THE USE OF NEXT GENERATION SEQUENCING IN RARE DISEASE

by

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ABSTRACT

Introduction: High throughput next generation sequencing (NGS) strategies such as whole exome sequencing (WES) are frequently used in medical research to identify the molecular cause of Mendelian genetic disease. WES, or clinical exome sequencing strategies are now being adopted into clinical genetics practice. This study focuses on the application of WES for genetic diagnosis in a group of mainly consanguineous families with rare phenotypes for which an autosomal recessively inherited disease was suspected but the molecular basis was unknown.

Materials and methods: Families were recruited retrospectively from a previous research cohort (the National Autozygosity Mapping study) and prospectively from the Birmingham Women's and Children's NHS Foundation Trust. WES was subsequently performed.

Results: 35 families with rare genetic disorders were studied by WES (in 9 families a single individual underwent sequencing). After bioinformatics analysis of WES data and detailed reassessment of the phenotype a molecular genetic diagnosis was reached in 15 families (42.9%).

Conclusion: WES is an effective strategy for identifying the molecular basis of recessively inherited disorders in consanguineous families. The combination of WES with detailed phenotyping significantly improved variant interpretation and diagnostic yield over WES alone.

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Table of Contents

LIST OF ILLUSTRATIONS

LIST OF TABLES

ABBREVIATIONS

ACMG	American College of Medical Genetics and Genomics
ADPKD	Autosomal dominant polycystic kidney disease
ALS4	Juvenile Amyotrophic Lateral Sclerosis type 4
ANA	Anti-nuclear antibodies
ANCA	Anti-neutrophil cytoplasmic antibodies
AOA2	Ataxia with Oculomotor Ataxia type 2
ARPKD	Autosomal recessive kidney disease
bp	Base pairs
CAPS	Cryopyrin-associated periodic syndromes
CRP	C-Reactive Protein
DDD	Deciphering Developmental Disorders study
EDS	Ehlers Danlos syndrome
EIEE	Early Infantile Epileptic Encephalopathy
ESRF	End Stage Renal Failure
FHL	Familial Haemophagocytic Lymphohistocytosis
FMF	Familial Mediterranean fever
FSGS	Focal Segmental Glomerulosclerosis

GeCIPs	Genomics England Clinical Interpretation Partnerships
HAART	Highly Active Antiretroviral Therapy
HIDS	Hyper-IgD syndrome
HIV	Human Immunodeficiency Virus
HPO	Human Phenotype Ontology
HUVS	Hypocomplementemic urticarial vasculitis syndrome
LUBAC	Linear Ubiquitin Chain Assembly Complex
MKD	Mevalonic kinase deficiency
MI	Myocardial Infarction
MSL	Multiple Symmetrical Lipomatosis
NCS	Nerve conduction studies
NF-κB	Nuclear factor κB
NHS	National Health Service
nt	Nucleotide
oe	Observed over expected ratio (constraint score)
OMIM	Online Mendelian Inheritance in Man database
ORAS	*OTULIN* related autoinflammatory syndrome
PCR	Polymerase chain reaction

pLI	The probability of being loss of function intolerant
SAVI	STING-associated vasculopathy with onset in infancy
SLE	Systemic lupus erythematosus
SMA	Spinal muscular atrophy
TPN	Total parenteral nutrition
TRAPS	TNF receptor-associated periodic syndrome
VCF	Variant Calling File
VLCFAs	Very Long Chain Fatty Acids
WCC	White Cell Count
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing

CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction and Overview

Clinical Geneticists diagnose and manage patients with rare genetic conditions. A condition is classed as a rare disease in the UK, if it affects 1 in 2000 people or less. Next generation sequencing has proved to be an invaluable tool in the identification of genetic rare diseases for both gene discovery and identification of disease-causing variants. This study will utilise next generation sequencing to identify the disease-causing variant in families identified from a clinical genetics department with presumed genetic rare disease.

The Human Genome project was a huge international collaborative project to sequence the entire human genome, which involved reading all 3.2 billion base pairs. It was completed in April 2003, after 10 years of global collaboration. This project provided the foundations to our current understanding of the human genome and genetic disease. With the advent of next generation sequencing, whole exome sequencing (WES), and even whole genome sequencing (WGS), can be performed in a matter of days rather than years. Human genetic rare disease is caused by rare variations within the genomic sequence. The challenge is to identify the needle in the haystack: the rare

disease-causing variant amongst the many thousands of genetic variants found in individuals, many of which represent benign normal variation.

The use of WES to identify novel genes causing human genetic disease was first described in 2009 (Ng et al., 2009). The same group subsequently used this method to prove that pathogenic variants in the *DHODH* gene cause Miller syndrome (Ng et al., 2010a). This was a major landmark for gene discovery of human genetic rare disease. Researchers can use WES variant prioritisation methods, based around this proof of concepts method, to identify novel genetic causes for rare disease. There are now 6,318 phenotypes for which the molecular basis is known on the Online Mendelian Inheritance in Man Morbid map ("OMIM - Online Mendelian Inheritance in Man," 19th December 2018), which has increased rapidly since this landmark paper in 2009. This study will utilise WES to identify the disease-causing variants in a group of patients with rare genetic disease.

Identifying the cause of genetic rare disease is extremely important. The patient is provided with a diagnosis, an explanation for the affected individual's condition. This may often provide the patient, and family, with more information regarding the clinical phenotype associated with the condition, appropriate management plan, prognostic information, and in some instances treatment. This can also be important to the entire family. Family relatives may choose to have predictive testing to clarify if they will also develop the condition in question, or they may want to clarify the chance of their

offspring having the same condition. Individuals may want to clarify if their baby is affected using prenatal testing, such as chorionic villous sampling or amniocentesis, to identify whether the fetus has the disease-causing variant in the family.

Newer technologies in the form of non-invasive prenatal diagnosis (NIPD) enable identification of the disease-causing variant from the free fetal DNA released into the mothers' blood stream during pregnancy from a maternal blood sample, without the need to an invasive test and the associated small risk of miscarriage. NIPD is regularly used for fetal sexing in X-linked recessive conditions, and diagnosis of conditions such as thanatophoric dysplasia, spinal muscular atrophy (SMA), and cystic fibrosis. Non-invasive prenatal testing or screening for the common trisomies 13, 18, and 21 is also available. Bespoke non-invasive prenatal diagnosis is also available in some instances, such as *de novo* heterozygous variants to clarify risk to subsequent offspring due to gonadal mosaicism. Individuals may also choose to have pre-implantation genetic testing. This utilises *in-vitro* fertilisation techniques to create an embryo. Only the unaffected embryos are then implanted back into the mother's uterus.

Importantly, identifying the genetic cause of rare diseases helps inform our understanding of biological pathway and subsequently our understanding of more common disease. In autoinflammatory conditions, for example, knowing which pathway is affected, can influence which immune modulating treatment is used. There are a handful of genetic rare diseases for which there is a targeted genetic therapy available.

One example of this is Leber's congenital amaurosis secondary to a variant in the RPE65 gene. Viral vectors containing the normal RPE65 genetic sequence are injected into the eye of affected individuals. This has been shown to improve visual function in this group of patients (Sharif and Sharif, 2017).

1.2 Deciphering Developmental Disorders (DDD) Study

The Deciphering Developmental Disorders (DDD) study is a landmark large-scale UK based research study delivered by the Wellcome Sanger institute in Cambridge with collaboration of the clinicians in the 24 NHS Regional Genetics Centres in the UK and Republic of Ireland. This ran during the time of my study and some of the lessons learnt from DDD have been relevant to this study. The aim of the DDD study was similarly, to use new genetic technologies to identify the genetic cause for a patient's developmental disorder. More than 12,000 patients, both adults and children, with an undiagnosed severe developmental disorder were recruited over a 4-year period in a trio with both parents ideally (PMC, 2017; Wright et al., 2018). The study employed high resolution array and then WES to identify the underlying cause of their disease (Wright et al., 2015). The DDD study reported back likely disease-causing variants for patients to the clinicians managing their care, as well as identifying new disease genes and novel genetic mechanisms (DDD study, PMC, 2017; Wright et al., 2018, 2015).

