%)	75,264,170 (96.5%)
M46	96,659,592	1,950,556 (2.0%)	1,820,194 (1.9%)	92,888,842 (96.1%)
M47	87,945,344	1,615,636 (1.8%)	1,499,358 (1.7%)	84,830,350 (96.5%)
M48	91,446,344	1,868,251 (2.0%)	1,747,261 (1.9%)	87,830,832 (96.0%)

Table A2.1 Sequence quality metrics per sample.

Total Reads: Total number of sequence reads analysed for each sample. LQ Reads: Number (percentage) of low quality reads. Single Reads: High quality reads without mates (2nd read). These are not included for further analysis. HQ Reads: Number (percentage) of high quality reads used for further analysis.

2. Alignment Metrics:

Mapping to the reference sequence (hg19) was performed by BWA with default parameters.

Sample	HQ Reads	Mapped Reads
M43	78,157,140	78,049,206 (99.86%)
M44	75,264,170	75,169,466 (99.87%)
M46	92,888,842	92,756,897 (99.86%)
M47	84,830,350	84,731,342 (99.88%)
M48	87,830,832	87,717,524 (99.87%)

Table A2.2. Mapped read metrics observed per sample.

[3]Adapted from the data analysis report provided by EuroFins GATC Biotech GmbH, Germany.

3. Alignment Classification:

The alignment classification table includes the following read categories:

•Mapped: Reads mapped to reference.

• Unique: Reads mapped to exactly one site on the reference.

• Non-unique: Reads mapped to more than one site on the reference.

• Singletons: Mapped reads without mates (read not paired).

• Cross-Contig: Read pairs with the mate mapped to a different contig.

• On target: Reads mapped to target +/- 100 bp extension.

Percentage of reads in Non-unique, Unique, Singletons, Cross-Contig were calculated based on the number of reads mapping to entire reference, while percentage of reads in On target was calculated based on the number of reads mapped uniquely.

Read category	M43	M44	M46
Mapped	78,049,206	75,169,466	92,756,897
Unique	75,039,729 (96.14%)	72,231,364 (96.09%)	89,101,891 (96.06%)
Non-unique	3,009,477 (3.86%)	2,938,102 (3.91%)	3,655,006 (3.94%)
Singletons	6,958 (0.01%)	6,003 (0.01%)	8,957 (0.01%)
Cross-Contig	297,274 (0.38%)	259,995 (0.35%)	299,031 (0.32%)
On target	66,947,146 (89.58%)	65,767,266 (91.39%)	79,422,475 (89.45%)

Table A2.3a Read metrics for M43, M44 and M46. M43 and M44 are patient identification code.

Read category	M47	M48
Mapped	84,731,342	87,717,524
Unique	81,424,207 (96.10%)	84,298,411 (96.10%)
Non-unique	3,307,135 (3.90%)	3,419,113 (3.90%)
Singletons	6,305 (0.01%)	9,145 (0.01%)
Cross-Contig	232,288 (0.27%)	235,797 (0.27%)
On target	73,829,169 (90.94%)	76,546,637 (91.07%)

Table A2.3b Read metrics for M47 and M48. M47and M48 are patient identification code.

4. Alignment Refinement Metrics

This includes removal of PCR duplicates to remove the artificial coverage and local realignment to transform regions with misalignments caused by indels. The number of high-quality reads after read mapping, alignment and refinement is shown in table A2.4.

Sample	Input Reads	Duplicate Reads	HQ Reads
M43	66,947,146	16,275,396 (24.31%)	50,671,750 (75.69%)
M44	65,767,266	14,337,336 (21.80%)	51,429,930 (78.20%)
M46	79,422,475	20,256,398 (25.50%)	59,166,077 (74.50%)
M47	73,829,169	18,310,924 (24.80%)	55,518,245 (75.20%)
M48	76,546,637	18,663,744 (24.38%)	57,882,893 (75.62%)

Table A2.4 High quality aligned reads per sample. M43, M44, M46, M47 and M48 are patient identification code.

Coverage Report

	Target coverage		% of target covered with at least							
Sample	total bases	average (×)	2×	5×	10×	20×	30×	60×	90×	120×
M43	5.82 GB	95.98	98.0	97.7	97.2	95.6	92.8	73.2	46.2	26.0
M44	5.90 GB	97.34	98.0	97.6	97.2	95.6	92.9	74.0	47.5	27.0
M46	6.80 GB	112.1	98.0	97.8	97.4	96.2	94.3	80.7	57.9	36.6
M47	6.36 GB	104.82	98.0	97.7	97.3	95.9	93.7	78.3	53.6	32.0
M48	6.63 GB	109.36	98.2	97.9	97.5	96.2	94.2	79.7	56.3	35.0

Table A2.5 Depth of coverage summary (excluding duplicated fragments). M43, M44, M46, M47 and M48 are patient identification code.

B. Second WES batch[4]

Statistics of mapping, coverage and depth

Sample	P3	P9	P12	P15	P18	N36
Total	105446718 (100%)	113694722 (100%)	102511730 (100%)	99820918 (100%)	98648972 (100%)	120677790 (100%)
Duplicate	28459785 (27.04%)	31805531 (28.01%)	28073552 (27.43%)	27212435 (27.31%)	25078322 (25.48%)	35075039 (29.11%)
Mapped	105257887 (99.82%)	113557088 (99.88%)	102341805 (99.83%)	99633728 (99.81%)	98419137 (99.77%)	120486346 (99.84%)
Properly mapped	104620704 (99.22%)	112838156 (99.25%)	101674642 (99.18%)	98976862 (99.15%)	97754512 (99.09%)	119710820 (99.20%)
PE mapped	105138560 (99.71%)	113453748 (99.79%)	102220014 (99.72%)	99496788 (99.68%)	98274904 (99.62%)	120355286 (99.73%)
SE mapped	238654 (0.23%)	206680 (0.18%)	243582 (0.24%)	273880 (0.27%)	288466 (0.29%)	262120 (0.22%)
With mate mapped to a different chr	448322 (0.43%)	545446 (0.48%)	479348 (0.47%)	454330 (0.46%)	455642 (0.46%)	562714 (0.47%)
With mate mapped to a different chr ((mapQ>=5))	378453 (0.36%)	471436 (0.41%)	412262 (0.40%)	386017 (0.39%)	389179 (0.39%)	482870 (0.40%)
Initial bases on target	60303837	60303837	60303837	60303837	60303837	60303837
Initial bases on/near target	135790180	135790180	135790180	135790180	135790180	135790180
Total effective yield (Mb)	15717.79	16958.4	15282.31	14874.43	14691.38	17990.72
Effective yield on target (Mb)	11125.93	12154.8	10843.73	10532.24	10427.19	12896.32
Fraction of effective bases on target	70.80%	71.70%	71.00%	70.80%	71.00%	71.70%
Fraction of effective bases on/or near target	90.40%	90.80%	90.60%	89.80%	89.90%	90.60%

Table A2.6 Statistics of mapping in each sample. P3, P9, P12, P15, P18 and N36 are patient identification code.

[4]Adapted from the data analysis report provided by Novogene Co., Ltd (China).

	Target Coverage			Fraction of target covered with at least				
Sample	Average depth	Bases covered	% Coverage	100x	50x	20x	10x	4x
P3	184.5	60113201	99.70%	68.60%	88.70%	96.90%	98.70%	99.40%
P9	201.56	59972507	99.50%	72.50%	90.00%	97.00%	98.50%	99.20%
P12	179.82	59967343	99.40%	69.50%	89.00%	96.80%	98.50%	99.20%
P15	174.65	60100677	99.70%	68.00%	88.40%	96.80%	98.60%	99.40%
P18	172.91	60104970	99.70%	65.60%	87.30%	96.60%	98.50%	99.40%
N36	213.86	59970468	99.40%	74.90%	90.90%	97.20%	98.60%	99.20%

Table A2. 7 Statistics of coverage and depth in each sample. P3, P9, P12, P15, P18 and N36 are patient identification code.

Note:

Total: The total number of clean reads. Duplicate: The number of duplicated reads (percentage: duplicated reads/clean reads). Mapped: The number of reads that mapped to the reference genome (percentage). Properly mapped: The number of reads that mapped to the reference genome and within the expected insert size (percentage). PE mapped: The number of pair-end reads that mapped to the reference genome (percentage). SE mapped: The number of single-end reads that mapped to the reference genome (percentage). With mate mapped to a different chr: The number of reads with mate reads mapped to diverse chromosomes (percentage). With mate mapped to a different chr (mapQ >= 5): The number of reads with mate reads mapped to diverse chromosomes and the MAQ > 5. Initial bases on target: The number of bases in the target region. Initial bases on/ near target: The number of bases in the target region or flanking region of the target. Total effective yield (Mb): The data size of the effective reads mapped to the reference genome (MB as a unit). Effective yield on target (Mb): The data size of the reads mapped to the target region. Fraction of effective bases on target: The percentage of bases mapped to the target region in all bases mapped to the reference genome. (Effective sequences on target/Total effective yield). Fraction of effective bases on/ near target: The percentage of bases mapped to the target or flanking region in all bases mapped to the reference

genome. Average sequencing depth on target: The average sequencing depth in the target region (Effective sequences on target * 1 million/Initial bases on target). Bases covered on target: The number of the bases covered in the target region. Coverage of target region: The coverage percentage of target regions (Base covered on target/Initial bases on target).

References

Adams, M., Smith, U. M., Logan, C. V., & Johnson, C. A. (2008). Recent advances in the molecular pathology, cell biology and genetics of ciliopathies. *Journal of Medical Genetics, 45*(5), 257-267. doi:10.1136/jmg.2007.054999

Adel Al-Lami, H., Barrell, W. B., & Liu, K. J. (2016). Micrognathia in mouse models of ciliopathies. *Biochem Soc Trans, 44*(6), 1753-1759. doi:10.1042/BST20160241

Adeva, M., El-Youssef, M., Rossetti, S., Kamath, P. S., Kubly, V., Consugar, M. B., . . . Harris, P. C. (2006). Clinical and molecular characterization defines a broadened spectrum of autosomal recessive polycystic kidney disease (ARPKD). *Medicine (Baltimore), 85*(1), 1-21. doi:10.1097/01.md.0000200165.90373.9a

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., . . . Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. *Nat Methods, 7*(4), 248-249. doi:10.1038/nmeth0410-248

Afzal, A. R., Rajab, A., Fenske, C. D., Oldridge, M., Elanko, N., Ternes-Pereira, E., . . . Jeffery, S. (2000). Recessive Robinow syndrome, allelic to dominant brachydactyly type B, is caused by mutation of ROR2. *Nat Genet, 25*(4), 419-422. doi:10.1038/78107

Aguiari, G., Varani, K., Bogo, M., Mangolini, A., Vincenzi, F., Durante, C., . . . Del Senno, L. (2009). Deficiency of polycystic kidney disease-1 gene (PKD1) expression increases A(3) adenosine receptors in human renal cells: implications for cAMP-dependent signalling and proliferation of PKD1-mutated cystic cells. *Biochim Biophys Acta, 1792*(6), 531-540. doi:10.1016/j.bbadis.2009.03.001

Aguilar, A. (2016). Ciliopathies: CPLANE regulates intraflagellar transport. *Nat Rev Nephrol, 12*(7), 376. doi:10.1038/nrneph.2016.74

Aguilar, A. (2016). Ciliopathies: Inpp5e links lipids, cysts and cilia. *Nat Rev Nephrol, 12*(9), 508. doi:10.1038/nrneph.2016.120

Akoh, J. A. (2015). Current management of autosomal dominant polycystic kidney disease. *World J Nephrol, 4*(4), 468-479. doi:10.5527/wjn.v4.i4.468

Al Alawi, I., Al Salmi, I., Al Mawali, A., Al Maimani, Y., & Sayer, J. A. (2017a). End-Stage Kidney Failure in Oman: An Analysis of Registry Data with an Emphasis on Congenital and Inherited Renal Diseases. *Int J Nephrol, 2017*, 6403985. doi:10.1155/2017/6403985

Al Alawi, I. H., Al Salmi, I., Al Mawali, A., & Sayer, J. A. (2017b). Kidney Disease in Oman: a View of the Current and Future Landscapes. *Iran J Kidney Dis, 11*(4), 263-270.

Al Alawi, I., Al Salmi, I., Al Rahbi, F., Al Riyami, M., Al Kalbani, N., Al Ghaithi, B., . . . Sayer, J. A. (2019). Molecular Genetic Diagnosis of Omani Patients With Inherited Cystic Kidney Disease. *Kidney International Reports*. doi:10.1016/j.ekir.2019.08.012

Al Ismaili, F., Al Salmi, I., Al Maimani, Y., Metry, A. M., Al Marhoobi, H., Hola, A., & Pisoni, R. L. (2017). Epidemiological Transition of End-Stage Kidney Disease in Oman. *Kidney Int Rep, 2*(1), 27-35. doi:10.1016/j.ekir.2016.09.001

Al Riyami, M. S., Al Shehhi, M., Al Sulaimi, T., Al Mamary, L., Al Maskari, A., Al Ghaithi, B., . . . Al Saidi, S. (2019). Epidemiology and Outcome of CKD in Omani Children. *Kidney Int Rep, 4*(5), 727-732. doi:10.1016/j.ekir.2019.02.014

Alashek, W. A., McIntyre, C. W., & Taal, M. W. (2013). Determinants of survival in patients receiving dialysis in Libya. *Hemodial Int, 17*(2), 249-255. doi:10.1111/j.1542-4758.2012.00728.x

Alby, C., Piquand, K., Huber, C., Megarbane, A., Ichkou, A., Legendre, M., . . . Thomas, S. (2015). Mutations in KIAA0586 Cause Lethal Ciliopathies Ranging from a Hydrolethalus Phenotype to Short-Rib Polydactyly Syndrome. *Am J Hum Genet, 97*(2), 311-318. doi:10.1016/j.ajhg.2015.06.003

Al-Gazali, L., Hamamy, H., & Al-Arrayad, S. (2006). Genetic disorders in the Arab world. *BMJ, 333*(7573), 831-834. doi:10.1136/bmj.38982.704931.AE

Al-Hamed, M. H., Kurdi, W., Alsahan, N., Alabdullah, Z., Abudraz, R., Tulbah, M., . . . Albaqumi, M. (2016). Genetic spectrum of Saudi Arabian patients with antenatal cystic kidney disease and ciliopathy phenotypes using a targeted renal gene panel. *J Med Genet, 53*(5), 338-347. doi:10.1136/jmedgenet-2015-103469

Al-Hamed, M. H., Alsahan, N., Rice, S. J., Edwards, N., Nooreddeen, E., Alotaibi, M., . . . Imtiaz, F. (2019). Bialleleic PKD1 mutations underlie early-onset autosomal dominant polycystic kidney disease in Saudi Arabian families. *Pediatr Nephrol, 34*(9), 1615-1623. doi:10.1007/s00467-019-04267-x

Alkanderi, S., Yates, L. M., Johnson, S. A., & Sayer, J. A. (2017). Lessons learned from a multidisciplinary renal genetics clinic. *QJM, 110*(7), 453-457. doi:10.1093/qjmed/hcx030

Al-Lawati, T. T. (2013). Fibropolycystic disease of the liver and kidney in Oman. *Arab J Gastroenterol, 14*(4), 173-175. doi:10.1016/j.ajg.2013.11.004

Allison, S. J. (2015). Ciliopathies: Replication stress-induced DNA damage in renal ciliopathies. *Nat Rev Nephrol, 11*(11), 632. doi:10.1038/nrneph.2015.151

Al-Marhoon, M. S., Bayoumi, R., Al-Farsi, Y., Al-Hinai, A., Al-Maskary, S., Venkiteswaran, K., . . . Al-Hashmi, I. (2015). Urinary stone composition in Oman: with high incidence of cystinuria. *Urolithiasis, 43*(3), 207-211. doi:10.1007/s00240-015-0763-7

Al-Marhuby, H. (1998). Renal replacement therapy in sultanate of oman. *Saudi J Kidney Dis Transpl, 9*(4), 459-460.

Al-Sayyari, A. A., & Shaheen, F. A. (2011). End stage chronic kidney disease in Saudi Arabia. A rapidly changing scene. *Saudi Med J, 32*(4), 339-346.

Anvarian, Z., Mykytyn, K., Mukhopadhyay, S., Pedersen, L. B., & Christensen, S. T. (2019). Cellular signalling by primary cilia in development, organ function and disease. *Nat Rev Nephrol, 15*(4), 199-219. doi:10.1038/s41581-019-0116-9

Armengot Carceller, M., Mata Roig, M., Milara Paya, X., & Cortijo Gimeno, J. (2010). [Primary ciliary dyskinesia. Ciliopathies]. *Acta Otorrinolaringol Esp, 61*(2), 149-159. doi:10.1016/j.otorri.2009.01.013

Armengot, M., Salom, D., Diaz-Llopis, M., Millan, J. M., Milara, J., Mata, M., & Cortijo, J. (2012). Nasal ciliary beat frequency and beat pattern in retinal ciliopathies. *Invest Ophthalmol Vis Sci, 53*(4), 2076-2079. doi:10.1167/iovs.11-8666

Arnaiz, O., Cohen, J., Tassin, A. M., & Koll, F. (2014). Remodeling Cildb, a popular database for cilia and links for ciliopathies. *Cilia, 3*, 9. doi:10.1186/2046-2530-3-9

Arts, H. H., & Knoers, N. V. (2013). Current insights into renal ciliopathies: what can genetics teach us? *Pediatr Nephrol, 28*(6), 863-874. doi:10.1007/s00467-012-2259-9

Asadollahi, R., Strauss, J. E., Zenker, M., Beuing, O., Edvardson, S., Elpeleg, O., . . . Rauch, A. (2018). Clinical and experimental evidence suggest a link between KIF7 and C5orf42-related ciliopathies through Sonic Hedgehog signaling. *Eur J Hum Genet, 26*(2), 197-209. doi:10.1038/s41431-017-0019-9

Ashe, A., Butterfield, N. C., Town, L., Courtney, A. D., Cooper, A. N., Ferguson, C., . . . Wicking, C. (2012). Mutations in mouse Ift144 model the craniofacial, limb and

rib defects in skeletal ciliopathies. *Hum Mol Genet, 21*(8), 1808-1823. doi:10.1093/hmg/ddr613
Attanasio, M. (2015). Ciliopathies and DNA damage: an emerging nexus. *Curr Opin Nephrol Hypertens, 24*(4), 366-370. doi:10.1097/MNH.0000000000000134
Audrezet, M. P., Cornec-Le Gall, E., Chen, J. M., Redon, S., Quere, I., Creff, J., . . . Ferec, C. (2012). Autosomal dominant polycystic kidney disease: comprehensive mutation analysis of PKD1 and PKD2 in 700 unrelated patients. *Hum Mutat, 33*(8), 1239-1250. doi:10.1002/humu.22103
Australian and New Zealand Dialysis and Transplant Registry (ANZDATA). (2012). The 35th Annual ANZDATA Report (2012). Retrieved from https://www.anzdata.org.au/report/anzdata-35th-annual-report-2012/
Ayme, S., Bockenhauer, D., Day, S., Devuyst, O., Guay-Woodford, L. M., Ingelfinger, J. R., . . . Winkelmayer, W. C. (2017). Common Elements in Rare Kidney Diseases: Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int, 92*(4), 796-808. doi:10.1016/j.kint.2017.06.018
Bachmann-Gagescu, R. (2014). [Genetic complexity of ciliopathies and novel genes identification]. *Med Sci (Paris), 30*(11), 1011-1023. doi:10.1051/medsci/20143011016
Bachmann-Gagescu, R., Phelps, I. G., Stearns, G., Link, B. A., Brockerhoff, S. E., Moens, C. B., & Doherty, D. (2011). The ciliopathy gene cc2d2a controls zebrafish photoreceptor outer segment development through a role in Rab8-dependent vesicle trafficking. *Hum Mol Genet, 20*(20), 4041-4055. doi:10.1093/hmg/ddr332
Badano, J. L., Mitsuma, N., Beales, P. L., & Katsanis, N. (2006). The Ciliopathies: An Emerging Class of Human Genetic Disorders. *Annual Review of Genomics and Human Genetics, 7*(1), 125-148. doi:10.1146/annurev.genom.7.080505.115610
Badano, J. L., Mitsuma, N., Beales, P. L., & Katsanis, N. (2006). The ciliopathies: an emerging class of human genetic disorders. *Annu Rev Genomics Hum Genet, 7*, 125-148. doi:10.1146/annurev.genom.7.080505.115610
Baker, K., & Beales, P. L. (2009). Making sense of cilia in disease: the human ciliopathies. *Am J Med Genet C Semin Med Genet, 151C*(4), 281-295. doi:10.1002/ajmg.c.30231
Bastos, A. P., & Onuchic, L. F. (2011). Molecular and cellular pathogenesis of autosomal dominant polycystic kidney disease. *Braz J Med Biol Res, 44*(7), 606-617. doi:10.1590/s0100-879x2011007500068
Batieha, A., Abdallah, S., Maghaireh, M., Awad, Z., Al-Akash, N., Batieneh, A., & Ajlouni, K. A. (2007). Epidemiology and cost of haemodialysis in Jordan. *East Mediterr Health J, 13*(3), 654-663.
Beisson, J., & Wright, M. (2003). Basal body/centriole assembly and continuity. *Curr Opin Cell Biol, 15*(1), 96-104. doi:10.1016/s0955-0674(02)00017-0
Belkadi, A., Pedergnana, V., Cobat, A., Itan, Y., Vincent, Q. B., Abhyankar, A., . . . Abel, L. (2016). Whole-exome sequencing to analyze population structure, parental inbreeding, and familial linkage. *Proc Natl Acad Sci U S A, 113*(24), 6713-6718. doi:10.1073/pnas.1606460113
Bellanne-Chantelot, C., Chauveau, D., Gautier, J. F., Dubois-Laforgue, D., Clauin, S., Beaufils, S., . . . Timsit, J. (2004). Clinical spectrum associated with hepatocyte nuclear factor-1beta mutations. *Ann Intern Med, 140*(7), 510-517. doi:10.7326/0003-4819-140-7-200404060-00009

Benmerah, A., Durand, B., Giles, R. H., Harris, T., Kohl, L., Laclef, C., . . . Bastin, P. (2015). The more we know, the more we have to discover: an exciting future for understanding cilia and ciliopathies. *Cilia, 4*, 5. doi:10.1186/s13630-015-0014-0

Berbari, N. F., O'Connor, A. K., Haycraft, C. J., & Yoder, B. K. (2009). The primary cilium as a complex signaling center. *Curr Biol, 19*(13), R526-535. doi:10.1016/j.cub.2009.05.025

Bergboer, J. G. M., Wyatt, C., Austin-Tse, C., Yaksi, E., & Drummond, I. A. (2018). Assaying sensory ciliopathies using calcium biosensor expression in zebrafish ciliated olfactory neurons. *Cilia, 7*, 2. doi:10.1186/s13630-018-0056-1

Bergmann, C. (2012). Educational paper: ciliopathies. *Eur J Pediatr, 171*(9), 1285-1300. doi:10.1007/s00431-011-1553-z

Bergmann, C. (2017). Genetics of Autosomal Recessive Polycystic Kidney Disease and Its Differential Diagnoses. *Front Pediatr, 5*, 221. doi:10.3389/fped.2017.00221

Bergmann, C. (2017). Genetics of Autosomal Recessive Polycystic Kidney Disease and Its Differential Diagnoses. *Front Pediatr, 5*, 221. doi:10.3389/fped.2017.00221

Bergmann, C. (2019). Early and Severe Polycystic Kidney Disease and Related Ciliopathies: An Emerging Field of Interest. *Nephron, 141*(1), 50-60. doi:10.1159/000493532

Bergmann, C., Bruchle, N. O., Frank, V., Rehder, H., & Zerres, K. (2008). Perinatal deaths in a family with autosomal dominant polycystic kidney disease and a PKD2 mutation. *N Engl J Med, 359*(3), 318-319. doi:10.1056/NEJMc0801868

Bergmann, C., Guay-Woodford, L. M., Harris, P. C., Horie, S., Peters, D. J. M., & Torres, V. E. (2018). Polycystic kidney disease. *Nat Rev Dis Primers, 4*(1), 50. doi:10.1038/s41572-018-0047-y

Bergmann, C., Senderek, J., Schneider, F., Dornia, C., Kupper, F., Eggermann, T., . . . Zerres, K. (2004). PKHD1 mutations in families requesting prenatal diagnosis for autosomal recessive polycystic kidney disease (ARPKD). *Hum Mutat, 23*(5), 487-495. doi:10.1002/humu.20019

Bergmann, C., Senderek, J., Sedlacek, B., Pegiazoglou, I., Puglia, P., Eggermann, T., . . . Zerres, K. (2003). Spectrum of mutations in the gene for autosomal recessive polycystic kidney disease (ARPKD/PKHD1). *J Am Soc Nephrol, 14*(1), 76-89. doi:10.1097/01.asn.0000039578.55705.6e

Bergmann, C., Senderek, J., Windelen, E., Kupper, F., Middeldorf, I., Schneider, F., . . . Zerres, K. (2005). Clinical consequences of PKHD1 mutations in 164 patients with autosomal-recessive polycystic kidney disease (ARPKD). *Kidney Int, 67*(3), 829-848. doi:10.1111/j.1523-1755.2005.00148.x

Bergmann, C., von Bothmer, J., Ortiz Bruchle, N., Venghaus, A., Frank, V., Fehrenbach, H., . . . Zerres, K. (2011). Mutations in multiple PKD genes may explain early and severe polycystic kidney disease. *J Am Soc Nephrol, 22*(11), 2047-2056. doi:10.1681/asn.2010101080

Bhogaraju, S., Engel, B. D., & Lorentzen, E. (2013). Intraflagellar transport complex structure and cargo interactions. *Cilia, 2*(1), 10. doi:10.1186/2046-2530-2-10

Bissler, J. J., & Christopher Kingswood, J. (2018). Renal manifestation of tuberous sclerosis complex. *Am J Med Genet C Semin Med Genet, 178*(3), 338-347. doi:10.1002/ajmg.c.31654

Blasius, T. L., Takao, D., & Verhey, K. J. (2019). NPHP proteins are binding partners of nucleoporins at the base of the primary cilium. *PLoS One, 14*(9), e0222924. doi:10.1371/journal.pone.0222924

Bloodgood, R. A. (2009). From central to rudimentary to primary: the history of an underappreciated organelle whose time has come. The primary cilium. *Methods Cell Biol, 94*, 3-52. doi:10.1016/s0091-679x(08)94001-2

Boycott, K. M., Rath, A., Chong, J. X., Hartley, T., Alkuraya, F. S., Baynam, G., . . . Lochmuller, H. (2017). International Cooperation to Enable the Diagnosis of All Rare Genetic Diseases. *Am J Hum Genet, 100*(5), 695-705. doi:10.1016/j.ajhg.2017.04.003

Boyle, B., Addor, M. C., Arriola, L., Barisic, I., Bianchi, F., Csáky-Szunyogh, M., . . . Dolk, H. (2018). Estimating Global Burden of Disease due to congenital anomaly: an analysis of European data. *Arch Dis Child Fetal Neonatal Ed, 103*(1), F22-f28. doi:10.1136/archdischild-2016-311845

Brancati, F., Camerota, L., Colao, E., Vega-Warner, V., Zhao, X., Zhang, R., . . . Otto, E. A. (2018). Biallelic variants in the ciliary gene TMEM67 cause RHYNS syndrome. *Eur J Hum Genet, 26*(9), 1266-1271. doi:10.1038/s41431-018-0183-6

Brancati, F., Iannicelli, M., Travaglini, L., Mazzotta, A., Bertini, E., Boltshauser, E., . . . Valente, E. M. (2009). MKS3/TMEM67 mutations are a major cause of COACH Syndrome, a Joubert Syndrome related disorder with liver involvement. *Hum Mutat, 30*(2), E432-442. doi:10.1002/humu.20924

Braun, D. A., & Hildebrandt, F. (2017). Ciliopathies. *Cold Spring Harb Perspect Biol, 9*(3). doi:10.1101/cshperspect.a028191

Braun, D. A., Schueler, M., Halbritter, J., Gee, H. Y., Porath, J. D., Lawson, J. A., . . . Hildebrandt, F. (2016). Whole exome sequencing identifies causative mutations in the majority of consanguineous or familial cases with childhood-onset increased renal echogenicity. *Kidney Int, 89*(2), 468-475. doi:10.1038/ki.2015.317

Bredrup, C., Saunier, S., Oud, M. M., Fiskerstrand, T., Hoischen, A., Brackman, D., . . . Arts, H. H. (2011). Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the IFT-A gene WDR19. *Am J Hum Genet, 89*(5), 634-643. doi:10.1016/j.ajhg.2011.10.001

Breslow, D. K., Hoogendoorn, S., Kopp, A. R., Morgens, D. W., Vu, B. K., Kennedy, M. C., . . . Nachury, M. V. (2018). A CRISPR-based screen for Hedgehog signaling provides insights into ciliary function and ciliopathies. *Nat Genet, 50*(3), 460-471. doi:10.1038/s41588-018-0054-7

Brugmann, S. A., Cordero, D. R., & Helms, J. A. (2010). Craniofacial ciliopathies: A new classification for craniofacial disorders. *Am J Med Genet A, 152A*(12), 2995-3006. doi:10.1002/ajmg.a.33727

Brun, A., Yu, X., Obringer, C., Ajoy, D., Haser, E., Stoetzel, C., . . . Marion, V. (2019). In vivo phenotypic and molecular characterization of retinal degeneration in mouse models of three ciliopathies. *Exp Eye Res, 186*, 107721. doi:10.1016/j.exer.2019.107721

Budny, B., Chen, W., Omran, H., Fliegauf, M., Tzschach, A., Wisniewska, M., . . . Ropers, H. H. (2006). A novel X-linked recessive mental retardation syndrome comprising macrocephaly and ciliary dysfunction is allelic to oral-facial-digital type I syndrome. *Hum Genet, 120*(2), 171-178. doi:10.1007/s00439-006-0210-5

Bujakowska, K. M., Liu, Q., & Pierce, E. A. (2017). Photoreceptor Cilia and Retinal Ciliopathies. *Cold Spring Harb Perspect Biol, 9*(10). doi:10.1101/cshperspect.a028274

Bullich, G., Domingo-Gallego, A., Vargas, I., Ruiz, P., Lorente-Grandoso, L., Furlano, M., . . . Ars, E. (2018). A kidney-disease gene panel allows a comprehensive genetic diagnosis of cystic and glomerular inherited kidney diseases. *Kidney Int, 94*(2), 363-371. doi:10.1016/j.kint.2018.02.027

Burgmaier, K., Kilian, S., Bammens, B., Benzing, T., Billing, H., Buscher, A., . . . Liebau, M. C. (2019). Clinical courses and complications of young adults with Autosomal Recessive Polycystic Kidney Disease (ARPKD). *Sci Rep, 9*(1), 7919. doi:10.1038/s41598-019-43488-w

Buscher, R., Buscher, A. K., Weber, S., Mohr, J., Hegen, B., Vester, U., & Hoyer, P. F. (2014). Clinical manifestations of autosomal recessive polycystic kidney disease (ARPKD): kidney-related and non-kidney-related phenotypes. *Pediatr Nephrol, 29*(10), 1915-1925. doi:10.1007/s00467-013-2634-1

Cabezas, O. R., Flanagan, S. E., Stanescu, H., García-Martínez, E., Caswell, R., Lango-Allen, H., . . . Bockenhauer, D. (2017). Polycystic Kidney Disease with Hyperinsulinemic Hypoglycemia Caused by a Promoter Mutation in Phosphomannomutase 2. *J Am Soc Nephrol, 28*(8), 2529-2539. doi:10.1681/asn.2016121312