The initial diagnostic yield from the DDD study was 27%, reaching a diagnosis in 311 out of the first 1133 patients recruited (Wright et al., 2015). Given the rapid rate of new gene discovery, DDD have regularly re-analysed the genomic data to constantly improve the diagnostic yield for clinicians, and ultimately the patients. However, there are logistical challenges to this, including the interval frequency of re-analysis, the logistics around re-analysis, the capacity of the clinicians to re-contact the recruited patients, and the timescale involved (Wright et al., 2018). Disease-causing variants may have been missed due to the limitation of our knowledge of the gene originally, inappropriate filtering of a variant, low depth of sequencing data and erroneous annotation or variant calling of the genomic data in the analysis pipeline (Wright et al., 2018). Importantly a percentage of patients' variants previously thought disease causing were re-classified as uncertain or benign likely related to the improved benign variant population databases (Wright et al., 2018). It can be challenging for clinicians and patients to receive a diagnostic result after an initial negative report, and even more challenging to remove a diagnostic label given to a patient. The subsequent diagnostic yield increased to 40%, with a full or partial diagnosis made in 454 out of 1133 initial patients (Wright et al., 2018).

1.3 Autosomal Recessive Disease

About 80% of the diagnoses made through DDD were *de novo* heterozygous variants, which were not present in either of the patient's parents (Wright et al., 2018). The main focus for this study is autosomal recessive disease in consanguineous families.

A consanguineous relationship is defined as a relationship between individuals biologically related as second cousins or closer (Black ML, 2015). First cousin marriage unions are the commonest form of consanguineous union worldwide, meaning that each parent shares 1/8 of their genome from a common ancestor. Their offspring will be homozygous at 6.25% of all gene loci analysed, over the baseline level in the general population (Black ML, 2015). In the UK as a whole, the frequency of consanguinity is reported to be less than 1%. Within the UK Pakistani community, an estimated 50-60% of marriages are reportedly consanguineous (Bittles, 2001). In a study by Bundey et al (1991), the consanguinity rates in the Birmingham Pakistani population interviewed were 69%.

Consanguinity is associated with double the baseline risk of congenital malformations (Sheridan et al., 2013). Bundey et al found a 1 in 100 risk of a lethal malformation for babies born to parents of Pakistani origin, mostly felt to be as a consequence of autosomal recessive disease (Bundey et al., 1991). The West Midlands population is therefore well suited to identify consanguineous families with autosomal recessive diseases.

Once a novel gene has been identified, functional work is often required to demonstrate causality. Animal models such as the mouse and zebrafish may be used to demonstrate the phenotypic effects of genetic variants in genes of interest. A knockout mouse model describes a mouse model with a biallelic loss of function variant in the

gene of interest. The loss of function variants disrupt the protein encoded by the gene causing complete loss of function of that protein. There are online databases describing some of the phenotypes seen in different mouse models. Similarly, researchers have reviewed population-based WES results from consanguineous populations to better understand the human knockout phenotype (MacArthur et al., 2012; Narasimhan et al., 2016; Saleheen et al., 2017; Sulem et al., 2015). In other words, studying humans with complete loss of function of a specific gene, and its phenotypic consequences, provides better insight into the function of these genes (Saleheen et al., 2017). One study described 253 genes with homozygous loss of function variants seen in at least one healthy individual, which they called LoF-tolerant genes (MacArthur et al., 2012). They found they were less well-conserved, common variants with an allele frequency of >1%, with minimal health consequences (MacArthur et al., 2012). In a study of 104,220 individuals in the Icelandic population, they identified 8,041 individuals (7.7%) with 1 gene completely knocked out by loss-of-function variants with a MAF under 2% (Sulem et al., 2015). Thus, studying population genomic data for human knockout model can be extremely informative in our understanding of the genome. Researchers can identify LoF tolerant genes which would not cause rare autosomal recessive disease.

1.4. Next generation sequencing methods

Genomics is the study of all 3.2 billion base pairs contained within the human genome. The original human genome project took many years to sequence using Sanger sequencing. The newer method of sequencing, next generation sequencing, has massively decreased the length of time to sequence the human genome. There are different mechanisms for next generation sequencing, which I have summarised in the table below:

Table 1.1: Comparison of next generation sequencing methods

Sequencing technology	Roche 454	Illumina	Ion Torrent	Nanopore
Amplification	Emulsion PCR: clusters on beads	Surface PCR: clusters by bridge amplification	Emulsion PCR: clusters on beads	Not required
Sequencing location	Beads on high density plate	Clusters arranged on flowcell	Beads in sensor wells on semi-conductor chip	DNA threaded through microscopic pore
Chemistry	Pyro -sequencing	Reversible chain terminator	Semi-conductive	Real time sequencing
Detection	Flash specific to nucleotide (nt)	Differentially coloured nt	Change in pH specific to nt	Voltage change specific to nt
Read length	>300bp	100bp	200bp	100,000bp
Advantage	Long read length	High throughput	De novo sequencing	High volume sequencing at low cost
Dis-advantages	High error rate	Short reads	Higher error rate	Higher error rate (improving)

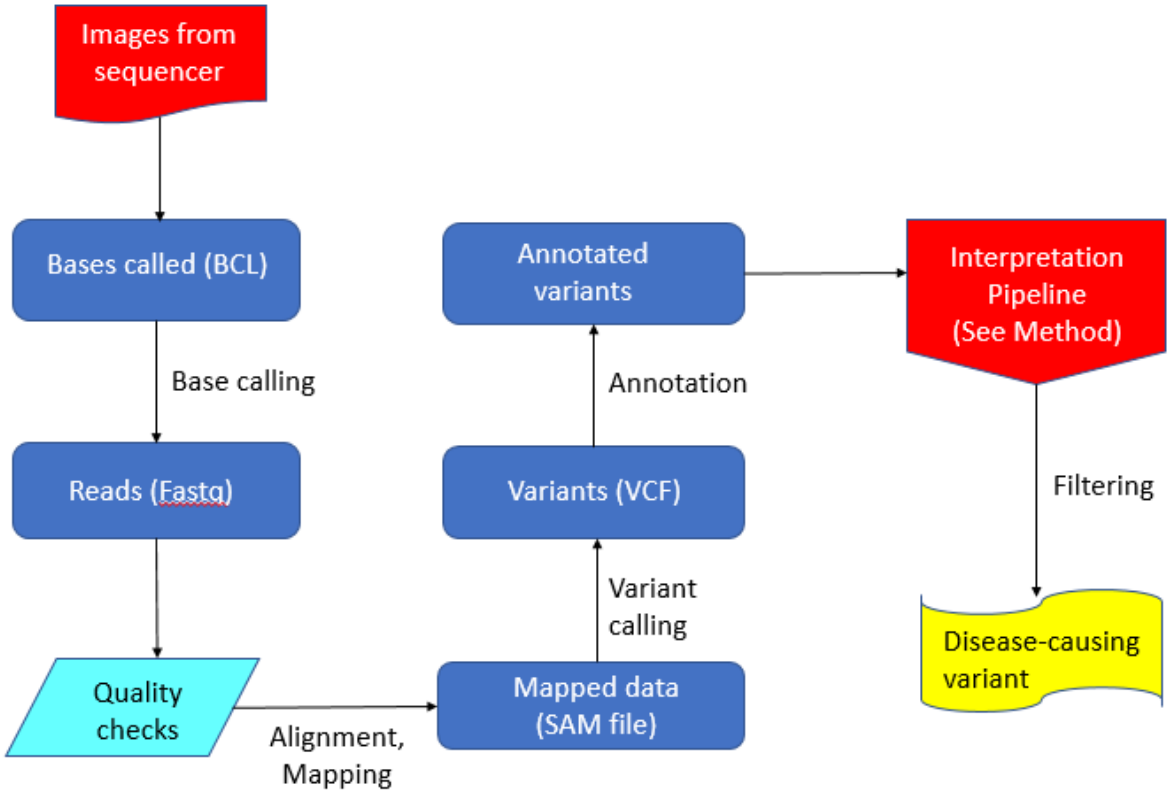
Illumina sequencing was used within this project. Library preparation is first required before sequencing can commence. The DNA for sequencing, is initially cleaved into short reads of about 100-150 base pairs (bp). These short reads are ligated with generic adaptors, which allow the reads to anneal to a slide. Polymerase chain reaction (PCR) is carried out to amplify each read, creating spots on the slide with many copies of each read. The reads are then separated into single strands to be sequenced.

To sequence the reads, the slide is then exposed to fluorescently labelled nucleotides, each base differentially labelled, and DNA polymerase. A chain terminator is also attached to each nucleotide to ensure only one base is added in each cycle. An image is taken of the slide demonstrating a fluorescent signal at each read location, which indicates the base added during that cycle. Preparation for the next cycle subsequently begins, including the removal of the chain terminators, and fluorescent signal. This is repeated until the reads have been re-sequenced, adding one nucleotide at a time with an image taken at the end of each cycle. The sequence reads are the same length, dictating the number of cycles carried out during sequencing. Base calling is then performed for each read based on the fluorescent image taken in each cycle. The DNA sequence is then constructed for each read.

The short reads subsequently need to be aligned to the correct place in the genome and calling of the variants for each individual sample made. This process is called a bioinformatic pipeline (see figure 1.1 below). At each step in the pipeline there are

many different tools that can be utilise depending on the required outcome. The tools used in this project are detailed in methods.

Figure 1.1: Bioinformatics pipeline summary



Legend: BCL base calling file, SAM sequence alignment mapping, VCF variant call file

When sequencing a human genome, there are different methods that can be employed. Whole Genome Sequencing (WGS) involves reading every 3.2 billion base pair. Whole Exome Sequencing (WES), which will be used in this project, reads the exome or the coding sequence of the genome. Other methods can include Clinical Exome

Sequencing, which focuses on reading the coding portion of the genome, but more specially focused on the genes for which a human disease phenotype is described (also known as Mendeliome). Finally, gene panels can be used to look at specific human phenotypes, for example an aortopathy gene panel would involve sequencing the known genes associated with an aortopathy clinical phenotype.

Identification of novel genes and phenotypes is potentially possible within this study, and therefore gene panels and clinical exome sequencing was not suitable. WES was considered overall to be the most cost-effective way of sequencing the genome to maximise the novel variant identification and minimise incidental findings.

1.5. Analysis of WES results

Analysing the results generated from WES required careful bioinformatic analysis. Each individual patient will have 20,000 to 30,000 variants detected on WES, the majority of which, are benign variants (Robinson et al., 2011). Bioinformatic methods for filtering variants need to be employed to identify the metaphorical “needle in a haystack”, the molecular cause for Mendelian disease in the patient amongst the many thousands of sequence variants seen in an individual (Ku et al, 2011; Bamshad et al, 2011). A careful line must be drawn between rigorously filtering of variants and potentially filtering out the disease-causing variant, without setting the filtering parameters so low that the investigator has too many variants to review. The filtering

strategies employed are well described in the literature. They rely on several assumptions, which means that it is still possible to inadvertently filter out the disease-causing variant (Bamshad et al., 2011; Ku et al., 2011; Wright et al., 2015). This process often includes filtering steps outlined below.

1. Population variant databases

   Everyone has many thousands of benign sequence variants. Population variant databases describe the frequency of these variants in the general population to aid sequence interpretation. These publically-available databases include dbSNP, 1000 Genomes, ESP, ExAC and more recently gnomAD (1000 Genomes Project Consortium et al., 2015; Fu et al., 2013; Lek et al., 2016; Sherry et al., 2001). They are an important resource for the interpretation of variants seen in patients undergoing WES for identification of their disease-causing variant (Lek et al., 2016). GnomAD found on average one sequence variant for every 8 bp within the exome due to the recurrence of variation (Lek et al., 2016). Sequence variants found commonly in the general population are more likely to be benign. Researchers are therefore able to filter out common variants by looking at the Minor Allele Frequency (MAF) (Bamshad et al., 2011; Ku et al., 2011). A MAF of less than 1% is commonly used (Wright et al., 2015). When looking for *de novo* heterozygous pathogenic variants a more rigorous MAF cut off less than <0.0005 (0.05%) may be employed (Wright et al., 2018).

Care needs to be taken when using this strategy. Pathological variants can also be found in these databases, potentially resulting in disease-causing variants being rule out erroneously. When utilising these datasets it is important to consider the population used for the sequence data. GnomAD excludes all individuals and their first-degree relatives with severe paediatric disease, but if the disease being studied is later onset, it is possible the variant may still be present in the database, albeit with a low frequency if studying rare disease (Lek et al., 2016). In addition, it may not include the ethnicity of the patient being studied. For autosomal recessive disorders, when using a MAF of less than 1%, it is important to consider whether the likely carrier frequency could be over this threshold in the population of interest, leading to erroneous exclusion of the disease-causing variant (Bamshad et al, 2011; Rabbani et al, 2012).

2. Disease Variant Databases

In addition to population variation databases, there are also a rich source of pathogenic variant databases. These may be disease or gene specific such as the UMD-FBN1 mutation database, or general including ClinVar and Human Gene Mutation Database (Collod-Béroud et al., 2003; Landrum et al., 2018; Stenson et al., 2014). ClinVar also rates the variants according to pathogenicity. If a variant is listed as pathogenic in one of these databases, this can be extremely useful for variant identification. Similarly, to the

population databases, the variants are not always classified correctly on the site, and therefore other sources of information should also be consulted.

3. Synonymous variants

Synonymous variants occur when the nucleotide substitution alters the codon to a different codon encoding the same amino acid and so resulting the same protein sequence (Livingstone et al., 2017). Synonymous changes are intuitively filtered out (Ku et al., 2011; Wright et al., 2015). There are however an increasing number of synonymous pathogenic variants reported in HGMD (Livingstone et al., 2017; Stenson et al., 2014). Synonymous variants may, for example, alter splicing, gene expression or DNA methylation patterns through altered CpG islands (Livingstone et al., 2017). There are a vast number of synonymous variants in an individual genome and predicting which synonymous variants are pathogenic can be very difficult.

4. Prediction software

Bioinformatic software tools exist to predict whether a non-synonymous substitution variant is benign or pathogenic. These include tools such as SIFT, POLYPHEN, and Mutation Taster (Adzhubei et al., 2010; Ku et al., 2011; Ng et al., 2010b; Rabbani et al., 2012; Schwarz et al., 2010; Wright et al., 2015). They look at different factors including species conservation at the nucleotide and amino acid level, the physio-chemical amino acid change involved, and the predicted effect on the structure and function of the protein

(Adzhubei et al., 2010; Ku et al., 2011; Ng et al., 2010b; Rabbani et al., 2012; Schwarz et al., 2010). These methods are predictive and therefore should be considered as supporting evidence for pathogenicity (Richards et al., 2015).

5. Group of patients with shared phenotype

Comparing WES results for groups of similarly affected, unrelated individuals to look for candidate pathogenic variants within a gene shared by all affected patients can be an effective mechanism to identify novel disease-causing genes (Ku et al, 2011; Rabbani et al, 2012). However, genetic (locus) heterogeneity exists in many Mendelian disorders, for example Coffin-Siris syndrome, and therefore variants within genes shared by the majority of affected individuals or genes within the same biological pathway may also need to be considered (Ku et al, 2011; Rabbani et al, 2012). Patients within this study had different and very rare phenotypes and were therefore unlikely to have variants within the same gene.