Cantagrel, V., Silhavy, J. L., Bielas, S. L., Swistun, D., Marsh, S. E., Bertrand, J. Y., . . . Gleeson, J. G. (2008). Mutations in the cilia gene ARL13B lead to the classical form of Joubert syndrome. *Am J Hum Genet, 83*(2), 170-179. doi:10.1016/j.ajhg.2008.06.023

Capisonda, R., Phan, V., Traubuci, J., Daneman, A., Balfe, J. W., & Guay-Woodford, L. M. (2003). Autosomal recessive polycystic kidney disease: outcomes from a single-center experience. *Pediatr Nephrol, 18*(2), 119-126. doi:10.1007/s00467-002-1021-0

Cardenas-Rodriguez, M., & Badano, J. L. (2009). Ciliary biology: understanding the cellular and genetic basis of human ciliopathies. *Am J Med Genet C Semin Med Genet, 151C*(4), 263-280. doi:10.1002/ajmg.c.30227

Carson, A. R., Smith, E. N., Matsui, H., Braekkan, S. K., Jepsen, K., Hansen, J. B., & Frazer, K. A. (2014). Effective filtering strategies to improve data quality from population-based whole exome sequencing studies. *BMC Bioinformatics, 15*, 125. doi:10.1186/1471-2105-15-125

Chaki, M., Airik, R., Ghosh, A. K., Giles, R. H., Chen, R., Slaats, G. G., . . . Hildebrandt, F. (2012). Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. *Cell, 150*(3), 533-548. doi:10.1016/j.cell.2012.06.028

Chaki, M., Hoefele, J., Allen, S. J., Ramaswami, G., Janssen, S., Bergmann, C., . . . Hildebrandt, F. (2011). Genotype-phenotype correlation in 440 patients with NPHP-related ciliopathies. *Kidney Int, 80*(11), 1239-1245. doi:10.1038/ki.2011.284

Chang, C. F., Chang, Y. T., Millington, G., & Brugmann, S. A. (2016). Craniofacial Ciliopathies Reveal Specific Requirements for GLI Proteins during Development of the Facial Midline. *PLoS Genet, 12*(11), e1006351. doi:10.1371/journal.pgen.1006351

Chapman, J. R., & Wong, G. (2016). Cancer in patients with inherited ciliopathies: polycystic kidney disease. *Lancet Oncol, 17*(10), 1343-1345. doi:10.1016/S1470-2045(16)30307-2

Chebib, F. T., Sussman, C. R., Wang, X., Harris, P. C., & Torres, V. E. (2015). Vasopressin and disruption of calcium signalling in polycystic kidney disease. *Nat Rev Nephrol, 11*(8), 451-464. doi:10.1038/nrneph.2015.39

Chen, X., Wang, X., Jiang, C., Xu, M., Liu, Y., Qi, R., . . . Zhao, C. (2018). IFT52 as a Novel Candidate for Ciliopathies Involving Retinal Degeneration. *Invest Ophthalmol Vis Sci, 59*(11), 4581-4589. doi:10.1167/iovs.17-23351

Choi, H. J., Lin, J. R., Vannier, J. B., Slaats, G. G., Kile, A. C., Paulsen, R. D., . . . Cimprich, K. A. (2013). NEK8 links the ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies. *Mol Cell, 51*(4), 423-439. doi:10.1016/j.molcel.2013.08.006

Chun, S., & Fay, J. C. (2009). Identification of deleterious mutations within three human genomes. *Genome Res, 19*(9), 1553-1561. doi:10.1101/gr.092619.109

Chung, E. M., Conran, R. M., Schroeder, J. W., Rohena-Quinquilla, I. R., & Rooks, V. J. (2014). From the radiologic pathology archives: pediatric polycystic kidney disease and other ciliopathies: radiologic-pathologic correlation. *Radiographics, 34*(1), 155-178. doi:10.1148/rg.341135179

Clarke, A. J. (2014). Managing the ethical challenges of next-generation sequencing in genomic medicine. *Br Med Bull, 111*(1), 17-30. doi:10.1093/bmb/ldu017

Collin, G. B., Cyr, E., Bronson, R., Marshall, J. D., Gifford, E. J., Hicks, W., . . . Naggert, J. K. (2005). Alms1-disrupted mice recapitulate human Alstrom syndrome. *Hum Mol Genet, 14*(16), 2323-2333. doi:10.1093/hmg/ddi235

Conduit, S. E., Dyson, J. M., & Mitchell, C. A. (2012). Inositol polyphosphate 5-phosphatases; new players in the regulation of cilia and ciliopathies. *FEBS Lett, 586*(18), 2846-2857. doi:10.1016/j.febslet.2012.07.037

Connaughton, D. M., Kennedy, C., Shril, S., Mann, N., Murray, S. L., Williams, P. A., . . . Hildebrandt, F. (2019). Monogenic causes of chronic kidney disease in adults. *Kidney Int, 95*(4), 914-928. doi:10.1016/j.kint.2018.10.031

Connor, T. M., Oygar, D. D., Gale, D. P., Steenkamp, R., Nitsch, D., Neild, G. H., & Maxwell, P. H. (2013). Incidence of end-stage renal disease in the Turkish-Cypriot population of Northern Cyprus: a population based study. *PLoS One, 8*(1), e54394. doi:10.1371/journal.pone.0054394

Coppieters, F., Lefever, S., Leroy, B. P., & De Baere, E. (2010). CEP290, a gene with many faces: mutation overview and presentation of CEP290base. *Hum Mutat, 31*(10), 1097-1108. doi:10.1002/humu.21337

Cornec-Le Gall, E., Audrezet, M. P., Rousseau, A., Hourmant, M., Renaudineau, E., Charasse, C., . . . Le Meur, Y. (2016). The PROPKD Score: A New Algorithm to Predict Renal Survival in Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol, 27*(3), 942-951. doi:10.1681/asn.2015010016

Cornec-Le Gall, E., Olson, R. J., Besse, W., Heyer, C. M., Gainullin, V. G., Smith, J. M., . . . Harris, P. C. (2018). Monoallelic Mutations to DNAJB11 Cause Atypical Autosomal-Dominant Polycystic Kidney Disease. *Am J Hum Genet, 102*(5), 832-844. doi:10.1016/j.ajhg.2018.03.013

Cornec-Le Gall, E., Torres, V. E., & Harris, P. C. (2018). Genetic Complexity of Autosomal Dominant Polycystic Kidney and Liver Diseases. *J Am Soc Nephrol, 29*(1), 13-23. doi:10.1681/asn.2017050483

Cornillie, F. J., Lauweryns, J. M., & Corbeel, L. (1984). Atypical bronchial cilia in children with recurrent respiratory tract infections. A comparative ultrastructural study. *Pathol Res Pract, 178*(6), 595-604. doi:10.1016/s0344-0338(84)80093-x

Cortes, C. R., Metzis, V., & Wicking, C. (2015). Unmasking the ciliopathies: craniofacial defects and the primary cilium. *Wiley Interdiscip Rev Dev Biol, 4*(6), 637-653. doi:10.1002/wdev.199

Crews, D. C., Bello, A. K., & Saadi, G. (2019). Burden, access, and disparities in kidney disease. *Braz J Med Biol Res, 52*(3), e8338. doi:10.1590/1414-431x20198338

D'Angelo, A., & Franco, B. (2009). The dynamic cilium in human diseases. *Pathogenetics, 2*(1), 3. doi:10.1186/1755-8417-2-3

Davenport, J. R., & Yoder, B. K. (2005). An incredible decade for the primary cilium: a look at a once-forgotten organelle. *Am J Physiol Renal Physiol, 289*(6), F1159-1169. doi:10.1152/ajprenal.00118.2005

Davis, E. E., & Katsanis, N. (2012). The ciliopathies: a transitional model into systems biology of human genetic disease. *Curr Opin Genet Dev, 22*(3), 290-303. doi:10.1016/j.gde.2012.04.006

Davydov, E. V., Goode, D. L., Sirota, M., Cooper, G. M., Sidow, A., & Batzoglou, S. (2010). Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput Biol, 6*(12), e1001025. doi:10.1371/journal.pcbi.1001025

de Haan, A., Eijgelsheim, M., Vogt, L., Knoers, N., & de Borst, M. H. (2019). Diagnostic Yield of Next-Generation Sequencing in Patients With Chronic Kidney Disease of Unknown Etiology. *Front Genet, 10*, 1264. doi:10.3389/fgene.2019.01264

Delgado-Escueta, A. V. (2007). Advances in genetics of juvenile myoclonic epilepsies. *Epilepsy Curr, 7*(3), 61-67. doi:10.1111/j.1535-7511.2007.00171.x

Denamur, E., Delezoide, A. L., Alberti, C., Bourillon, A., Gubler, M. C., Bouvier, R., . . . Wann, A. R. (2010). Genotype-phenotype correlations in fetuses and neonates with autosomal recessive polycystic kidney disease. *Kidney Int, 77*(4), 350-358. doi:10.1038/ki.2009.440

Devlin, L. A., & Sayer, J. A. (2019). Renal ciliopathies. *Curr Opin Genet Dev, 56*, 49-60. doi:10.1016/j.gde.2019.07.005

Devuyst, O., Knoers, N. V., Remuzzi, G., & Schaefer, F. (2014). Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet, 383*(9931), 1844-1859. doi:10.1016/s0140-6736(14)60659-0

Dewey, F. E., Grove, M. E., Pan, C., Goldstein, B. A., Bernstein, J. A., Chaib, H., . . . Quertermous, T. (2014). Clinical interpretation and implications of whole-genome sequencing. *JAMA, 311*(10), 1035-1045. doi:10.1001/jama.2014.1717

Di Gioia, S. A., Letteboer, S. J., Kostic, C., Bandah-Rozenfeld, D., Hetterschijt, L., Sharon, D., . . . Rivolta, C. (2012). FAM161A, associated with retinitis pigmentosa, is a component of the cilia-basal body complex and interacts with proteins involved in ciliopathies. *Hum Mol Genet, 21*(23), 5174-5184. doi:10.1093/hmg/dds368

Dillman, J. R., Trout, A. T., Smith, E. A., & Towbin, A. J. (2017). Hereditary Renal Cystic Disorders: Imaging of the Kidneys and Beyond. *Radiographics, 37*(3), 924-946. doi:10.1148/rg.2017160148

Ding, W. Y., Beresford, M. W., Saleem, M. A., & Ramanan, A. V. (2019). Big data and stratified medicine: what does it mean for children? *Arch Dis Child, 104*(4), 389-394. doi:10.1136/archdischild-2018-315125

Ebner, K., Dafinger, C., Ortiz-Bruechle, N., Koerber, F., Schermer, B., Benzing, T., . . . Liebau, M. C. (2017). Challenges in establishing genotype-phenotype correlations in ARPKD: case report on a toddler with two severe PKHD1 mutations. *Pediatr Nephrol, 32*(7), 1269-1273. doi:10.1007/s00467-017-3648-x

Ebner, K., Feldkoetter, M., Ariceta, G., Bergmann, C., Buettner, R., Doyon, A., . . . Group, G. P. N. S. (2015). Rationale, design and objectives of ARegPKD, a European ARPKD registry study. *BMC Nephrology, 16*(1), 22. doi:10.1186/s12882-015-0002-z

Edghill, E. L., Bingham, C., Ellard, S., & Hattersley, A. T. (2006). Mutations in hepatocyte nuclear factor-1beta and their related phenotypes. *J Med Genet, 43*(1), 84-90. doi:10.1136/jmg.2005.032854

Edrees, B. M., Athar, M., Al-Allaf, F. A., Taher, M. M., Khan, W., Bouazzaoui, A., . . . Abduljaleel, Z. (2016). Next-generation sequencing for molecular diagnosis of autosomal recessive polycystic kidney disease. *Gene, 591*(1), 214-226. doi:10.1016/j.gene.2016.07.021

Ellingford, J. M., Barton, S., Bhaskar, S., Williams, S. G., Sergouniotis, P. I., O'Sullivan, J., . . . Black, G. C. (2016). Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease. *Ophthalmology, 123*(5), 1143-1150. doi:10.1016/j.ophtha.2016.01.009

Estrada-Cuzcano, A., Roepman, R., Cremers, F. P., den Hollander, A. I., & Mans, D. A. (2012). Non-syndromic retinal ciliopathies: translating gene discovery into therapy. *Hum Mol Genet, 21*(R1), R111-124. doi:10.1093/hmg/dds298

European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Registry. (2015). ERA-EDTA Registry Annual Report 2013. *Academic Medical Center, Department of Medical Informatics, Amsterdam, The Netherlands*. Retrieved from https://era-edta-reg.org/files/annualreports/pdf/AnnRep2013.pdf

Fain, P. R., McFann, K. K., Taylor, M. R., Tison, M., Johnson, A. M., Reed, B., & Schrier, R. W. (2005). Modifier genes play a significant role in the phenotypic expression of PKD1. *Kidney Int, 67*(4), 1256-1267. doi:10.1111/j.1523-1755.2005.00203.x

Faus Perez, A., Sanchis Calvo, A., Codoner Franch, P., Camarasa Lillo, N., & Alcover Barrachina, I. (2015). [Ciliopathies: a journey through the cilium]. *An Pediatr (Barc), 82*(2), 104-105. doi:10.1016/j.anpedi.2014.07.025

Fehrenbach, H., Decker, C., Eisenberger, T., Frank, V., Hampel, T., Walden, U., . . . Bergmann, C. (2014). Mutations in WDR19 encoding the intraflagellar transport component IFT144 cause a broad spectrum of ciliopathies. *Pediatr Nephrol, 29*(8), 1451-1456. doi:10.1007/s00467-014-2762-2

Ferkol, T. W., & Leigh, M. W. (2012). Ciliopathies: the central role of cilia in a spectrum of pediatric disorders. *J Pediatr, 160*(3), 366-371. doi:10.1016/j.jpeds.2011.11.024

Fernando, M. R., Dent, H., McDonald, S. P., & Rangan, G. K. (2017). Incidence and survival of end-stage kidney disease due to polycystic kidney disease in Australia and New Zealand (1963-2014). *Popul Health Metr, 15*(1), 7. doi:10.1186/s12963-017-0123-7

Fischer, D. C., Jacoby, U., Pape, L., Ward, C. J., Kuwertz-Broeking, E., Renken, C., . . . Haffner, D. (2009). Activation of the AKT/mTOR pathway in autosomal recessive polycystic kidney disease (ARPKD). *Nephrol Dial Transplant, 24*(6), 1819-1827. doi:10.1093/ndt/gfn744

Fonck, C., Chauveau, D., Gagnadoux, M. F., Pirson, Y., & Grunfeld, J. P. (2001). Autosomal recessive polycystic kidney disease in adulthood. *Nephrol Dial Transplant, 16*(8), 1648-1652. doi:10.1093/ndt/16.8.1648

Furu, L., Onuchic, L. F., Gharavi, A., Hou, X., Esquivel, E. L., Nagasawa, Y., . . . Somlo, S. (2003). Milder presentation of recessive polycystic kidney disease requires presence of amino acid substitution mutations. *J Am Soc Nephrol, 14*(8), 2004-2014. doi:10.1097/01.asn.0000078805.87038.05

Gabriel, G. C., Pazour, G. J., & Lo, C. W. (2018). Congenital Heart Defects and Ciliopathies Associated With Renal Phenotypes. *Front Pediatr, 6*, 175. doi:10.3389/fped.2018.00175

Gee, H. Y., Otto, E. A., Hurd, T. W., Ashraf, S., Chaki, M., Cluckey, A., . . . Hildebrandt, F. (2014). Whole-exome resequencing distinguishes cystic kidney diseases from

phenocopies in renal ciliopathies. *Kidney Int, 85*(4), 880-887. doi:10.1038/ki.2013.450