6. Family segregation studies

Family segregation studies can be very useful to aid identification of the disease-causing variant within a family. Identification of the variant within multiply affected individuals in the same family, especially when these are more distantly related individuals, can be very effective (Ku et al, 2011; Rabbani et al, 2012). Autozygosity mapping studies can be utilised in consanguineous families by mapping the shared regions of homozygosity in

the affected family members and then focussing variant identification from WES within these regions (Bolze et al., 2010). This also includes identifying variants not present in unaffected family members.

7. Trio analysis

Trio analysis is a specific type of family segregation studies. Trio analysis typically involves sequencing the affected individual and both parents. Trio WES analysis is an extremely effective filtering mechanism for identification of likely pathogenic *de novo* heterozygous sequence variants for patients with unaffected parents. It can also be used for other inheritance patterns, such as autosomal recessive, and autosomal dominant with an affected parent, but is most effective for disorders caused by *de novo* heterozygous variants. Trio analysis has been very effectively employed in the DDD study (Wright et al., 2018, 2015). The majority of patients recruited to my project were suspected to have autosomal recessive disease, therefore family segregation studies in general were employed.

8. Phenotype analysis

This involves phenotype-based analysis returning variants that are specific to the patient's disease. Genotype-phenotype based analysis can be key to variant prioritisation. This is a key part of this project and is discussed in more detail in the next section.

9. Constraint score

More recently constraint scores provided in ExaC, and subsequently updated in GnomAD, provide additional information for the interpretation of WES data by quantifying the level of tolerance each gene has to variation (Lek et al., 2016; Samocha et al., 2014). The constraint score used in gnomAD is gene specific. It looks at synonymous, missense and LoF variants separately. For each variant category within each gene the database details the expected number of variants and the observed number of variants. This is used to create a constraint score and Z score from the observed over expected ratio (oe) with a 90% confidence interval (Lek et al., 2016; Samocha et al., 2014).

Benign variants have a higher frequency in the population when compared to disease-causing variants due to natural selection (Lek et al., 2016). A low oe score for a specific variant type means that the gene is under greater selection for that type of variant than a gene with a higher score (Lek et al., 2016). This must be taken in the context of the confidence interval. Positive Z scores suggests the gene has fewer variants than expected and is therefore, more intolerant to variation (Lek et al., 2016). A negative Z scores indicates a gene has more variants than expected and is therefore more tolerant to variation (Lek et al., 2016).

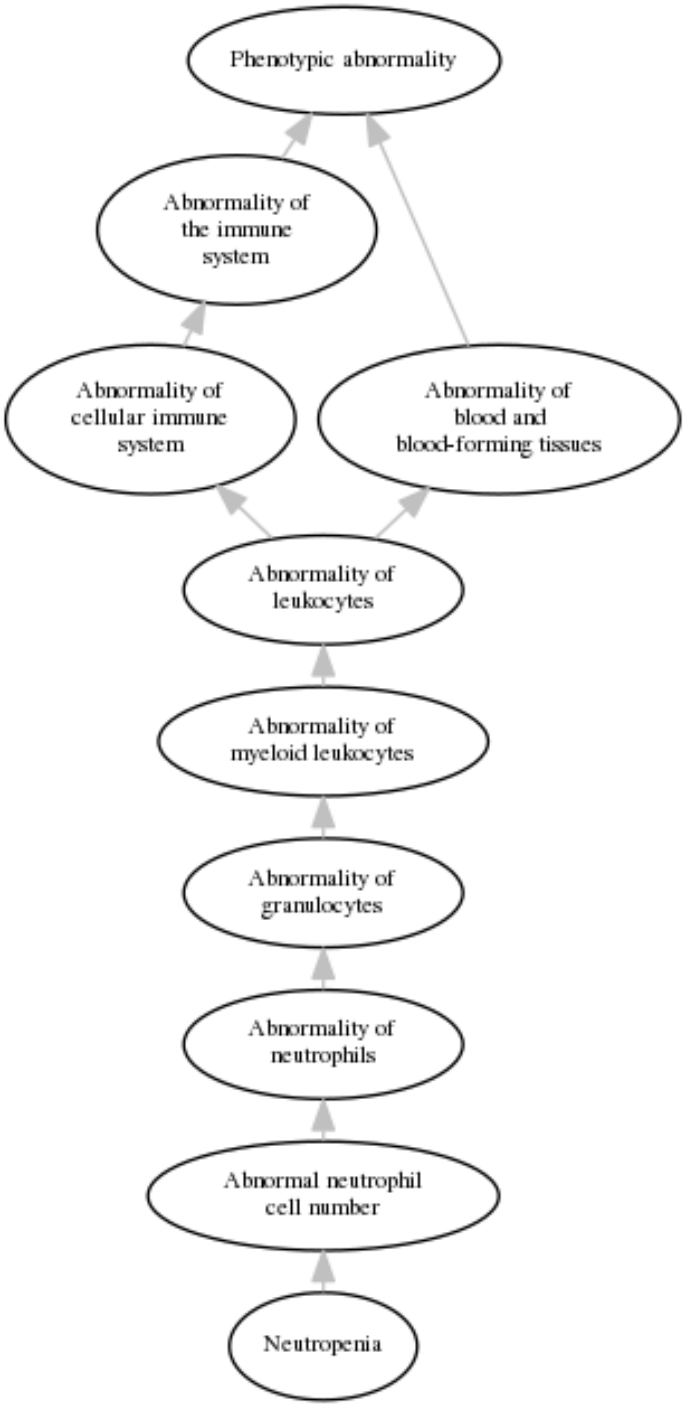
The probability of being loss of function intolerant (pLI) is used for LoF variants and has 3 classes: null where loss of function of one or both copies of the gene is tolerated, recessive where loss of function if both copies of the gene is not tolerated, and haploinsufficient where loss of a single copy of the gene is not tolerated (Lek et al., 2016). Null genes had the expected number of LoF variants, recessive genes had a Z score of around 0.5 or less and severe haploinsufficient genes observed LoF variants less than 10% of the expected variation level with a Z score of 0.1 (Lek et al., 2016). This score has been used more latterly in this project.

1.6. Phenotyping and HPO terms

It was postulated that as next generation sequencing becomes mainstream in medicine, we will see the demise of the importance of accurate phenotyping that has been a key part of clinical genetics practice (particularly for dysmorphology) (Hennekam and Biesecker, 2012). Good phenotyping, however, is still needed to ensure that each disease is fully characterised to allow the patients to receive accurate information about their condition. Expert phenotyping can also aid the interpretation of WES results, requiring a medical geneticists' unique skill of combining clinical and genetic knowledge to make the correct diagnosis (Hennekam and Biesecker, 2012). This was an important manual step within the DDD project pipeline (Wright et al., 2015).

To accurately phenotype on a large scale, precise standardised, universal phenotypic terms are important. The Human Phenotype Ontology (HPO) was created for this purpose (Köhler et al., 2017; Robinson et al., 2008). HPO is now widely used in the medical literature, phenotypic databases including OMIM, and WES/WGS bioinformatic prioritisation software (Köhler et al., 2017; "OMIM - Online Mendelian Inheritance in Man,"; Wright et al., 2015; Zemojtel et al., 2014). Each term within the ontology falls into one of 5 sub-ontologies (Köhler et al., 2017). The main sub-ontology is for phenotypic abnormalities describing a specific phenotypic trait in humans within the ontology (Köhler et al., 2017). This term is part of a tree of terms (ontology), which relates back to the parent term (Köhler et al., 2017). Less specific terms, such as abnormality of the immune system (parent), are found closer to the root, and more specific terms such as neutropenia (child), are found in more distant branches (see figure 1.2)(Köhler et al., 2017). Each sub-ontology or child term can relate back to more than one parent term such as abnormality of leucocytes below (see figure 1.2) (Köhler et al., 2017). The other main sub-ontology trees are mode of inheritance, clinical modifiers such as severity, clinical course and frequency (Köhler et al., 2017). Each term in the ontology is given a unique number starting with HP: followed by 7 digits (Köhler et al., 2017). The HPO is regularly updated. HPO terms are utilised within this project.

Figure 1.2: Example of HPO sub-ontology from phenomizer (Köhler et al., 2017, 2009)



1.7. Incidental findings

An incidental finding in the context of NGS data means the identification of a likely disease-causing sequence variant in a gene not associated with the primary indication for testing. In other words, if a patient has hypertrophic cardiomyopathy and a variant is identified in the *BRCA1* gene, this would be considered an incidental finding. This can create ethical challenges for the researcher and patient regarding the reporting and management of these findings. Extra caution is advised when evaluating and reporting variants identified in healthy or asymptomatic individuals, because these are less likely to be pathogenic, than if detected in a disease specific gene panel (Richards et al., 2015). It is predicted that the penetrance of pathogenic variants would be lower than expected, in an individual with no clinical phenotype for that condition and with no relevant family history (Richards et al., 2015). Despite this, the American College of Medical Genetics and Genomics recommended actively looking for and reporting incidental findings pertaining to a list of 56 genes considered to be clinically actionable, without seeking patient preference (Green et al., 2013). This list has subsequently been updated, the incidental findings referred to as secondary findings because variants in these genes were actively being sought, and an opt-out preference for patient receiving these incidental findings unanimously added (Kalia et al., 2017).

The DDD study has not reported back incidental findings (Wright et al., 2015). A sub-study of the DDD project performed an online survey looking at peoples' views in the reporting of incidental findings. It was completed by 6944 people from 75 different

countries from four groups: 4961 members of the public, 607 genomic researchers, 533 genetic health professionals and 843 other health professionals (Middleton et al., 2015). 88% thought that incidental findings should be made available to research participants, and 31% thought that researchers should actively look for these incidental findings (Middleton et al., 2015). The US Presidential Commission for the Study of Bioethical Issues recommends informing research participants of the findings that might arise from the project, as well as the findings that will not be returned, and thus ensuring informed consent (Weiner, 2014). However, reporting of incidental findings was outside the ethical approval used for my project (this is specified in the consent form) and therefore incidental findings were not to be reported back to the patient in this project.

1.8. Clinical Application of Next Generation Sequencing

WES is being increasingly used in the clinical setting. It can be hugely beneficial by reducing the diagnostic odyssey for patients, particularly those patients with highly genetically heterogeneous conditions, such as intellectual disability syndromes. This diagnostic odyssey is often a slow and costly journey, with patients with rare genetic disorders seeing multiple specialists and undergoing a diverse range of investigations (e.g. imaging, metabolic investigations, genetic tests and, in some patients, invasive testing such as a skin biopsy) (Sawyer et al., 2016). The global rare disease commission quote an average time of about 4.8 years between the onset of symptoms and the diagnosis of a rare disease ("Global Commission to End the Diagnostic Odyssey for Children with a Rare Disease,"). Currently, Sanger sequencing for

individual genes is no longer the baseline, and generally gene panels using next generation sequencing are performed for clinical diagnostic testing in NHS genetics centres. This can become costly if the disease-causing variant is not identified in the first gene panel performed. In this instance clinical exome sequencing or WES are more beneficial as, in the event of negative results for a specific gene panel, additional gene panels can be interrogated *in silico* without having to undertake further molecular genetic studies.

In one of the first studies looking into the clinical application of WES to the clinical setting, Need *et al* identified the likely genetic diagnosis in 6 out of 12 patients recruited from a general genetics clinic (Need et al., 2012). Worthey et al also used WES in the clinical setting to make a diagnosis in a single patient with inflammatory bowel disease (Worthey et al., 2011). Similarly, there have subsequently been many studies demonstrating the utility if WES in the clinical setting. Yang et al had a diagnostic rate of 25.2% in 504 patients using WES in the clinical setting (Yang et al., 2014). Clinical exome sequencing had a diagnostic yield of 26% in 814 patients in California (Lee et al., 2014). Retterer et al reported a diagnostic rate of 28.8% in 3,040 patients using WES in a clinical laboratory (Retterer et al., 2016). This rate would have been higher if WES had been used earlier in the investigation of the patients, because many had been extensively investigated including metabolic, karyotype, microarray, single gene or gene panel testing (Retterer et al., 2016). Several patients actually had an immediate change of management including dietary fructose elimination in a patient with hereditary fructose intolerance, pyridoxine phosphate medication in a patient with pyridoxamine 5-

prime-phosphate oxidase deficiency, and treatment with L-dopa and selegiline in a patient with Segawa syndrome (Retterer et al., 2016).

When considering WES in the clinical setting, it is important that patients are consented appropriately, which should include discussion with families regarding the reporting of incidental findings, the potential for complex results, and identification of non paternity. It is important for the clinican to consider when it is best to use WES over gene panels. Laboratories must have clear guidelines for reporting of WES data (Bamshad et al., 2011). There are of course limitations to the detection of the causative variant with WES such as non-coding variants, copy number variants, expansion mutations and, in the clinical setting, the ability to investigate novel genes and genomic mechanisms further. It is however, highly likely that whole exome sequencing will become increasingly used in the clinical setting for the patient's benefit where used appropriately. This project will consider its clinical utility further.

1.9. Conclusion

WES is an extremely useful tool for identification of the disease-causing variant in patients. WES generates many sequence variants. Filtering strategies need to be carefully considered to help identify the pathogenic variant explaining the patient's phenotype. The clinical application of WES and the impact of phenotyping in this process will be carefully considered throughout the project.

1.10. Aims of the Project

1. Identify the most efficient process for rare variant interpretation and disease gene identification.

2. Define the role of deep clinical phenotyping in the interpretation of comprehensive NGS analysis.

CHAPTER 2: METHOD

2.1. Patient Acquisition

2.1.1. Phenotypes of Interest

Families in this study were selected for Whole Exome Sequencing based on whether their phenotype included one of the diseases of interest (see table 2.1). I mainly focused on phenotypes involving the immune, neurological, bone and renal systems and cases with likely autosomal recessive inheritance.

Table 2.1: Disease and Phenotypes of Interest

Disorder Type	Disease of Interest
Neurological	Neurodegenerative
	Neuro-inflammatory
	Early Onset Epilepsy including Early Infantile Epileptic Encephalopathy (EIEE)
	Seizures particularly responsive to certain drugs
Immune-Related	Immunodeficiency Disorders
	Familial Autoimmune Conditions
	Inflammatory Conditions including Inflammatory Bowel Disease
Bone-related	Autosomal Recessive Osteogenesis imperfecta
	Osteopetrosis
Renal	Glomerulonephritis
	Focal Segmental Glomerulosclerosis (FSGS)
Other	Multi-system disorders

2.1.2. Family Prioritisation

To increase the likelihood of identifying a disease-causing variant, I prioritised consanguineous families with likely autosomal recessive diseases and families in whom a de novo heterozygous mutation could be identified with trio analysis (analysis of the proband and both parents).

Families were also prioritised for inclusion if they had a genetic condition for which the molecular basis was unknown and the potential for identification of a novel disease gene or phenotype.

2.1.3. Patient Recruitment

I employed several different strategies to identify patients for recruitment within this project:

1. I manually reviewed 366 research files of families previously recruited to the National Autozygosity Mapping Research (NAMR) study in Birmingham. I identified 21 families who had a phenotype of interest who had already consented to the Molecular Pathology of Human Disease study. I identified 6 further novel families of interest who had not been recruited to the Molecular Pathology of Human Genetic Disease project. Of these, 3 families were recruited to the study, but the other 3 families did not respond to the invitation to participate.
2. Families were identified through the West Midlands Regional Clinical Genetics department retrospectively by identifying those coded with diseases of interest listed above:

a. Of the 23 clinical genetics files with a diagnosis of Osteopetrosis, I could identify 2 suitable families based on the above criteria. These families were contacted via letter but did not respond.
b. Of the 6 families with Pulmonary Fibrosis, only 1 family was suitable. After recontacting and visiting the family it transpired that sadly all affected family members had died in the intervening period.
c. There were 12 possible patients with Inflammatory Bowel Disease, of which only 1 family were suitable, but again both affected family members were now deceased.
d. 152 patients were identified with neuro-degenerative of childhood unspecified, but only 1 family were appropriate for inclusion in the study. This family were contacted and recruited to the study.