Geoffroy, V., Stoetzel, C., Scheidecker, S., Schaefer, E., Perrault, I., Bar, S., . . . Muller, J. (2018). Whole-genome sequencing in patients with ciliopathies uncovers a novel recurrent tandem duplication in IFT140. *Hum Mutat, 39*(7), 983-992. doi:10.1002/humu.23539

Georges, B., Cosyns, J. P., Dahan, K., Snyers, B., Carlier, B., Loute, G., & Pirson, Y. (2000). Late-onset renal failure in Senior-Loken syndrome. *Am J Kidney Dis, 36*(6), 1271-1275. doi:10.1053/ajkd.2000.19845

Gerhardt, C. (2019). Treatment of Ciliopathies: Current Perspectives. *Curr Med Chem, 26*(17), 3080. doi:10.2174/0929867326171908201236 48

Gerhardt, C., Leu, T., Lier, J. M., & Ruther, U. (2016). The cilia-regulated proteasome and its role in the development of ciliopathies and cancer. *Cilia, 5*, 14. doi:10.1186/s13630-016-0035-3

Gerth-Kahlert, C., & Koller, S. (2018). [Ciliopathies]. *Klin Monbl Augenheilkd, 235*(3), 264-272. doi:10.1055/a-0573-9199

Ghouse, J., Have, C. T., Skov, M. W., Andreasen, L., Ahlberg, G., Nielsen, J. B., . . . Olesen, M. S. (2017). Numerous Brugada syndrome-associated genetic variants have no effect on J-point elevation, syncope susceptibility, malignant cardiac arrhythmia, and all-cause mortality. *Genet Med, 19*(5), 521-528. doi:10.1038/gim.2016.151

Giles, R. H., Ajzenberg, H., & Jackson, P. K. (2014). 3D spheroid model of mIMCD3 cells for studying ciliopathies and renal epithelial disorders. *Nat Protoc, 9*(12), 2725-2731. doi:10.1038/nprot.2014.181

Gimpel, C., Bergmann, C., Bockenhauer, D., Breysem, L., Cadnapaphornchai, M. A., Cetiner, M., . . . Schaefer, F. (2019). International consensus statement on the diagnosis and management of autosomal dominant polycystic kidney disease in children and young people. *Nat Rev Nephrol, 15*(11), 713-726. doi:10.1038/s41581-019-0155-2

Goetz, S. C., & Anderson, K. V. (2010). The primary cilium: a signalling centre during vertebrate development. *Nat Rev Genet, 11*(5), 331-344. doi:10.1038/nrg2774

Goggolidou, P. (2014). Wnt and planar cell polarity signaling in cystic renal disease. *Organogenesis, 10*(1), 86-95. doi:10.4161/org.26766

Gong, S., Ji, F., Wang, B., Zhang, Y., Xu, X., & Sun, M. (2018). Tectonic Proteins Are Important Players in Non-Motile Ciliopathies. *Cell Physiol Biochem, 50*(1), 398-409. doi:10.1159/000494017

Green, R. C., Berg, J. S., Grody, W. W., Kalia, S. S., Korf, B. R., Martin, C. L., . . . Biesecker, L. G. (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med, 15*(7), 565-574. doi:10.1038/gim.2013.73

Gritti, A. P., & Boletta, A. (2015). [The ciliopathies--Polycystic Kidney Disease]. *G Ital Nefrol, 32 Suppl 64*. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26479061

Groopman, E., Goldstein, D., & Gharavi, A. (2019). Diagnostic Utility of Exome Sequencing for Kidney Disease. Reply. *N Engl J Med, 380*(21), 2080-2081. doi:10.1056/NEJMc1903250

Groopman, E. E., Marasa, M., Cameron-Christie, S., Petrovski, S., Aggarwal, V. S., Milo-Rasouly, H., . . . Gharavi, A. G. (2019). Diagnostic Utility of Exome Sequencing for Kidney Disease. *N Engl J Med, 380*(2), 142-151. doi:10.1056/NEJMoa1806891

Guan, P., & Sung, W. K. (2016). Structural variation detection using next-generation sequencing data: A comparative technical review. *Methods, 102*, 36-49. doi:10.1016/j.ymeth.2016.01.020

Guay-Woodford, L. M., Bissler, J. J., Braun, M. C., Bockenhauer, D., Cadnapaphornchai, M. A., Dell, K. M., . . . Moxey-Mims, M. (2014). Consensus expert recommendations for the diagnosis and management of autosomal recessive polycystic kidney disease: report of an international conference. *J Pediatr, 165*(3), 611-617. doi:10.1016/j.jpeds.2014.06.015

Gunay-Aygun, M. (2009). Liver and kidney disease in ciliopathies. *Am J Med Genet C Semin Med Genet, 151C*(4), 296-306. doi:10.1002/ajmg.c.30225

Gunay-Aygun, M., Font-Montgomery, E., Lukose, L., Tuchman, M., Graf, J., Bryant, J. C., . . . Gahl, W. A. (2010). Correlation of kidney function, volume and imaging findings, and PKHD1 mutations in 73 patients with autosomal recessive polycystic kidney disease. *Clin J Am Soc Nephrol, 5*(6), 972-984. doi:10.2215/CJN.07141009

Gunay-Aygun, M., Turkbey, B. I., Bryant, J., Daryanani, K. T., Gerstein, M. T., Piwnica-Worms, K., . . . Gahl, W. A. (2011). Hepatorenal findings in obligate heterozygotes for autosomal recessive polycystic kidney disease. *Mol Genet Metab, 104*(4), 677-681. doi:10.1016/j.ymgme.2011.09.001

Guo, J., Higginbotham, H., Li, J., Nichols, J., Hirt, J., Ghukasyan, V., & Anton, E. S. (2015). Developmental disruptions underlying brain abnormalities in ciliopathies. *Nat Commun, 6*, 7857. doi:10.1038/ncomms8857

Habbig, S., & Liebau, M. C. (2015). Ciliopathies - from rare inherited cystic kidney diseases to basic cellular function. *Mol Cell Pediatr, 2*(1), 8. doi:10.1186/s40348-015-0019-1

Halbritter, J., Porath, J. D., Diaz, K. A., Braun, D. A., Kohl, S., Chaki, M., . . . Otto, E. A. (2013). Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. *Hum Genet, 132*(8), 865-884. doi:10.1007/s00439-013-1297-0

Hammarsjo, A., Wang, Z., Vaz, R., Taylan, F., Sedghi, M., Girisha, K. M., . . . Ikegawa, S. (2017). Novel KIAA0753 mutations extend the phenotype of skeletal ciliopathies. *Sci Rep, 7*(1), 15585. doi:10.1038/s41598-017-15442-1

Harambat, J., van Stralen, K. J., Kim, J. J., & Tizard, E. J. (2012). Epidemiology of chronic kidney disease in children. *Pediatr Nephrol, 27*(3), 363-373. doi:10.1007/s00467-011-1939-1

Harris, P. C. (2009). 2008 Homer W. Smith Award: insights into the pathogenesis of polycystic kidney disease from gene discovery. *J Am Soc Nephrol, 20*(6), 1188-1198. doi:10.1681/ASN.2009010014

Harris, P. C., Bae, K. T., Rossetti, S., Torres, V. E., Grantham, J. J., Chapman, A. B., . . . Miller, J. P. (2006). Cyst number but not the rate of cystic growth is associated with the mutated gene in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol, 17*(11), 3013-3019. doi:10.1681/asn.2006080835

Harris, P. C., & Rossetti, S. (2010). Molecular diagnostics for autosomal dominant polycystic kidney disease. *Nat Rev Nephrol, 6*(4), 197-206. doi:10.1038/nrneph.2010.18

Harris, P. C., & Torres, V. E. (2009). Polycystic kidney disease. *Annu Rev Med, 60*, 321-337. doi:10.1146/annurev.med.60.101707.125712

Harrison, J. L., Hildreth, C. M., Callahan, S. M., Goodchild, A. K., & Phillips, J. K. (2010). Cardiovascular autonomic dysfunction in a novel rodent model of

polycystic kidney disease. *Auton Neurosci, 152*(1-2), 60-66. doi:10.1016/j.autneu.2009.09.019

Hartill, V., Szymanska, K., Sharif, S. M., Wheway, G., & Johnson, C. A. (2017). Meckel-Gruber Syndrome: An Update on Diagnosis, Clinical Management, and Research Advances. *Front Pediatr, 5*, 244. doi:10.3389/fped.2017.00244

Hartung, E. A., & Guay-Woodford, L. M. (2014). Autosomal recessive polycystic kidney disease: a hepatorenal fibrocystic disorder with pleiotropic effects. *Pediatrics, 134*(3), e833-845. doi:10.1542/peds.2013-3646

Hassanien, A. A., Al-Shaikh, F., Vamos, E. P., Yadegarfar, G., & Majeed, A. (2012). Epidemiology of end-stage renal disease in the countries of the Gulf Cooperation Council: a systematic review. *JRSM Short Rep, 3*(6), 38. doi:10.1258/shorts.2012.011150

Hayden, E. C. (2008). Biological tools revamp disease classification. *Nature, 453*(7196), 709. doi:10.1038/453709a

Hecking, M., Bieber, B. A., Ethier, J., Kautzky-Willer, A., Sunder-Plassmann, G., Saemann, M. D., . . . Port, F. K. (2014). Sex-specific differences in hemodialysis prevalence and practices and the male-to-female mortality rate: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *PLoS Med, 11*(10), e1001750. doi:10.1371/journal.pmed.1001750

Heuser, T., Raytchev, M., Krell, J., Porter, M. E., & Nicastro, D. (2009). The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. *J Cell Biol, 187*(6), 921-933. doi:10.1083/jcb.200908067

Heyer, C. M., Sundsbak, J. L., Abebe, K. Z., Chapman, A. B., Torres, V. E., Grantham, J. J., . . . Harris, P. C. (2016). Predicted Mutation Strength of Nontruncating PKD1 Mutations Aids Genotype-Phenotype Correlations in Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol, 27*(9), 2872-2884. doi:10.1681/asn.2015050583

Hildebrandt, F., Attanasio, M., & Otto, E. (2009). Nephronophthisis: disease mechanisms of a ciliopathy. *J Am Soc Nephrol, 20*(1), 23-35. doi:10.1681/asn.2008050456

Hildebrandt, F., Benzing, T., & Katsanis, N. (2011). Ciliopathies. *N Engl J Med, 364*(16), 1533-1543. doi:10.1056/NEJMra1010172

Hildebrandt, F., Otto, E., Rensing, C., Nothwang, H. G., Vollmer, M., Adolphs, J., . . . Brandis, M. (1997). A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. *Nat Genet, 17*(2), 149-153. doi:10.1038/ng1097-149

Hoefele, J., Nayir, A., Chaki, M., Imm, A., Allen, S. J., Otto, E. A., & Hildebrandt, F. (2011). Pseudodominant inheritance of nephronophthisis caused by a homozygous NPHP1 deletion. *Pediatr Nephrol, 26*(6), 967-971. doi:10.1007/s00467-011-1761-9

Hogg, R. J., Furth, S., Lemley, K. V., Portman, R., Schwartz, G. J., Coresh, J., . . . Levey, A. S. (2003). National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. *Pediatrics, 111*(6 Pt 1), 1416-1421. doi:10.1542/peds.111.6.1416

Hopp, K., Ward, C. J., Hommerding, C. J., Nasr, S. H., Tuan, H. F., Gainullin, V. G., . . . Harris, P. C. (2012). Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J Clin Invest, 122*(11), 4257-4273. doi:10.1172/jci64313

Hopp, K., Ward, C. J., Hommerding, C. J., Nasr, S. H., Tuan, H. F., Gainullin, V. G., . . . Harris, P. C. (2012). Functional polycystin-1 dosage governs autosomal dominant

polycystic kidney disease severity. *J Clin Invest, 122*(11), 4257-4273. doi:10.1172/jci64313
Hor, C. H., & Goh, E. L. (2019). Small GTPases in hedgehog signalling: emerging insights into the disease mechanisms of Rab23-mediated and Arl13b-mediated ciliopathies. *Curr Opin Genet Dev, 56*, 61-68. doi:10.1016/j.gde.2019.07.009
Horani, A., & Ferkol, T. W. (2016). Primary ciliary dyskinesia and associated sensory ciliopathies. *Expert Rev Respir Med, 10*(5), 569-576. doi:10.1586/17476348.2016.1165612
Horani, A., & Ferkol, T. W. (2018). Advances in the Genetics of Primary Ciliary Dyskinesia: Clinical Implications. *Chest, 154*(3), 645-652. doi:10.1016/j.chest.2018.05.007
Horani, A., Ferkol, T. W., Dutcher, S. K., & Brody, S. L. (2016). Genetics and biology of primary ciliary dyskinesia. *Paediatr Respir Rev, 18*, 18-24. doi:10.1016/j.prrv.2015.09.001
Hsiao, Y.-C., Tuz, K., & Ferland, R. J. (2012). Trafficking in and to the primary cilium. *Cilia, 1*(1), 4. doi:10.1186/2046-2530-1-4
Hsu, K. S., Chuang, J. Z., & Sung, C. H. (2017). The Biology of Ciliary Dynamics. *Cold Spring Harb Perspect Biol, 9*(4). doi:10.1101/cshperspect.a027904
Hua, K., & Ferland, R. J. (2018). Primary Cilia Reconsidered in the Context of Ciliopathies: Extraciliary and Ciliary Functions of Cilia Proteins Converge on a Polarity theme? *Bioessays, 40*(8), e1700132. doi:10.1002/bies.201700132
Huangfu, D., Liu, A., Rakeman, A. S., Murcia, N. S., Niswander, L., & Anderson, K. V. (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature, 426*(6962), 83-87. doi:10.1038/nature02061
Hurd, T. W., & Hildebrandt, F. (2011). Mechanisms of nephronophthisis and related ciliopathies. *Nephron Exp Nephrol, 118*(1), e9-14. doi:10.1159/000320888
Hwang, Y. H., Conklin, J., Chan, W., Roslin, N. M., Liu, J., He, N., . . . Pei, Y. (2016). Refining Genotype-Phenotype Correlation in Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol, 27*(6), 1861-1868. doi:10.1681/asn.2015060648
Iannicelli, M., Brancati, F., Mougou-Zerelli, S., Mazzotta, A., Thomas, S., Elkhartoufi, N., . . . Valente, E. M. (2010). Novel TMEM67 mutations and genotype-phenotype correlates in meckelin-related ciliopathies. *Hum Mutat, 31*(5), E1319-1331. doi:10.1002/humu.21239
Iliuta, I. A., Kalatharan, V., Wang, K., Cornec-Le Gall, E., Conklin, J., Pourafkari, M., . . . Pei, Y. (2017). Polycystic Kidney Disease without an Apparent Family History. *J Am Soc Nephrol, 28*(9), 2768-2776. doi:10.1681/asn.2016090938
Irfanullah, Khan, S., Ullah, I., Nasir, A., Meijer, C. A., Laurense-Bik, M., . . . Ahmad, W. (2016). Hypomorphic MKS1 mutation in a Pakistani family with mild Joubert syndrome and atypical features: Expanding the phenotypic spectrum of MKS1-related ciliopathies. *Am J Med Genet A, 170*(12), 3289-3293. doi:10.1002/ajmg.a.37934
Ishikawa, H., & Marshall, W. F. (2011). Ciliogenesis: building the cell's antenna. *Nat Rev Mol Cell Biol, 12*(4), 222-234. doi:10.1038/nrm3085
Jackson, P. K. (2013). Nek8 couples renal ciliopathies to DNA damage and checkpoint control. *Mol Cell, 51*(4), 407-408. doi:10.1016/j.molcel.2013.08.013
Jalali Sefid Dashti, M., & Gamieldien, J. (2017). A practical guide to filtering and prioritizing genetic variants. *Biotechniques, 62*(1), 18-30. doi:10.2144/000114492

Jordan, D. M., Frangakis, S. G., Golzio, C., Cassa, C. A., Kurtzberg, J., Davis, E. E., . . . Katsanis, N. (2015). Identification of cis-suppression of human disease mutations by comparative genomics. *Nature, 524*(7564), 225-229. doi:10.1038/nature14497

Kaariainen, H. (1987). Polycystic kidney disease in children: a genetic and epidemiological study of 82 Finnish patients. *J Med Genet, 24*(8), 474-481.

Kääriäinen, H. (1987). Polycystic kidney disease in children: a genetic and epidemiological study of 82 Finnish patients. *J Med Genet, 24*(8), 474-481.

Kagan, K. O., Dufke, A., & Gembruch, U. (2017). Renal cystic disease and associated ciliopathies. *Curr Opin Obstet Gynecol, 29*(2), 85-94. doi:10.1097/GCO.0000000000000348

Karp, N., Grosse-Wortmann, L., & Bowdin, S. (2012). Severe aortic stenosis, bicuspid aortic valve and atrial septal defect in a child with Joubert Syndrome and Related Disorders (JSRD) - a case report and review of congenital heart defects reported in the human ciliopathies. *Eur J Med Genet, 55*(11), 605-610. doi:10.1016/j.ejmg.2012.07.010

Katabathina, V. S., Kota, G., Dasyam, A. K., Shanbhogue, A. K., & Prasad, S. R. (2010). Adult renal cystic disease: a genetic, biological, and developmental primer. *Radiographics, 30*(6), 1509-1523. doi:10.1148/rg.306105513

Kempeneers, C., & Chilvers, M. A. (2018). To beat, or not to beat, that is question! The spectrum of ciliopathies. *Pediatr Pulmonol, 53*(8), 1122-1129. doi:10.1002/ppul.24078

Kennedy, M. P., Omran, H., Leigh, M. W., Dell, S., Morgan, L., Molina, P. L., . . . Knowles, M. R. (2007). Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation, 115*(22), 2814-2821. doi:10.1161/circulationaha.106.649038

Khaddour, R., Smith, U., Baala, L., Martinovic, J., Clavering, D., Shaffiq, R., . . . Attie-Bitach, T. (2007). Spectrum of MKS1 and MKS3 mutations in Meckel syndrome: a genotype-phenotype correlation. Mutation in brief #960. Online. *Hum Mutat, 28*(5), 523-524. doi:10.1002/humu.9489

Khanna, H., & Baehr, W. (2012). Retina ciliopathies: from genes to mechanisms and treatment. *Vision Res, 75*, 1. doi:10.1016/j.visres.2012.11.007

Khanna, H., Davis, E. E., Murga-Zamalloa, C. A., Estrada-Cuzcano, A., Lopez, I., den Hollander, A. I., . . . Katsanis, N. (2009). A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet, 41*(6), 739-745. doi:10.1038/ng.366

Kim, I., Fu, Y., Hui, K., Moeckel, G., Mai, W., Li, C., . . . Wu, G. (2008). Fibrocystin/polyductin modulates renal tubular formation by regulating polycystin-2 expression and function. *J Am Soc Nephrol, 19*(3), 455-468. doi:10.1681/ASN.2007070770

Kirby, A., Gnirke, A., Jaffe, D. B., Baresova, V., Pochet, N., Blumenstiel, B., . . . Daly, M. J. (2013). Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in MUC1 missed by massively parallel sequencing. *Nat Genet, 45*(3), 299-303. doi:10.1038/ng.2543

Kirk, R. (2014). Genetics: Causative genes identified in rare renal ciliopathies. *Nat Rev Nephrol, 10*(2), 64. doi:10.1038/nrneph.2013.261

Klena, N. T., Gibbs, B. C., & Lo, C. W. (2017). Cilia and Ciliopathies in Congenital Heart Disease. *Cold Spring Harb Perspect Biol, 9*(8). doi:10.1101/cshperspect.a028266

Knopp, C., Rudnik-Schoneborn, S., Eggermann, T., Bergmann, C., Begemann, M., Schoner, K., . . . Ortiz Bruchle, N. (2015). Syndromic ciliopathies: From single

gene to multi gene analysis by SNP arrays and next generation sequencing. *Mol Cell Probes, 29*(5), 299-307. doi:10.1016/j.mcp.2015.05.008

Kong, S. W., Lee, I. H., Liu, X., Hirschhorn, J. N., & Mandl, K. D. (2018). Measuring coverage and accuracy of whole-exome sequencing in clinical context. *Genet Med, 20*(12), 1617-1626. doi:10.1038/gim.2018.51

Konig, J., Kranz, B., Konig, S., Schlingmann, K. P., Titieni, A., Tonshoff, B., . . . Gesellschaft fur Padiatrische, N. (2017). Phenotypic Spectrum of Children with Nephronophthisis and Related Ciliopathies. *Clin J Am Soc Nephrol, 12*(12), 1974-1983. doi:10.2215/CJN.01280217

Krall, P., Pineda, C., Ruiz, P., Ejarque, L., Vendrell, T., Camacho, J. A., . . . Ars, E. (2014). Cost-effective PKHD1 genetic testing for autosomal recessive polycystic kidney disease. *Pediatr Nephrol, 29*(2), 223-234. doi:10.1007/s00467-013-2657-7

Kunova Bosakova, M., Varecha, M., Hampl, M., Duran, I., Nita, A., Buchtova, M., . . . Krejci, P. (2018). Regulation of ciliary function by fibroblast growth factor signaling identifies FGFR3-related disorders achondroplasia and thanatophoric dysplasia as ciliopathies. *Hum Mol Genet, 27*(6), 1093-1105. doi:10.1093/hmg/ddy031

Lambie, L., Amin, R., Essop, F., Cnaan, A., Krause, A., & Guay-Woodford, L. M. (2015). Clinical and genetic characterization of a founder PKHD1 mutation in Afrikaners with ARPKD. *Pediatr Nephrol, 30*(2), 273-279. doi:10.1007/s00467-014-2917-1

Lancaster, M. A., & Gleeson, J. G. (2009). The primary cilium as a cellular signaling center: lessons from disease. *Curr Opin Genet Dev, 19*(3), 220-229. doi:10.1016/j.gde.2009.04.008

Lancaster, M. A., Gopal, D. J., Kim, J., Saleem, S. N., Silhavy, J. L., Louie, C. M., . . . Gleeson, J. G. (2011). Defective Wnt-dependent cerebellar midline fusion in a mouse model of Joubert syndrome. *Nat Med, 17*(6), 726-731. doi:10.1038/nm.2380

Lanktree, M. B., Iliuta, I. A., Haghighi, A., Song, X., & Pei, Y. (2019). Evolving role of genetic testing for the clinical management of autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant, 34*(9), 1453-1460. doi:10.1093/ndt/gfy261

Lata, S., Marasa, M., Li, Y., Fasel, D. A., Groopman, E., Jobanputra, V., . . . Gharavi, A. G. (2018). Whole-Exome Sequencing in Adults With Chronic Kidney Disease: A Pilot Study. *Ann Intern Med, 168*(2), 100-109. doi:10.7326/m17-1319

Lazarin, G. A., Haque, I. S., Nazareth, S., Iori, K., Patterson, A. S., Jacobson, J. L., . . . Srinivasan, B. S. (2013). An empirical estimate of carrier frequencies for 400+ causal Mendelian variants: results from an ethnically diverse clinical sample of 23,453 individuals. *Genet Med, 15*(3), 178-186. doi:10.1038/gim.2012.114

Lee, J. H., & Gleeson, J. G. (2010). The role of primary cilia in neuronal function. *Neurobiol Dis, 38*(2), 167-172. doi:10.1016/j.nbd.2009.12.022

Lee, J. H., Silhavy, J. L., Lee, J. E., Al-Gazali, L., Thomas, S., Davis, E. E., . . . Gleeson, J. G. (2012). Evolutionarily assembled cis-regulatory module at a human ciliopathy locus. *Science, 335*(6071), 966-969. doi:10.1126/science.1213506

Lee, S., & Weatherbee, S. D. (2010). Synergistic interaction between ciliary genes reflects the importance of mutational load in ciliopathies. *J Am Soc Nephrol, 21*(5), 724-726. doi:10.1681/ASN.2010030301

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., . . . MacArthur, D. G. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature, 536*(7616), 285-291. doi:10.1038/nature19057

Li, L., Guthridge, S., Li, S. Q., Zhao, Y., Lawton, P., & Cass, A. (2018). Estimating the total prevalence and incidence of end-stage kidney disease among Aboriginal and non-Aboriginal populations in the Northern Territory of Australia, using multiple data sources. *BMC Nephrol, 19*(1), 15. doi:10.1186/s12882-017-0791-3

Li, Y., Ling, K., & Hu, J. (2012). The emerging role of Arf/Arl small GTPases in cilia and ciliopathies. *J Cell Biochem, 113*(7), 2201-2207. doi:10.1002/jcb.24116

Liang, G., Yang, J., Wang, Z., Li, Q., Tang, Y., & Chen, X. Z. (2008). Polycystin-2 down-regulates cell proliferation via promoting PERK-dependent phosphorylation of eIF2alpha. *Hum Mol Genet, 17*(20), 3254-3262. doi:10.1093/hmg/ddn221

Lienkamp, S., Ganner, A., & Walz, G. (2012). Inversin, Wnt signaling and primary cilia. *Differentiation, 83*(2), S49-55. doi:10.1016/j.diff.2011.11.012

Linck, R. W., Chemes, H., & Albertini, D. F. (2016). The axoneme: the propulsive engine of spermatozoa and cilia and associated ciliopathies leading to infertility. *J Assist Reprod Genet, 33*(2), 141-156. doi:10.1007/s10815-016-0652-1

Lindstrand, A., Davis, E. E., Carvalho, C. M., Pehlivan, D., Willer, J. R., Tsai, I. C., . . . Katsanis, N. (2014). Recurrent CNVs and SNVs at the NPHP1 locus contribute pathogenic alleles to Bardet-Biedl syndrome. *Am J Hum Genet, 94*(5), 745-754. doi:10.1016/j.ajhg.2014.03.017

Lindstrand, A., Frangakis, S., Carvalho, C. M., Richardson, E. B., McFadden, K. A., Willer, J. R., . . . Katsanis, N. (2016). Copy-Number Variation Contributes to the Mutational Load of Bardet-Biedl Syndrome. *Am J Hum Genet, 99*(2), 318-336. doi:10.1016/j.ajhg.2015.04.023

Liu, K. J. (2016). Craniofacial Ciliopathies and the Interpretation of Hedgehog Signal Transduction. *PLoS Genet, 12*(12), e1006460. doi:10.1371/journal.pgen.1006460

Liyanage, T., Ninomiya, T., Jha, V., Neal, B., Patrice, H. M., Okpechi, I., . . . Perkovic, V. (2015). Worldwide access to treatment for end-stage kidney disease: a systematic review. *Lancet, 385*(9981), 1975-1982. doi:10.1016/s0140-6736(14)61601-9

Logan, C. V., Abdel-Hamed, Z., & Johnson, C. A. (2011). Molecular genetics and pathogenic mechanisms for the severe ciliopathies: insights into neurodevelopment and pathogenesis of neural tube defects. *Mol Neurobiol, 43*(1), 12-26. doi:10.1007/s12035-010-8154-0

Lonergan, G. J., Rice, R. R., & Suarez, E. S. (2000). Autosomal recessive polycystic kidney disease: radiologic-pathologic correlation. *Radiographics, 20*(3), 837-855. doi:10.1148/radiographics.20.3.g00ma20837

Lopes, C. A., Prosser, S. L., Romio, L., Hirst, R. A., O'Callaghan, C., Woolf, A. S., & Fry, A. M. (2011). Centriolar satellites are assembly points for proteins implicated in human ciliopathies, including oral-facial-digital syndrome 1. *J Cell Sci, 124*(Pt 4), 600-612. doi:10.1242/jcs.077156

Lopez, E., Thauvin-Robinet, C., Reversade, B., Khartoufi, N. E., Devisme, L., Holder, M., . . . Attie-Bitach, T. (2014). C5orf42 is the major gene responsible for OFD syndrome type VI. *Hum Genet, 133*(3), 367-377. doi:10.1007/s00439-013-1385-1

Losekoot, M., Haarloo, C., Ruivenkamp, C., White, S. J., Breuning, M. H., & Peters, D. J. (2005). Analysis of missense variants in the PKHD1-gene in patients with autosomal recessive polycystic kidney disease (ARPKD). *Hum Genet, 118*(2), 185-206. doi:10.1007/s00439-005-0027-7

Losekoot, M., Ruivenkamp, C. A., Tholens, A. P., Grimbergen, J. E., Vijfhuizen, L., Vermeer, S., . . . Peters, D. J. (2012). Neonatal onset autosomal dominant polycystic kidney disease (ADPKD) in a patient homozygous for a PKD2

missense mutation due to uniparental disomy. *J Med Genet, 49*(1), 37-40. doi:10.1136/jmedgenet-2011-100452
Louie, C. M., Caridi, G., Lopes, V. S., Brancati, F., Kispert, A., Lancaster, M. A., . . . Gleeson, J. G. (2010). AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet, 42*(2), 175-180. doi:10.1038/ng.519
Lu, H., Galeano, M. C. R., Ott, E., Kaeslin, G., Kausalya, P. J., Kramer, C., . . . Bergmann, C. (2017). Mutations in DZIP1L, which encodes a ciliary-transition-zone protein, cause autosomal recessive polycystic kidney disease. *Nat Genet, 49*(7), 1025-1034. doi:10.1038/ng.3871
Luo, F., & Tao, Y. H. (2018). Nephronophthisis: A review of genotype-phenotype correlation. *Nephrology (Carlton), 23*(10), 904-911. doi:10.1111/nep.13393
Ma, M., Gallagher, A. R., & Somlo, S. (2017). Ciliary Mechanisms of Cyst Formation in Polycystic Kidney Disease. *Cold Spring Harb Perspect Biol, 9*(11). doi:10.1101/cshperspect.a028209
MacArthur, D. G., Balasubramanian, S., Frankish, A., Huang, N., Morris, J., Walter, K., . . . Tyler-Smith, C. (2012). A systematic survey of loss-of-function variants in human protein-coding genes. *Science, 335*(6070), 823-828. doi:10.1126/science.1215040
MacArthur, D. G., Manolio, T. A., Dimmock, D. P., Rehm, H. L., Shendure, J., Abecasis, G. R., . . . Gunter, C. (2014). Guidelines for investigating causality of sequence variants in human disease. *Nature, 508*(7497), 469-476. doi:10.1038/nature13127
Madhivanan, K., & Aguilar, R. C. (2014). Ciliopathies: the trafficking connection. *Traffic, 15*(10), 1031-1056. doi:10.1111/tra.12195
Malicki, J. J., & Johnson, C. A. (2017). The Cilium: Cellular Antenna and Central Processing Unit. *Trends Cell Biol, 27*(2), 126-140. doi:10.1016/j.tcb.2016.08.002
Mallawaarachchi, A. C., Hort, Y., Cowley, M. J., McCabe, M. J., Minoche, A., Dinger, M. E., . . . Furlong, T. J. (2016). Whole-genome sequencing overcomes pseudogene homology to diagnose autosomal dominant polycystic kidney disease. *Eur J Hum Genet, 24*(11), 1584-1590. doi:10.1038/ejhg.2016.48
Mallett, A., Fowles, L. F., McGaughran, J., Healy, H., & Patel, C. (2016). A multidisciplinary renal genetics clinic improves patient diagnosis. *Med J Aust, 204*(2), 58-59. doi:10.5694/mja15.01157
Mallett, A., Patel, C., Salisbury, A., Wang, Z., Healy, H., & Hoy, W. (2014). The prevalence and epidemiology of genetic renal disease amongst adults with chronic kidney disease in Australia. *Orphanet J Rare Dis, 9*, 98. doi:10.1186/1750-1172-9-98
Mallett, A. J., McCarthy, H. J., Ho, G., Holman, K., Farnsworth, E., Patel, C., . . . Alexander, S. I. (2017). Massively parallel sequencing and targeted exomes in familial kidney disease can diagnose underlying genetic disorders. *Kidney Int, 92*(6), 1493-1506. doi:10.1016/j.kint.2017.06.013
Mann, N., Braun, D. A., Amann, K., Tan, W., Shril, S., Connaughton, D. M., . . . Hildebrandt, F. (2019). Whole-Exome Sequencing Enables a Precision Medicine Approach for Kidney Transplant Recipients. *J Am Soc Nephrol, 30*(2), 201-215. doi:10.1681/asn.2018060575
Mans, D. A., & Arts, H. H. (2014). Medical genetics of ciliopathies. *J Pediatr Genet, 3*(2), 47-48. doi:10.3233/PGE-14087
Mantere, T., Kersten, S., & Hoischen, A. (2019). Long-Read Sequencing Emerging in Medical Genetics. *Front Genet, 10*, 426. doi:10.3389/fgene.2019.00426