3. I reviewed the medical records of 16 families with an inherited cerebral palsy like condition, previously phenotyped by a colleague in the department. A molecular diagnosis of Aicardi Goutiere syndrome had since been made in 1 family and another was being investigated for Refsum syndrome. 4 families were already having WES as part of other research studies. 2 families had requested no further involvement in research documented in their notes. For 1 family, the genetics file was missing. This left a total of 7 families who were initially contacted through their clinicians (due to the time that had lapsed). 1 family were interested in meeting to discuss the study but declined involvement because the parents felt that a molecular diagnosis would not impact their day-to-day care.

4. I applied for a Research Passport with Leicester Royal Infirmary to recruit patients to my study. After reviewing files coded as Early Encephalopathy. I was able to identify 4 suitable familes not currently involved in other studies. These patients were contacted by the local clinical geneticist involved in their care, but unfortunately, we did not hear back from them.
5. Families were identified through the West Midlands Regional Clinical Genetics department prospectively by referral from the clinician involved in their care. I raised awareness for this project by presenting at the whole department laboratory meeting and sending out intermittent email reminders of the inclusion criteria afterwards. We identified 9 suitable families from the department. These families were contacted by letter or telephone and invited to participate. 7 families were recruited to the study. 1 family responded and later decided not to proceed after completing their family, and 1 family were unsuitable after further discussion. 1 of the 7 recruited families were not sent for WES analysis because they received a molecular diagnosis soon after recruitment and clinical phenotyping, leaving 6 families recruited via this method.
6. I regularly attended a neurometabolic clinic at the Birmingham Children's Hospital. I identified 1 suitable family who were not already known to the West Midlands Regional Genetics department. This family were recruited to the study.
7. The Regional Immunology service is based at Birmingham Heartland's Hospital. I contacted the department directly, but unfortunately, they had already sent all patients without a molecular diagnosis for further investigation in Newcastle-upon-Tyne.

8. The Regional Paediatric Endocrinology department were also contacted to identify families of interest. They kindly invited me to present at their departmental meeting at the Birmingham Children's Hospital. They were unable to identify any families for inclusion into this study.
9. The Paediatric Rheumatology department at the Birmingham Children's Hospital identified 3 families not already known to the project. These families were recruited to the project. Subsequently a joint clinic was established with Dr Eslam Al-Abadi to identify patients with autoinflammatory disease requiring clinical genetic input.

Table 2.2: Number of patients identified, contacted and recruited to the study

Patients Identified	Number of Patients Contacted	Number of Patients Recruited
NAMR Already Recruited	21	21
NAMR Required Recruiting	6	3
Department Retrospectively	5	1
Department Prospectively	9	6 (7)
Birmingham Children's Hospital	4	4
Leicester Royal Infirmary	4	0
Total	49	35 (36)

2.1.4. Informed Consent

This project was part of the Molecular Pathology of Human Genetic Disease study. The study design, protocol, patient information leaflets, and consent forms had already been approved by the relevant Research Ethics Committee (South Birmingham REC reference number 5175). The relevant forms can be found in Appendix11.1 and 11.2. Written informed consent was obtained from all participants and all their family members. This clinical research adhered to principles outlined by the Declaration of Helsinki.

For adult patients who lacked capacity, the General Medical Council guidance on Consent in Research and Mental Health Act 2005 were adhered to. Their parents were

asked if they thought the patient would wish to be involved in this project if they had capacity to decide for themselves.

2.2. Human Phenotype Ontology and Phenotyping

For the patients seen by Clinical Geneticists, consent for inclusion in the Molecular of Pathology of Human Genetic Disease was obtained at a routine clinic appointment.

Patients who were referred to the study by other specialties or had been seen in Clinical Genetics previously and were later referred for inclusion in the study, were invited to attend a clinic appointment by letter and a follow up telephone call. For these patients, I undertook deep clinical phenotyping in clinic or during a home visit, took consent to join the research study and a blood sample if required for DNA extraction (see Methods 2.3).

For all patients, medical records were requested from their local hospital or tertiary hospital where relevant. The medical records and clinical genetics records for the recruited patients were thoroughly reviewed. The patients were then phenotyped using Human Phenotype Ontology (HPO) terms. These terms were documented within an excel spreadsheet to allow easy comparison of the phenotypes for the recruited patients and their families. A summary of this table can be seen in the Appendix (11.3 and 11.4).

For patients in whom we identified a putative causative variant, further phenotyping was performed where relevant to aid interpretation of the data. This included clinical assessment and where relevant, further clinical investigation.

2.3. DNA Analysis

2.3.1. Sample Collection and Extraction

1. For individuals with an adequate volume of stored DNA in the local regional genetics laboratory, we used this preferentially.
2. For each adult participant with no available stored DNA, a 10ml blood sample was taken in an EDTA blood tube bottle. For each child participant with no stored DNA sample, a 5ml of blood sample was taken in an EDTA tube. In some individuals a saliva sample was taken instead using an Oragene DNA kit. The DNA was then extracted from the blood or saliva using standard techniques by staff of the West Midlands Regional Genetics Laboratory.
3. Aliquots of DNA of sufficient quantity (minimum of 3 microgram samples) and quality for WES were shipped to The Centre for Human Genetic Variation (CHGV) at Duke University, USA, by international courier (e.g. FedEx) for families 1-10, and to the University of Cambridge, UK, for families 11-35.

2.3.2. Whole Exome Sequencing Studies

For the first 10 families WES studies were undertaken at the Genomic Analysis Facility at the Duke Centre for Human Genome Variation, USA. Exons were captured using the Agilent exon capture platform and massively parallel sequencing was undertaken on the Illumina HiSeq 2000 system. Reads were aligned to the reference sequence using Novoalign alignment tool. Depth of capture and coverage of the exome was assessed using custom analytical tools built using the BED tools package. Quality filtering was undertaken using the Sam tools and Picard tools packages. Single nucleotide substitutions and small insertion deletion variants were called using the Sam tools package. All identified variants were annotated with respect to open reading frames and cross referenced with publically available variant databases and internal control samples using custom scripts built around the Annovar tool.

The remaining families had WES studies performed at the Department of Medical Genetics in the University of Cambridge. Similar methods were utilised. In brief, DNA was prepared using the TruSeq rapid exome library prep kit (Illumina, United States) following the manufacturer's protocol. Pooled libraries were analysed on an Illumina HiSeq 2500 using Illumina HiSeq Reagent v.2 – paired end 150 bp (Illumina, United States). FASTQ files were aligned to genome reference GRCh38 using BWA-MEM (version 0.7.15-r1140) in ALT-contig aware mode. PCR duplicates were flagged by SAMtools rmdup (version 1.4.1). Variant calling was carried out using GATK unified genotyper (version 3.7-0-gcfedb67). VCFtools was employed to filter variants. Annovar

was used to annotate the variants using various databases including refGene, 1000g2015aug_all, exac03, avsnp150, dbnsfp35a, clinvar_20180603, cosmic70, nci60, dbscsnv11. Variants were filtered by global minor allele frequencies present in both the 1000 genomes project and ExAC cohorts. Variants with heterozygous call rates >15% of the total cohort were classed as technical artefacts or undocumented common SNPs and therefore removed.

2.4. Identification of candidate pathogenic mutations

Filtering of variants was performed using standard methods. Briefly, common variants with a MAF of greater than 1% were removed using data from publically available databases such as dbSNP, the 1000 genome project (http://browser.1000genomes.org/index.html) and NHLBI Exome Sequencing Project, exome variant server (http://evs.gs.washington.edu/EVS/). Synonymous variants and intronic variants more than 4 base pairs from the splice site were removed. I also removed genes starting with MUC, HLA, LINC, LAIR.