McDonald, S. P., & Craig, J. C. (2004). Long-term survival of children with end-stage renal disease. *N Engl J Med, 350*(26), 2654-2662. doi:10.1056/NEJMoa031643
McIntyre, J. C., Davis, E. E., Joiner, A., Williams, C. L., Tsai, I. C., Jenkins, P. M., . . . Martens, J. R. (2012). Gene therapy rescues cilia defects and restores olfactory function in a mammalian ciliopathy model. *Nat Med, 18*(9), 1423-1428. doi:10.1038/nm.2860
McIntyre, J. C., Williams, C. L., & Martens, J. R. (2013). Smelling the roses and seeing the light: gene therapy for ciliopathies. *Trends Biotechnol, 31*(6), 355-363. doi:10.1016/j.tibtech.2013.03.005
Michel-Calemard, L., Dijoud, F., Till, M., Lambert, J. C., Vercherat, M., Tardy, V., . . . Morel, Y. (2009). Pseudoexon activation in the PKHD1 gene: a French founder intronic mutation IVS46+653A>G causing severe autosomal recessive polycystic kidney disease. *Clin Genet, 75*(2), 203-206. doi:10.1111/j.1399-0004.2008.01106.x
Milla, C. E. (2016). The evolving spectrum of ciliopathies and respiratory disease. *Curr Opin Pediatr, 28*(3), 339-347. doi:10.1097/MOP.0000000000000358
Mitchell, A., Chang, H. Y., Daugherty, L., Fraser, M., Hunter, S., Lopez, R., . . . Finn, R. D. (2015). The InterPro protein families database: the classification resource after 15 years. *Nucleic Acids Res, 43*(Database issue), D213-221. doi:10.1093/nar/gku1243
Mitchison, H. M., & Valente, E. M. (2017). Motile and non-motile cilia in human pathology: from function to phenotypes. *J Pathol, 241*(2), 294-309. doi:10.1002/path.4843
Moalem, S., Keating, S., Shannon, P., Thompson, M., Millar, K., Nykamp, K., . . . Chitayat, D. (2013). Broadening the ciliopathy spectrum: motile cilia dyskinesia, and nephronophthisis associated with a previously unreported homozygous mutation in the INVS/NPHP2 gene. *Am J Med Genet A, 161a*(7), 1792-1796. doi:10.1002/ajmg.a.36036
Mockel, A., Perdomo, Y., Stutzmann, F., Letsch, J., Marion, V., & Dollfus, H. (2011). Retinal dystrophy in Bardet-Biedl syndrome and related syndromic ciliopathies. *Prog Retin Eye Res, 30*(4), 258-274. doi:10.1016/j.preteyeres.2011.03.001
Mok, C. A., & Heon, E. (2012). Caenorhabditis elegans as a model organism for ciliopathies and related forms of photoreceptor degeneration. *Adv Exp Med Biol, 723*, 533-538. doi:10.1007/978-1-4614-0631-0_67
Molinari, E., Decker, E., Mabillard, H., Tellez, J., Srivastava, S., Raman, S., . . . Sayer, J. A. (2018). Human urine-derived renal epithelial cells provide insights into kidney-specific alternate splicing variants. *Eur J Hum Genet, 26*(12), 1791-1796. doi:10.1038/s41431-018-0212-5
Mougou-Zerelli, S., Thomas, S., Szenker, E., Audollent, S., Elkhartoufi, N., Babarit, C., . . . Attie-Bitach, T. (2009). CC2D2A mutations in Meckel and Joubert syndromes indicate a genotype-phenotype correlation. *Hum Mutat, 30*(11), 1574-1582. doi:10.1002/humu.21116
Mukherjee, K., & Brocchieri, L. (2013). Ancient Origin of Chaperonin Gene Paralogs Involved in Ciliopathies. *J Phylogenetics Evol Biol, 1*(1). doi:10.4172/2329-9002.1000107
Mukhopadhyay, S. (2015). TCTEX1D2, a potential link to skeletal ciliopathies. *Cell Cycle, 14*(3), 293-294. doi:10.1080/15384101.2015.1006548
Murdoch, J. N., & Copp, A. J. (2010). The relationship between sonic Hedgehog signaling, cilia, and neural tube defects. *Birth Defects Res A Clin Mol Teratol, 88*(8), 633-652. doi:10.1002/bdra.20686

Murga-Zamalloa, C. A., Ghosh, A. K., Patil, S. B., Reed, N. A., Chan, L. S., Davuluri, S., . . . Khanna, H. (2011). Accumulation of the Raf-1 kinase inhibitory protein (Rkip) is associated with Cep290-mediated photoreceptor degeneration in ciliopathies. *J Biol Chem, 286*(32), 28276-28286. doi:10.1074/jbc.M111.237560

Nash, B. M., Wright, D. C., Grigg, J. R., Bennetts, B., & Jamieson, R. V. (2015). Retinal dystrophies, genomic applications in diagnosis and prospects for therapy. *Transl Pediatr, 4*(2), 139-163. doi:10.3978/j.issn.2224-4336.2015.04.03

Neumann, H. P., Jilg, C., Bacher, J., Nabulsi, Z., Malinoc, A., Hummel, B., . . . Eng, C. (2013). Epidemiology of autosomal-dominant polycystic kidney disease: an in-depth clinical study for south-western Germany. *Nephrol Dial Transplant, 28*(6), 1472-1487. doi:10.1093/ndt/gfs551

Nevers, Y., Prasad, M. K., Poidevin, L., Chennen, K., Allot, A., Kress, A., . . . Lecompte, O. (2017). Insights into Ciliary Genes and Evolution from Multi-Level Phylogenetic Profiling. *Mol Biol Evol, 34*(8), 2016-2034. doi:10.1093/molbev/msx146

Ng, P. C., & Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res, 31*(13), 3812-3814. doi:10.1093/nar/gkg509

Noda, K., Kitami, M., Kitami, K., Kaku, M., & Komatsu, Y. (2016). Canonical and noncanonical intraflagellar transport regulates craniofacial skeletal development. *Proc Natl Acad Sci U S A, 113*(19), E2589-2597. doi:10.1073/pnas.1519458113

Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., . . . Hirokawa, N. (1998). Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell, 95*(6), 829-837. doi:10.1016/s0092-8674(00)81705-5

Norris, D. P., & Grimes, D. T. (2012). Mouse models of ciliopathies: the state of the art. *Dis Model Mech, 5*(3), 299-312. doi:10.1242/dmm.009340

Novarino, G., Akizu, N., & Gleeson, J. G. (2011). Modeling human disease in humans: the ciliopathies. *Cell, 147*(1), 70-79. doi:10.1016/j.cell.2011.09.014

Obeidova, L., Seeman, T., Elisakova, V., Reiterova, J., Puchmajerova, A., & Stekrova, J. (2015). Molecular genetic analysis of PKHD1 by next-generation sequencing in Czech families with autosomal recessive polycystic kidney disease. *BMC Med Genet, 16*, 116. doi:10.1186/s12881-015-0261-3

Office for National Statistics (ONS). (2019). An overview of the UK population: how it has changed, why it has changed and how it is projected to change in the future. Retrieved from www.ons.gov.uk

National Center for Statistics and Information. Sultanate of Oman. (2015). Statistical Yearbook. Retrieved from https://www.ncsi.gov.om/Pages/NCSI.aspx

Ong, A. C., & Harris, P. C. (2015). A polycystin-centric view of cyst formation and disease: the polycystins revisited. *Kidney Int, 88*(4), 699-710. doi:10.1038/ki.2015.207

Ong, A. C., & Harris, P. C. (2015). A polycystin-centric view of cyst formation and disease: the polycystins revisited. *Kidney Int, 88*(4), 699-710. doi:10.1038/ki.2015.207

Onuchic, L. F., Furu, L., Nagasawa, Y., Hou, X., Eggermann, T., Ren, Z., . . . Germino, G. G. (2002). PKHD1, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats. *Am J Hum Genet, 70*(5), 1305-1317. doi:10.1086/340448

Orskov, B., Romming Sorensen, V., Feldt-Rasmussen, B., & Strandgaard, S. (2010). Improved prognosis in patients with autosomal dominant polycystic kidney

disease in Denmark. *Clin J Am Soc Nephrol, 5*(11), 2034-2039. doi:10.2215/cjn.01460210

Osborn, D. P., Roccasecca, R. M., McMurray, F., Hernandez-Hernandez, V., Mukherjee, S., Barroso, I., . . . Christou-Savina, S. (2014). Loss of FTO antagonises Wnt signaling and leads to developmental defects associated with ciliopathies. *PLoS One, 9*(2), e87662. doi:10.1371/journal.pone.0087662

O'Toole, J. F., Liu, Y., Davis, E. E., Westlake, C. J., Attanasio, M., Otto, E. A., . . . Hildebrandt, F. (2010). Individuals with mutations in XPNPEP3, which encodes a mitochondrial protein, develop a nephronophthisis-like nephropathy. *J Clin Invest, 120*(3), 791-802. doi:10.1172/jci40076

Otto, E. A., Hurd, T. W., Airik, R., Chaki, M., Zhou, W., Stoetzel, C., . . . Hildebrandt, F. (2010). Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. *Nat Genet, 42*(10), 840-850. doi:10.1038/ng.662

Oud, M. M., Lamers, I. J., & Arts, H. H. (2017). Ciliopathies: Genetics in Pediatric Medicine. *J Pediatr Genet, 6*(1), 18-29. doi:10.1055/s-0036-1593841

Pampliega, O., Orhon, I., Patel, B., Sridhar, S., Diaz-Carretero, A., Beau, I., . . . Cuervo, A. M. (2013). Functional interaction between autophagy and ciliogenesis. *Nature, 502*(7470), 194-200. doi:10.1038/nature12639

Parisi, M. A. (2019). The molecular genetics of Joubert syndrome and related ciliopathies: The challenges of genetic and phenotypic heterogeneity. *Transl Sci Rare Dis, 4*(1-2), 25-49. doi:10.3233/trd-190041

Parnell, S. C., Magenheimer, B. S., Maser, R. L., Zien, C. A., Frischauf, A. M., & Calvet, J. P. (2002). Polycystin-1 activation of c-Jun N-terminal kinase and AP-1 is mediated by heterotrimeric G proteins. *J Biol Chem, 277*(22), 19566-19572. doi:10.1074/jbc.M201875200

Patnaik, S. R., Raghupathy, R. K., Zhang, X., Mansfield, D., & Shu, X. (2015). The Role of RPGR and Its Interacting Proteins in Ciliopathies. *J Ophthalmol, 2015*, 414781. doi:10.1155/2015/414781

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B., & Cole, D. G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol, 151*(3), 709-718. doi:10.1083/jcb.151.3.709

Pei, Y., Obaji, J., Dupuis, A., Paterson, A. D., Magistroni, R., Dicks, E., . . . Ravine, D. (2009). Unified criteria for ultrasonographic diagnosis of ADPKD. *J Am Soc Nephrol, 20*(1), 205-212. doi:10.1681/asn.2008050507

Pei, Y., Paterson, A. D., Wang, K. R., He, N., Hefferton, D., Watnick, T., . . . St George-Hyslop, P. (2001). Bilineal disease and trans-heterozygotes in autosomal dominant polycystic kidney disease. *Am J Hum Genet, 68*(2), 355-363. doi:10.1086/318188

Penchev, V., Boueva, A., Kamenarova, K., Roussinov, D., Tzveova, R., Ivanova, M., . . . Beltcheva, O. (2017). A familial case of severe infantile nephronophthisis explained by oligogenic inheritance. *Eur J Med Genet, 60*(6), 321-325. doi:10.1016/j.ejmg.2017.04.002

Pennekamp, P., Menchen, T., Dworniczak, B., & Hamada, H. (2015). Situs inversus and ciliary abnormalities: 20 years later, what is the connection? *Cilia, 4*(1), 1. doi:10.1186/s13630-014-0010-9

Perugorria, M. J., Bujanda, L., & Banales, J. M. (2016). More insight into the diversity of cholangiocyte ciliopathies. *J Hepatol, 65*(6), 1083-1085. doi:10.1016/j.jhep.2016.08.015

Petersen, B. S., Fredrich, B., Hoeppner, M. P., Ellinghaus, D., & Franke, A. (2017). Opportunities and challenges of whole-genome and -exome sequencing. *BMC Genet, 18*(1), 14. doi:10.1186/s12863-017-0479-5

Piatti, G., De Santi, M. M., Brogi, M., Castorina, P., & Ambrosetti, U. (2014). Emerging ciliopathies: are respiratory cilia compromised in Usher syndrome? *Am J Otolaryngol, 35*(3), 340-346. doi:10.1016/j.amjoto.2014.01.010

Pigino, G., Geimer, S., Lanzavecchia, S., Paccagnini, E., Cantele, F., Diener, D. R., . . . Lupetti, P. (2009). Electron-tomographic analysis of intraflagellar transport particle trains in situ. *J Cell Biol, 187*(1), 135-148. doi:10.1083/jcb.200905103

Piontek, K., Menezes, L. F., Garcia-Gonzalez, M. A., Huso, D. L., & Germino, G. G. (2007). A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. *Nat Med, 13*(12), 1490-1495. doi:10.1038/nm1675

Piperno, G., LeDizet, M., & Chang, X. J. (1987). Microtubules containing acetylated alpha-tubulin in mammalian cells in culture. *J Cell Biol, 104*(2), 289-302. doi:10.1083/jcb.104.2.289

Pollard, K. S., Hubisz, M. J., Rosenbloom, K. R., & Siepel, A. (2010). Detection of nonneutral substitution rates on mammalian phylogenies. *Genome Res, 20*(1), 110-121. doi:10.1101/gr.097857.109

Porath, B., Gainullin, V. G., Cornec-Le Gall, E., Dillinger, E. K., Heyer, C. M., Hopp, K., . . . Harris, P. C. (2016). Mutations in GANAB, Encoding the Glucosidase IIalpha Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. *Am J Hum Genet, 98*(6), 1193-1207. doi:10.1016/j.ajhg.2016.05.004

Porter, M. E., & Sale, W. S. (2000). The 9 + 2 axoneme anchors multiple inner arm dyneins and a network of kinases and phosphatases that control motility. *J Cell Biol, 151*(5), F37-42. doi:10.1083/jcb.151.5.f37

Powles-Glover, N. (2014). Cilia and ciliopathies: classic examples linking phenotype and genotype-an overview. *Reprod Toxicol, 48*, 98-105. doi:10.1016/j.reprotox.2014.05.005

Powles-Glover, N. (2014). Cilia and ciliopathies: classic examples linking phenotype and genotype-an overview. *Reprod Toxicol, 48*, 98-105. doi:10.1016/j.reprotox.2014.05.005

Prakash, S., & Gharavi, A. G. (2015). Diagnosing kidney disease in the genetic era. *Curr Opin Nephrol Hypertens, 24*(4), 380-387. doi:10.1097/mnh.0000000000000139

Prattichizzo, C., Macca, M., Novelli, V., Giorgio, G., Barra, A., & Franco, B. (2008). Mutational spectrum of the oral-facial-digital type I syndrome: a study on a large collection of patients. *Hum Mutat, 29*(10), 1237-1246. doi:10.1002/humu.20792

Praveen, K., Davis, E. E., & Katsanis, N. (2015). Unique among ciliopathies: primary ciliary dyskinesia, a motile cilia disorder. *F1000Prime Rep, 7*, 36. doi:10.12703/P7-36

Priya, S., Nampoothiri, S., Sen, P., & Sripriya, S. (2016). Bardet-Biedl syndrome: Genetics, molecular pathophysiology, and disease management. *Indian J Ophthalmol, 64*(9), 620-627. doi:10.4103/0301-4738.194328

Qian, Q., Hunter, L. W., Du, H., Ren, Q., Han, Y., & Sieck, G. C. (2007). Pkd2+/- vascular smooth muscles develop exaggerated vasocontraction in response to phenylephrine stimulation. *J Am Soc Nephrol, 18*(2), 485-493. doi:10.1681/ASN.2006050501

Quinlan, R. J., Tobin, J. L., & Beales, P. L. (2008). Modeling ciliopathies: Primary cilia in development and disease. *Curr Top Dev Biol, 84*, 249-310. doi:10.1016/S0070-2153(08)00605-4

Quint, A., Sagi, M., Carmi, S., Daum, H., Macarov, M., Ben Neriah, Z., . . . Lerer, I. (2016). An Ashkenazi founder mutation in the PKHD1 gene. *Eur J Med Genet, 59*(2), 86-90. doi:10.1016/j.ejmg.2015.12.013

Rachel, R. A., Li, T., & Swaroop, A. (2012). Photoreceptor sensory cilia and ciliopathies: focus on CEP290, RPGR and their interacting proteins. *Cilia, 1*(1), 22. doi:10.1186/2046-2530-1-22

Rachel, R. A., Yamamoto, E. A., Dewanjee, M. K., May-Simera, H. L., Sergeev, Y. V., Hackett, A. N., . . . Swaroop, A. (2015). CEP290 alleles in mice disrupt tissue-specific cilia biogenesis and recapitulate features of syndromic ciliopathies. *Hum Mol Genet, 24*(13), 3775-3791. doi:10.1093/hmg/ddv123

Raja, J. V., Asha, M. L., Kumar, G. A., Sattigeri, A. V., & Malhotra, D. (2016). Craniofacial ciliopathies: An expanding oral disease spectrum - a review of literature and a case report. *Indian J Dent, 7*(3), 153-157. doi:10.4103/0975-962X.180315

Rajab, A., Al Rashdi, I., & Al Salmi, Q. (2013). Genetic services and testing in the Sultanate of Oman. Sultanate of Oman steps into modern genetics. *J Community Genet, 4*(3), 391-397. doi:10.1007/s12687-013-0153-1

Rajab, A., Bappal, B., Al-Shaikh, H., Al-Khusaibi, S., & Mohammed, A. J. (2005). Common autosomal recessive diseases in Oman derived from a hospital-based registry. *Community Genet, 8*(1), 27-30. doi:10.1159/000083334

Rajab, A., Hamza, N., Al Harasi, S., Al Lawati, F., Gibbons, U., Al Alawi, I., . . . Robinson, P. (2015). Repository of mutations from Oman: The entry point to a national mutation database. *F1000Res, 4*, 891. doi:10.12688/f1000research.6938.1

Rajab, A., & Patton, M. A. (2000). A study of consanguinity in the Sultanate of Oman. *Ann Hum Biol, 27*(3), 321-326. doi:10.1080/030144600282208

Reed, B., McFann, K., Kimberling, W. J., Pei, Y., Gabow, P. A., Christopher, K., . . . Schrier, R. W. (2008). Presence of de novo mutations in autosomal dominant polycystic kidney disease patients without family history. *Am J Kidney Dis, 52*(6), 1042-1050. doi:10.1053/j.ajkd.2008.05.015

Reiter, J. F., & Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol, 18*(9), 533-547. doi:10.1038/nrm.2017.60

Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., & Kircher, M. (2019). CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res, 47*(D1), D886-d894. doi:10.1093/nar/gky1016

Report., M. o. H. A. H. (2016). Health Facts. Retrieved from https://www.moh.gov.om/en/web/statistics/annual-reports

Rhee, E. P. (2018). How Omics Data Can Be Used in Nephrology. *Am J Kidney Dis, 72*(1), 129-135. doi:10.1053/j.ajkd.2017.12.008

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., . . . Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med, 17*(5), 405-424. doi:10.1038/gim.2015.30

Rock, N., & McLin, V. (2014). Liver involvement in children with ciliopathies. *Clin Res Hepatol Gastroenterol, 38*(4), 407-414. doi:10.1016/j.clinre.2014.04.001

Rohatgi, R., & Snell, W. J. (2010). The ciliary membrane. *Curr Opin Cell Biol, 22*(4), 541-546. doi:10.1016/j.ceb.2010.03.010

Rossetti, S., Consugar, M. B., Chapman, A. B., Torres, V. E., Guay-Woodford, L. M., Grantham, J. J., . . . Consortium, C. (2007). Comprehensive molecular diagnostics

in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol, 18*(7), 2143-2160. doi:10.1681/ASN.2006121387

Rossetti, S., Hopp, K., Sikkink, R. A., Sundsbak, J. L., Lee, Y. K., Kubly, V., . . . Harris, P. C. (2012). Identification of gene mutations in autosomal dominant polycystic kidney disease through targeted resequencing. *J Am Soc Nephrol, 23*(5), 915-933. doi:10.1681/asn.2011101032

Rossetti, S., Kubly, V. J., Consugar, M. B., Hopp, K., Roy, S., Horsley, S. W., . . . Harris, P. C. (2009). Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. *Kidney Int, 75*(8), 848-855. doi:10.1038/ki.2008.686

Rossetti, S., Torra, R., Coto, E., Consugar, M., Kubly, V., Malaga, S., . . . Harris, P. C. (2003). A complete mutation screen of PKHD1 in autosomal-recessive polycystic kidney disease (ARPKD) pedigrees. *Kidney Int, 64*(2), 391-403. doi:10.1046/j.1523-1755.2003.00111.x

Ruat, M., Roudaut, H., Ferent, J., & Traiffort, E. (2012). Hedgehog trafficking, cilia and brain functions. *Differentiation, 83*(2), S97-104. doi:10.1016/j.diff.2011.11.011

Ryan, R., Failler, M., Reilly, M. L., Garfa-Traore, M., Delous, M., Filhol, E., . . . Saunier, S. (2018). Functional characterization of tektin-1 in motile cilia and evidence for TEKT1 as a new candidate gene for motile ciliopathies. *Hum Mol Genet, 27*(2), 266-282. doi:10.1093/hmg/ddx396

Saigusa, T., & Bell, P. D. (2015). Molecular pathways and therapies in autosomal-dominant polycystic kidney disease. *Physiology (Bethesda), 30*(3), 195-207. doi:10.1152/physiol.00032.2014

Saito, M., & Sato, T. (2019). [Current situation of researches on a sensor organelle, primary cilium, to understand the pathogenesis of ciliopathy]. *Nihon Yakurigaku Zasshi, 153*(3), 117-123. doi:10.1254/fpj.153.117

Sakhuja, V., & Sud, K. (2003). End-stage renal disease in India and Pakistan: burden of disease and management issues. *Kidney Int Suppl*(83), S115-118. doi:10.1046/j.1523-1755.63.s83.24.x

Sampson, J. R., Maheshwar, M. M., Aspinwall, R., Thompson, P., Cheadle, J. P., Ravine, D., . . . Harris, P. C. (1997). Renal cystic disease in tuberous sclerosis: role of the polycystic kidney disease 1 gene. *Am J Hum Genet, 61*(4), 843-851. doi:10.1086/514888

Sanchez-Navarro, I., L, R. J. d. S., Blanco-Kelly, F., Zurita, O., Sanchez-Bolivar, N., Villaverde, C., . . . Ayuso, C. (2018). Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. *Sci Rep, 8*(1), 5285. doi:10.1038/s41598-018-23520-1

Sasai, N., Toriyama, M., & Kondo, T. (2019). Hedgehog Signal and Genetic Disorders. *Front Genet, 10*, 1103. doi:10.3389/fgene.2019.01103

Satir, P., & Christensen, S. T. (2007). Overview of structure and function of mammalian cilia. *Annu Rev Physiol, 69*, 377-400. doi:10.1146/annurev.physiol.69.040705.141236

Satir, P., & Christensen, S. T. (2008). Structure and function of mammalian cilia. *Histochem Cell Biol, 129*(6), 687-693. doi:10.1007/s00418-008-0416-9

Sattar, S., & Gleeson, J. G. (2011). The ciliopathies in neuronal development: a clinical approach to investigation of Joubert syndrome and Joubert syndrome-related disorders. *Dev Med Child Neurol, 53*(9), 793-798. doi:10.1111/j.1469-8749.2011.04021.x

Sawardekar, K. P. (2005). Profile of major congenital malformations at Nizwa Hospital, Oman: 10-year review. *J Paediatr Child Health, 41*(7), 323-330. doi:10.1111/j.1440-1754.2005.00625.x

Schmidts, M., Frank, V., Eisenberger, T., Al Turki, S., Bizet, A. A., Antony, D., . . . Bergmann, C. (2013). Combined NGS approaches identify mutations in the intraflagellar transport gene IFT140 in skeletal ciliopathies with early progressive kidney Disease. *Hum Mutat, 34*(5), 714-724. doi:10.1002/humu.22294

Schock, E. N., & Brugmann, S. A. (2017). Discovery, Diagnosis, and Etiology of Craniofacial Ciliopathies. *Cold Spring Harb Perspect Biol, 9*(9). doi:10.1101/cshperspect.a028258

Schock, E. N., Chang, C. F., Youngworth, I. A., Davey, M. G., Delany, M. E., & Brugmann, S. A. (2016). Utilizing the chicken as an animal model for human craniofacial ciliopathies. *Dev Biol, 415*(2), 326-337. doi:10.1016/j.ydbio.2015.10.024

Schueler, M., Halbritter, J., Phelps, I. G., Braun, D. A., Otto, E. A., Porath, J. D., . . . Hildebrandt, F. (2016). Large-scale targeted sequencing comparison highlights extreme genetic heterogeneity in nephronophthisis-related ciliopathies. *J Med Genet, 53*(3), 208-214. doi:10.1136/jmedgenet-2015-103304

Schwarz, J. M., Rodelsperger, C., Schuelke, M., & Seelow, D. (2010). MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods, 7*(8), 575-576. doi:10.1038/nmeth0810-575

Seo, S., & Datta, P. (2017). Photoreceptor outer segment as a sink for membrane proteins: hypothesis and implications in retinal ciliopathies. *Hum Mol Genet, 26*(R1), R75-R82. doi:10.1093/hmg/ddx163

Shaheen, R., Schmidts, M., Faqeih, E., Hashem, A., Lausch, E., Holder, I., . . . Alkuraya, F. S. (2015). A founder CEP120 mutation in Jeune asphyxiating thoracic dystrophy expands the role of centriolar proteins in skeletal ciliopathies. *Hum Mol Genet, 24*(5), 1410-1419. doi:10.1093/hmg/ddu555

Shaheen, R., Szymanska, K., Basu, B., Patel, N., Ewida, N., Faqeih, E., . . . Alkuraya, F. S. (2016). Characterizing the morbid genome of ciliopathies. *Genome Biol, 17*(1), 242. doi:10.1186/s13059-016-1099-5

Sharp, A. M., Messiaen, L. M., Page, G., Antignac, C., Gubler, M. C., Onuchic, L. F., . . . Guay-Woodford, L. M. (2005). Comprehensive genomic analysis of PKHD1 mutations in ARPKD cohorts. *J Med Genet, 42*(4), 336-349. doi:10.1136/jmg.2004.024489

Shi, Y., Su, Y., Lipschutz, J. H., & Lobo, G. P. (2017). Zebrafish as models to study ciliopathies of the eye and kidney. *Clin Nephrol Res, 1*(1), 6-9. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29553143

Shivanna, M., Anand, M., Chakrabarti, S., & Khanna, H. (2019). Ocular Ciliopathies: Genetic and Mechanistic Insights into Developing Therapies. *Curr Med Chem, 26*(17), 3120-3131. doi:10.2174/0929867325666180917102557

Silva, E. D., & Pinazo-Duran, M. D. (2013). The ciliopathies and their relationship with ophthalmology. *Arch Soc Esp Oftalmol, 88*(5), 165-167. doi:10.1016/j.oftal.2013.04.002

Slaats, G. G., & Giles, R. H. (2015). Are renal ciliopathies (replication) stressed out? *Trends Cell Biol, 25*(6), 317-319. doi:10.1016/j.tcb.2015.03.005

Smolović, B., D, M., A, H., G, B., & B, P. (2018). The Role of Next Generation Sequencing in the Differential Diagnosis of Caroli's Syndrome. *Balkan J Med Genet, 21*(2), 49-53. doi:10.2478/bjmg-2018-0027

Soliman, N. A., Hildebrandt, F., Otto, E. A., Nabhan, M. M., Allen, S. J., Badr, A. M., . . . El-Kiky, H. (2012). Clinical characterization and NPHP1 mutations in nephronophthisis and associated ciliopathies: a single center experience. *Saudi J Kidney Dis Transpl, 23*(5), 1090-1098. doi:10.4103/1319-2442.100968

Song, Z., Zhang, X., Jia, S., Yelick, P. C., & Zhao, C. (2016). Zebrafish as a Model for Human Ciliopathies. *J Genet Genomics, 43*(3), 107-120. doi:10.1016/j.jgg.2016.02.001

Sorokin, S. P. (1968). Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J Cell Sci, 3*(2), 207-230. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/5661997

Sorusch, N., Wunderlich, K., Bauss, K., Nagel-Wolfrum, K., & Wolfrum, U. (2014). Usher syndrome protein network functions in the retina and their relation to other retinal ciliopathies. *Adv Exp Med Biol, 801*, 527-533. doi:10.1007/978-1-4614-3209-8_67

Srivastava, S., Molinari, E., Raman, S., & Sayer, J. A. (2017). Many Genes-One Disease? Genetics of Nephronophthisis (NPHP) and NPHP-Associated Disorders. *Front Pediatr, 5*, 287. doi:10.3389/fped.2017.00287

Srivastava, S., & Sayer, J. A. (2014). Nephronophthisis. *J Pediatr Genet, 3*(2), 103-114. doi:10.3233/pge-14086

Steyaert, W., Callens, S., Coucke, P., Dermaut, B., Hemelsoet, D., Terryn, W., & Poppe, B. (2018). Future perspectives of genome-scale sequencing. *Acta Clin Belg, 73*(1), 7-10. doi:10.1080/17843286.2017.1413809

Stokman, M. F., Bijnsdorp, I. V., Schelfhorst, T., Pham, T. V., Piersma, S. R., Knol, J. C., . . . Renkema, K. Y. (2019). Changes in the urinary extracellular vesicle proteome are associated with nephronophthisis-related ciliopathies. *J Proteomics, 192*, 27-36. doi:10.1016/j.jprot.2018.07.008

Stokman, M. F., Renkema, K. Y., Giles, R. H., Schaefer, F., Knoers, N. V., & van Eerde, A. M. (2016). The expanding phenotypic spectra of kidney diseases: insights from genetic studies. *Nat Rev Nephrol, 12*(8), 472-483. doi:10.1038/nrneph.2016.87

Stokman, M. F., van der Zwaag, B., van de Kar, N., van Haelst, M. M., van Eerde, A. M., van der Heijden, J. W., . . . Lilien, M. R. (2018). Clinical and genetic analyses of a Dutch cohort of 40 patients with a nephronophthisis-related ciliopathy. *Pediatr Nephrol, 33*(10), 1701-1712. doi:10.1007/s00467-018-3958-7