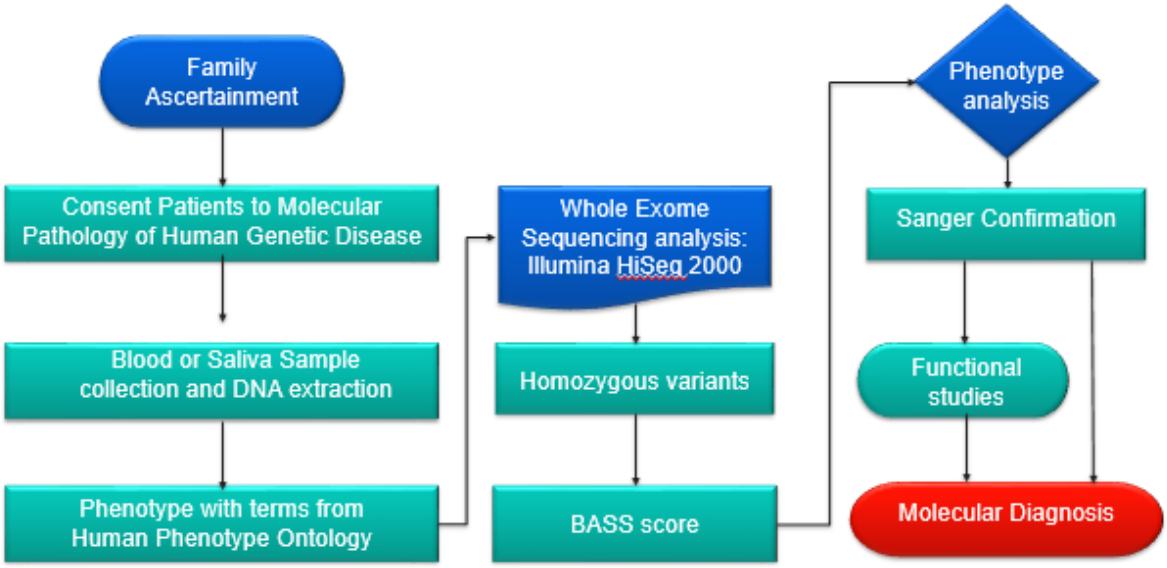
Initially known variants that had been previously reported as pathogenic in HGMD, ClinVar or the medical literature were prioritised. For the consanguineous families, heterozygous variants were then removed to focus on homozygous variants. Novel truncating or nonsense variants were prioritised next, when present in all affected family members. Missense substitutions were considered for further analysis if they were

predicted to affect protein function or structure, were evolutionary conserved, and were absent in the homozygous state in ethnically matched controls. The remaining candidate variants were then considered depending on the function of the protein, the biological pathways and interactions of that protein, and any known associated phenotypes. For consanguineous families, in whom no putative homozygous variants were identified, I then considered other types of variants, such as de novo heterozygous variants in families with a single affected patient and variants on the X chromosome if only affected males in the family.

2.5. Molecular Confirmations

Sanger sequencing confirmation and segregation analysis was performed in families in whom a putative causative variant was identified. Verification of candidate pathogenic variants was undertaken by a postgraduate research student (Atif Al-Saedi) at the University of Birmingham, including segregation analysis within the family using standard methods. Primers were designed using Primer3 tool to cover the specific mutation and the surrounding area of the gene. PCR amplification was performed on the DNA samples. Each set of PCR reactions included a negative control to check for contamination. PCR products were sequenced using standard BigDye Terminator v3.1 cycle sequencing method. Sequencing products were loaded on ABI 3730 automated sequencer and the sequences were processed using Bioedit and/or mutation surveyor. Sequence traces from each of the DNAs analysed was compared to the reference sequence from the ENSEMBL database.

Figure 2.1: Summary of Flow through the study



2.6. Website Addresses for Internet resources

Several different online tools and databases were utilised to analyse the WES data.

These resources are listed in table 2.3.

Table 2.3 Internet Resources

Name of Internet Resource	Website address
Autoinflammatory Alliance	http://www.autoinflammatory-search.org/search/index
ClinVar	https://www.ncbi.nlm.nih.gov/clinvar/
dbSNP	https://www.ncbi.nlm.nih.gov/snp
Decipher	https://decipher.sanger.ac.uk/
Ensembl	http://www.ensembl.org/index.html
Exome Aggregation Consortium	http://exac.broadinstitute.org/
Exome Variant Server	http://evs.gs.washington.edu/EVS/
GeneCards	http://www.genecards.org/
HGMD	http://www.hgmd.cf.ac.uk/ac/index.php
Mouse Genome Informatics	http://www.informatics.jax.org/
OMIM	http://omim.org/
Orphanet	http://www.orpha.net/consor/cgi-bin/index.php
Phenomizer	http://compbio.charite.de/phenomizer/
Pubmed	https://www.ncbi.nlm.nih.gov/pubmed/
UCSC Genome Browser	https://genome.ucsc.edu/

CHAPTER 3: RESULTS; IMMUNE-RELATED DISORDERS

This chapter includes findings that have been published previously in a journal article (Damgaard et al., 2016).

3.1. Overview

Table 3.1: Immune Related Disorders results overview

Family number	Clinical Diagnosis	Molecular Diagnosis
1	Familial Haemophagocytic Lymphohistocytosis (FHL)	*UNC13D* homozygous missense variant
2	Relapsing Nodular Panniculitis (Weber Christian Disease)	*OTULIN* homozygous missense variant
5	Immunodeficiency Syndrome	No candidate mutation identified
8	T Cell Immunodeficiency and Photoreceptor Deficiency	No candidate mutation identified
10	Autosomal Recessive Inflammatory Bowel Disease- like	No candidate mutation identified
12	Acute disseminated encephalomyelitis in 2 siblings	No candidate mutation identified
15	Phenotypic diarrhoea and autoinflammatory conditions	No candidate mutation identified
32	Autoinflammatory Condition	No candidate mutation identified
33	Autoinflammatory Condition	Candidate *NLRC3* heterozygous missense variant TNFRSF1A heterozygous missense variant – conflicting reports
34	Autoinflammatory Condition	*DNASE1L3* homozygous frameshift deletion variant

I studied 10 families with immune-related disorders. I identified the likely pathogenic disease-causing variant in 3 out of the 10 families (30%). A further family (Family 33) had a candidate *NLRC3* variant identified, as well as a variant in TNFRSF1A with conflicting pathogenicity data on ClinVar. Interestingly, the clinical phenotype would fit well with a TRAPS phenotype. *NLRC3* is an immune-related gene with no human phenotype reported, which will be further investigated after this project has ended.

The proband in Family 1 presented with clinical features consistent with Familial Haemophagocytic Lymphohistiocytosis (FHL). She was the only affected individual in a multiply consanguineous family, who originally presented to the local paediatric department at 9 months of age with febrile seizures. The clinical features included generalised seizures, jaundice, hepatosplenomegaly with deranged liver function tests, cytopenia, raised ferritin, hypertriglyceridaemia, microcephaly, short stature, global developmental delay, cerebellar ataxia, diarrhoea, and hypopigmentation of the skin. A bone marrow biopsy demonstrated haemophagocytosis without any evidence of myeloproliferative disease, which confirmed the diagnosis of haemophagocytic lymphohistiocytosis. She went on to receive a successful bone marrow transplant, although she continued to have neurological sequelae, including learning difficulties and cerebellar ataxia. Exome sequencing identified a homozygous missense variant in *UNC13D* c.3053G>T (p.Arg1018Gly), predicted to be deleterious, found within a highly conserved region of the gene, and not present in population variation databases. This gene is also known as *FHL3* and accounts for around 20% of all cases of FHL. It is therefore likely to explain the clinical phenotype seen in the patient, which will therefore

not be discussed further within this report. This chapter will instead focus on the autoinflammatory disorders as exemplified by Family 2 and Family 34.