Szymanska, K., Berry, I., Logan, C. V., Cousins, S. R., Lindsay, H., Jafri, H., . . . Johnson, C. A. (2012). Founder mutations and genotype-phenotype correlations in Meckel-Gruber syndrome and associated ciliopathies. *Cilia, 1*(1), 18. doi:10.1186/2046-2530-1-18

Tabler, J. M., Barrell, W. B., Szabo-Rogers, H. L., Healy, C., Yeung, Y., Perdiguero, E. G., . . . Liu, K. J. (2013). Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF-related syndromes. *Dev Cell, 25*(6), 623-635. doi:10.1016/j.devcel.2013.05.021

Tadmouri, G. O., Nair, P., Obeid, T., Al Ali, M. T., Al Khaja, N., & Hamamy, H. A. (2009). Consanguinity and reproductive health among Arabs. *Reprod Health, 6*, 17. doi:10.1186/1742-4755-6-17

Tan, K., Liu, P., Pang, L., Yang, W., & Hou, F. (2018). A human ciliopathy with polycystic ovarian syndrome and multiple subcutaneous cysts: A rare case report. *Medicine (Baltimore), 97*(50), e13531. doi:10.1097/md.0000000000013531

Tan, P. L., Barr, T., Inglis, P. N., Mitsuma, N., Huang, S. M., Garcia-Gonzalez, M. A., . . . Katsanis, N. (2007). Loss of Bardet Biedl syndrome proteins causes defects in

peripheral sensory innervation and function. *Proc Natl Acad Sci U S A, 104*(44), 17524-17529. doi:10.1073/pnas.0706618104

Tan, Y. C., Michaeel, A., Blumenfeld, J., Donahue, S., Parker, T., Levine, D., & Rennert, H. (2012). A novel long-range PCR sequencing method for genetic analysis of the entire PKD1 gene. *J Mol Diagn, 14*(4), 305-313. doi:10.1016/j.jmoldx.2012.02.007

Taub, M. A., Corrada Bravo, H., & Irizarry, R. A. (2010). Overcoming bias and systematic errors in next generation sequencing data. *Genome Medicine, 2*(12), 87. doi:10.1186/gm208

The International Polycystic Kidney Disease Consortium. (1995). Polycystic kidney disease: the complete structure of the PKD1 gene and its protein. The International Polycystic Kidney Disease Consortium. *Cell, 81*(2), 289-298. doi:10.1016/0092-8674(95)90339-9

Tobin, J. L., & Beales, P. L. (2008). Restoration of renal function in zebrafish models of ciliopathies. *Pediatr Nephrol, 23*(11), 2095-2099. doi:10.1007/s00467-008-0898-7

Toriello, H. V., & Parisi, M. A. (2009). Cilia and the ciliopathies: an introduction. *Am J Med Genet C Semin Med Genet, 151C*(4), 261-262. doi:10.1002/ajmg.c.30230

Torres, V. E., & Harris, P. C. (2009). Autosomal dominant polycystic kidney disease: the last 3 years. *Kidney Int, 76*(2), 149-168. doi:10.1038/ki.2009.128

Torres, V. E., & Harris, P. C. (2019). Progress in the understanding of polycystic kidney disease. *Nat Rev Nephrol, 15*(2), 70-72. doi:10.1038/s41581-018-0108-1

Tory, K., Lacoste, T., Burglen, L., Moriniere, V., Boddaert, N., Macher, M. A., . . . Saunier, S. (2007). High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of NPHP6 and AHI1 mutations in patients with NPHP1 mutations. *J Am Soc Nephrol, 18*(5), 1566-1575. doi:10.1681/asn.2006101164

Trujillano, D., Bullich, G., Ossowski, S., Ballarin, J., Torra, R., Estivill, X., & Ars, E. (2014). Diagnosis of autosomal dominant polycystic kidney disease using efficient PKD1 and PKD2 targeted next-generation sequencing. *Mol Genet Genomic Med, 2*(5), 412-421. doi:10.1002/mgg3.82

United Kingdom Renal Registry. (2013). 15th annual report: Appendix A the UK Renal Registry statement of purpose. *Nephron Clin Pract, 123 Suppl 1*, 195-199. doi:10.1159/000353329

United States Renal Data System (USRD). (2013). Chapter 10: International Comparison. Retrieved from http://www.usrds.org/2014/view/v2_10.aspx

Uytingco, C. R., Green, W. W., & Martens, J. R. (2019). Olfactory Loss and Dysfunction in Ciliopathies: Molecular Mechanisms and Potential Therapies. *Curr Med Chem, 26*(17), 3103-3119. doi:10.2174/0929867325666180105102447

van Reeuwijk, J., Arts, H. H., & Roepman, R. (2011). Scrutinizing ciliopathies by unraveling ciliary interaction networks. *Hum Mol Genet, 20*(R2), R149-157. doi:10.1093/hmg/ddr354

Vilboux, T., Doherty, D. A., Glass, I. A., Parisi, M. A., Phelps, I. G., Cullinane, A. R., . . . Gunay-Aygun, M. (2017). Molecular genetic findings and clinical correlations in 100 patients with Joubert syndrome and related disorders prospectively evaluated at a single center. *Genet Med, 19*(8), 875-882. doi:10.1038/gim.2016.204

Vivante, A., Hwang, D. Y., Kohl, S., Chen, J., Shril, S., Schulz, J., . . . Hildebrandt, F. (2017). Exome Sequencing Discerns Syndromes in Patients from Consanguineous Families with Congenital Anomalies of the Kidneys and Urinary Tract. *J Am Soc Nephrol, 28*(1), 69-75. doi:10.1681/ASN.2015080962

Vujic, M., Heyer, C. M., Ars, E., Hopp, K., Markoff, A., Orndal, C., . . . Harris, P. C. (2010). Incompletely penetrant PKD1 alleles mimic the renal manifestations of ARPKD. *J Am Soc Nephrol, 21*(7), 1097-1102. doi:10.1681/asn.2009101070
Walentek, P., & Quigley, I. K. (2017). What we can learn from a tadpole about ciliopathies and airway diseases: Using systems biology in Xenopus to study cilia and mucociliary epithelia. *Genesis, 55*(1-2). doi:10.1002/dvg.23001
Wang, A. (2011). TMEM216 joins its ciliary cousins in ciliopathies. *Clin Genet, 79*(1), 45-47. doi:10.1111/j.1399-0004.2010.01556_2.x
Wang, S., Zhang, J., Nauli, S. M., Li, X., Starremans, P. G., Luo, Y., . . . Zhou, J. (2007). Fibrocystin/polyductin, found in the same protein complex with polycystin-2, regulates calcium responses in kidney epithelia. *Mol Cell Biol, 27*(8), 3241-3252. doi:10.1128/mcb.00072-07
Wang, X., Harris, P. C., Somlo, S., Batlle, D., & Torres, V. E. (2009). Effect of calcium-sensing receptor activation in models of autosomal recessive or dominant polycystic kidney disease. *Nephrol Dial Transplant, 24*(2), 526-534. doi:10.1093/ndt/gfn527
Wang, X., Ward, C. J., Harris, P. C., & Torres, V. E. (2010). Cyclic nucleotide signaling in polycystic kidney disease. *Kidney Int, 77*(2), 129-140. doi:10.1038/ki.2009.438
Wang, Y., Chen, F., Wang, J., Zhao, Y., & Liu, F. (2019). Two novel homozygous mutations in NPHP1 lead to late onset end-stage renal disease: a case report of an adult nephronophthisis in a Chinese intermarriage family. *BMC Nephrol, 20*(1), 173. doi:10.1186/s12882-019-1372-4
Ward, C. J., Hogan, M. C., Rossetti, S., Walker, D., Sneddon, T., Wang, X., . . . Harris, P. C. (2002). The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet, 30*(3), 259-269. doi:10.1038/ng833
Ward, C. J., Wu, Y., Johnson, R. A., Woollard, J. R., Bergstralh, E. J., Cicek, M. S., . . . Boardman, L. A. (2011). Germline PKHD1 mutations are protective against colorectal cancer. *Hum Genet, 129*(3), 345-349. doi:10.1007/s00439-011-0950-8
Waters, A. M., & Beales, P. L. (2011). Ciliopathies: an expanding disease spectrum. *Pediatr Nephrol, 26*(7), 1039-1056. doi:10.1007/s00467-010-1731-7
Wheatley, D. N. (1995). Primary cilia in normal and pathological tissues. *Pathobiology, 63*(4), 222-238. doi:10.1159/000163955
Wheway, G., Genomics England Research, C., & Mitchison, H. M. (2019). Corrigendum: Opportunities and Challenges for Molecular Understanding of Ciliopathies-The 100,000 Genomes Project. *Front Genet, 10*, 569. doi:10.3389/fgene.2019.00569
Wheway, G., Lord, J., & Baralle, D. (2019). Splicing in the pathogenesis, diagnosis and treatment of ciliopathies. *Biochim Biophys Acta Gene Regul Mech*, 194433. doi:10.1016/j.bbagrm.2019.194433
Wheway, G., Mitchison, H. M., & Genomics England Research, C. (2019). Opportunities and Challenges for Molecular Understanding of Ciliopathies-The 100,000 Genomes Project. *Front Genet, 10*, 127. doi:10.3389/fgene.2019.00127
Wheway, G., Nazlamova, L., Turner, D., & Cross, S. (2019). 661W Photoreceptor Cell Line as a Cell Model for Studying Retinal Ciliopathies. *Front Genet, 10*, 308. doi:10.3389/fgene.2019.00308
Wheway, G., Parry, D. A., & Johnson, C. A. (2014). The role of primary cilia in the development and disease of the retina. *Organogenesis, 10*(1), 69-85. doi:10.4161/org.26710
White, D. R., Ganesh, A., Nishimura, D., Rattenberry, E., Ahmed, S., Smith, U. M., . . . Maher, E. R. (2007). Autozygosity mapping of Bardet-Biedl syndrome to 12q21.2

and confirmation of FLJ23560 as BBS10. *Eur J Hum Genet, 15*(2), 173-178. doi:10.1038/sj.ejhg.5201736
Wingfield, J. L., Lechtreck, K. F., & Lorentzen, E. (2018). Trafficking of ciliary membrane proteins by the intraflagellar transport/BBSome machinery. *Essays Biochem, 62*(6), 753-763. doi:10.1042/ebc20180030
Wolf, M. T. (2015). Nephronophthisis and related syndromes. *Curr Opin Pediatr, 27*(2), 201-211. doi:10.1097/mop.0000000000000194
Wood, C. R., Huang, K., Diener, D. R., & Rosenbaum, J. L. (2013). The cilium secretes bioactive ectosomes. *Curr Biol, 23*(10), 906-911. doi:10.1016/j.cub.2013.04.019
Yamamura, T., Morisada, N., Nozu, K., Minamikawa, S., Ishimori, S., Toyoshima, D., . . . Iijima, K. (2017). Rare renal ciliopathies in non-consanguineous families that were identified by targeted resequencing. *Clin Exp Nephrol, 21*(1), 136-142. doi:10.1007/s10157-016-1256-x
Yannakoudakis, B. Z., & Liu, K. J. (2013). Common skeletal features in rare diseases: New links between ciliopathies and FGF-related syndromes. *Rare Dis, 1*, e27109. doi:10.4161/rdis.27109
Yildiz, O., & Khanna, H. (2012). Ciliary signaling cascades in photoreceptors. *Vision Res, 75*, 112-116. doi:10.1016/j.visres.2012.08.007
Yis, U., Dirik, M. A., & Dirik, E. (2014). Clinical syndromes or ciliopathies associated with molar tooth sign. *Pediatr Neurol, 50*(6), e16. doi:10.1016/j.pediatrneurol.2013.11.023
Yoshiba, S., Shiratori, H., Kuo, I. Y., Kawasumi, A., Shinohara, K., Nonaka, S., . . . Hamada, H. (2012). Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science, 338*(6104), 226-231. doi:10.1126/science.1222538
Yu, F., Ran, J., & Zhou, J. (2016). Ciliopathies: Does HDAC6 Represent a New Therapeutic Target? *Trends Pharmacol Sci, 37*(2), 114-119. doi:10.1016/j.tips.2015.11.002
Zacchia, M., Marchese, E., Trani, E. M., Caterino, M., Capolongo, G., Perna, A., . . . Capasso, G. (2019). Proteomics and metabolomics studies exploring the pathophysiology of renal dysfunction in autosomal dominant polycystic kidney disease and other ciliopathies. *Nephrol Dial Transplant*. doi:10.1093/ndt/gfz121
Zaghloul, N. A., & Katsanis, N. (2011). Zebrafish assays of ciliopathies. *Methods Cell Biol, 105*, 257-272. doi:10.1016/B978-0-12-381320-6.00011-4
Zaucke, F., Boehnlein, J. M., Steffens, S., Polishchuk, R. S., Rampoldi, L., Fischer, A., . . . Wolf, M. T. (2010). Uromodulin is expressed in renal primary cilia and UMOD mutations result in decreased ciliary uromodulin expression. *Hum Mol Genet, 19*(10), 1985-1997. doi:10.1093/hmg/ddq077
Zerres, K., Mucher, G., Becker, J., Steinkamm, C., Rudnik-Schoneborn, S., Heikkila, P., . . . Guay-Woodford, L. M. (1998). Prenatal diagnosis of autosomal recessive polycystic kidney disease (ARPKD): molecular genetics, clinical experience, and fetal morphology. *Am J Med Genet, 76*(2), 137-144.
Zhang, J., Wu, M., Wang, S., Shah, J. V., Wilson, P. D., & Zhou, J. (2010). Polycystic kidney disease protein fibrocystin localizes to the mitotic spindle and regulates spindle bipolarity. *Hum Mol Genet, 19*(17), 3306-3319. doi:10.1093/hmg/ddq233
Zhang, M. Z., Mai, W., Li, C., Cho, S. Y., Hao, C., Moeckel, G., . . . Wu, G. (2004). PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. *Proc Natl Acad Sci U S A, 101*(8), 2311-2316. doi:10.1073/pnas.0400073101

Zhang, Q., Hu, J., & Ling, K. (2013). Molecular views of Arf-like small GTPases in cilia and ciliopathies. *Exp Cell Res, 319*(15), 2316-2322. doi:10.1016/j.yexcr.2013.03.024

Zhang, W., Taylor, S. P., Ennis, H. A., Forlenza, K. N., Duran, I., Li, B., . . . Cohn, D. H. (2018). Expanding the genetic architecture and phenotypic spectrum in the skeletal ciliopathies. *Hum Mutat, 39*(1), 152-166. doi:10.1002/humu.23362

Zhang, Y., Seo, S., Bhattarai, S., Bugge, K., Searby, C. C., Zhang, Q., . . . Sheffield, V. C. (2014). BBS mutations modify phenotypic expression of CEP290-related ciliopathies. *Hum Mol Genet, 23*(1), 40-51. doi:10.1093/hmg/ddt394

Zhao, C., & Malicki, J. (2011). Nephrocystins and MKS proteins interact with IFT particle and facilitate transport of selected ciliary cargos. *EMBO J, 30*(13), 2532-2544. doi:10.1038/emboj.2011.165

Zhao, X., Paterson, A. D., Zahirieh, A., He, N., Wang, K., & Pei, Y. (2008). Molecular diagnostics in autosomal dominant polycystic kidney disease: utility and limitations. *Clin J Am Soc Nephrol, 3*(1), 146-152. doi:10.2215/CJN.03430807

Zur Lage, P., Newton, F. G., & Jarman, A. P. (2019). Survey of the Ciliary Motility Machinery of Drosophila Sperm and Ciliated Mechanosensory Neurons Reveals Unexpected Cell-Type Specific Variations: A Model for Motile Ciliopathies. *Front Genet, 10*, 24. doi:10.3389/fgene.2019.00024