3.2. Family 2

3.2.1. Introduction

Systemic autoinflammatory disorders are a group of rare conditions caused by a dysregulation of the innate immune system reflected by a neutrophilic, monocytic or macrophagic response with an abnormal cytokine profile (Broderick, 2016; Hoffman and Broderick, 2016; Kastner et al., 2010). They are characterised by systemic autoinflammation in the absence of infection, autoantibodies and antigen specific T cells with features which may include recurrent fevers, rash, serositis, uveitis, meningitis, arthralgia or arthritis, sensorineural hearing loss and lymphadenopathy (Broderick, 2016) (Hoffman and Broderick, 2016). This is generally associated with a neutrophilic leukocytosis and raised acute inflammatory markers. These episodes may be chronic or recurrent in nature. Some of these episodes may be triggered by external stimuli such as cold exposure (Hoffman and Broderick, 2016). It is important to distinguish these patients from those with an infectious cause with or without an immunodeficiency. Failure to treat these autoinflammatory conditions can lead in many cases to AA amyloidosis and the consequences of this such as renal failure leading to death.

The first systemic autoinflammatory conditions described were characterized by recurrent fevers, rash, raised inflammatory markers, a neutrophilia, and with no identifiable source of infection (Broderick, 2016). These conditions included Familial Mediterranean fever (FMF) where patients may have had repeated normal laparotomies for an acute abdomen, Cryopyrin-associated periodic syndromes (CAPS), TNF receptor-associated periodic syndrome (TRAPS), and Hyper-IgD syndrome (HIDS) also known as mevalonic kinase deficiency (MKD) (Broderick, 2016). There are three distinct phenotypes seen within CAPS, starting with the mildest cold induced symptoms with Familial Cold Autoinflammatory Syndrome (FCAS), to the recurrent unprovoked episodes in Muckle Wells syndrome (MWS) and finally with continuous symptoms in Neonatal-Onset Multisystem Inflammatory Disorder (NOMID). Please see table 3.1. for a description of these conditions amongst other autoinflammatory conditions.

The type 1 interferonopathies were subsequently described. There are different components to the type I interferon (IFN) family including 13 different types of IFN-α, as well as IFN-β, IFN-ε, IFN-κ and IFN-ω (Eloranta and Rönnblom, 2016). The levels of IFN typically increase in response to an infection, particularly a viral infection, after recognition of foreign nucleic acids by the pattern recognition receptors (PRRs), such as certain Toll-like receptors (TLRs) mainly present in immune cells (Eloranta and Rönnblom, 2016). The recently named relopathies, or NF-kB-related autoinflammatory diseases, are relatively new members of the autoinflammatory groups of conditions.

Table 3.2. Overview of Known Autoinflammatory Conditions

Condition	Gene	Age of Onset	Duration attack	Clinical Features	Standard Treatment
Interleukin 1 cytokine based pathology					
FMF	MEFV (AR)	First decade	1-3 days	Erysipeloid rash LL Monoarthritis Serositis Splenomegaly	Colchicine
HIDS /MKD	MVK (AR)	Infancy	3-7 days (imms may trigger)	Maculopapular rash Aphthous ulcers Abdominal pain, D, V Arthralgia, Symmetrical polyarthritis lymphadenopathy	NSAIDs Anti-IL-1
TRAPS	TNFRSF1A (AD)	Varied (0-53 years)	7-21 days	Daily fevers Periorbital oedema Migratory rash +/- underlying myalgia Large joint arthritis Serositis, CBH Conjunctivitis Splenomegaly	Steroids Anti-IL-1
CAPS (FCAS)	NLRP3 (AD)	Infancy	<1 day	Cold induced fever Urticarial like rash Arthralgia	Anti-IL-1
CAPS (MWS)	NLRP3 (AD)	Infancy	2-3 days	Urticarial like rash Abdominal pain Polyarthralgia Conjunctivitis, episcleritis SNHL	Anti-IL-1
CAPS (NOMID)	NLRP3 (AD)	Neo -nate	Constant	Urticarial like rash Epiphyseal overgrowth Chronic arthritis Uveitis, visual loss Chronic aseptic meningitis Mental retardation Hepatospenomegaly	Anti-IL-1
PAPA	PSTPIP1 (AD)	Child	Induced mild trauma or infection	**P**yogenic Sterile **A**rthritis **P**yoderma Gangrenosum **A**cne syndrome	Steroid Anti-IL-1

				Recurrent ulcers Lymphadenopathy	
NLRC4	NLRC4 (AD – de novo)	Varied	Variable	Prolonged episode fever Urticarial like Rash Arthritis Raised ferritin Severe GI symptoms - including enterocolitis	Anti-IL-1 Anti -IFNγ
Interferonopathy					
CANDLE	PSMB8 PSMB4 PSMB9 PSMA3 POMP (AR)	Infancy	Frequent	**C**hronic **A**typical **N**eutrophilic **D**ermatosis **L**ipodystrophy **E**levated temperature Violaceous eyelid swelling	(partial) TNFα blocker Anti-IL-1
SAVI: STING Associated Vasculo -pathy onset in Infancy	TMEM173 encodes STING	Infancy		Fever Neutrophilic vasculopathy Interstitial lung disease	Nil
AGS: Aicardi Goutiere Syndrome	ADAR, RNASEH2A RNASEH2B RNASEH2C SAMHD1 TREX1 IFIH1	Infancy	Constant	Fevers and chilblains Encephalopathy Severe LD/ regression Dystonia and spasticity Calcification basal ganglia Hepatosplenomegaly	Nil
Inhibitory Receptor Mutations					
DIRA: Deficiency of IL-1 Receptor Antagonist	IL1RN (AR)	Birth	Constant	Neutrophilic pustular rash Osteopenia Sterile lytic bone lesions	Anti-IL-1
DITRA: Deficiency of IL-36 Receptor Antagonist	IL36RN (AR)	Varied	Flares days to weeks	Generalised Pustular Erythematous rash flaring with physiological stressors	Anti-IL-1
Complex Autoinflammatory disorders					
PFAPA	Unknown	<5 years	~monthly	**P**eriodic **F**ever **A**phthous Stomatitis **P**haryngitis **A**denitis Syndrome	
Autoinflammatory Disease with Immunodeficiency					

HOIL-1 deficiency	HOIL-1 (AR)			Pyogenic bacterial infections Autoinflammation Amylopectinosis (CM, sm muscle myopathy)	
HOIP Deficiency	HOIP (AR)			Immunodeficiency Autoinflammation Amylopectinosis Multiple lymphanectasia	
DADA2 Deficiency of ADA2	CERC1 encodes ADA2 (AR)	<5 years	Episodic	Recurrent fever Livedo reticularis Early lacunar strokes Low IgM >50% lymphopenia and hypogammaglobinaemia	
Other					
FHL	PRF1 STX11 STXBP2 UNC13D RAB27A SH2D1A BIRC4	<1 year	>2 weeks	Maculopapular/ nodular rash Liver disease CNS inflammation Blindness Lymphoma Haemophagocytosis bone marrow	
Majeed	LPIN2 (AR)	<2 years	Few days 1-4/m	Neutrophilic dermatosis Congenital Dyserythropoietic Anaemia Multifocal osteomyelitis Delayed growth	
Blau syndrome	NOD2 (AD)	<5 years	Episodic or constant	Non-caseating granulomatous rash Arthritis and vasculitis Cranial neuropathies Uveitis, cataracts Pericarditis Lymphadenopathy	

AD autosomal dominant, AR autosomal recessive, CBH change in bowel habit, CM cardiomyopathy, D diarrhea, DIRA Deficiency of IL-1 Receptor Antagonist, DITRA Deficiency of the Interleukin-36 Receptor Antagonist, imms immunisations, LL lower leg, m month, sm smooth, SNHL sensorineural hearing loss, temp temperature, V vomiting, (Broderick, 2016) (Pagon et al., 1993) ("Systemic Autoinflammatory Diseases (SAID) Database," n.d.) (Hoffman and Broderick, 2016)

Family 2 and 34 both had features consistent with a systemic autoinflammatory disorder, although the clinical presentations were quite different. Neither fell typically into the above known systemic autoinflammatory disorders.

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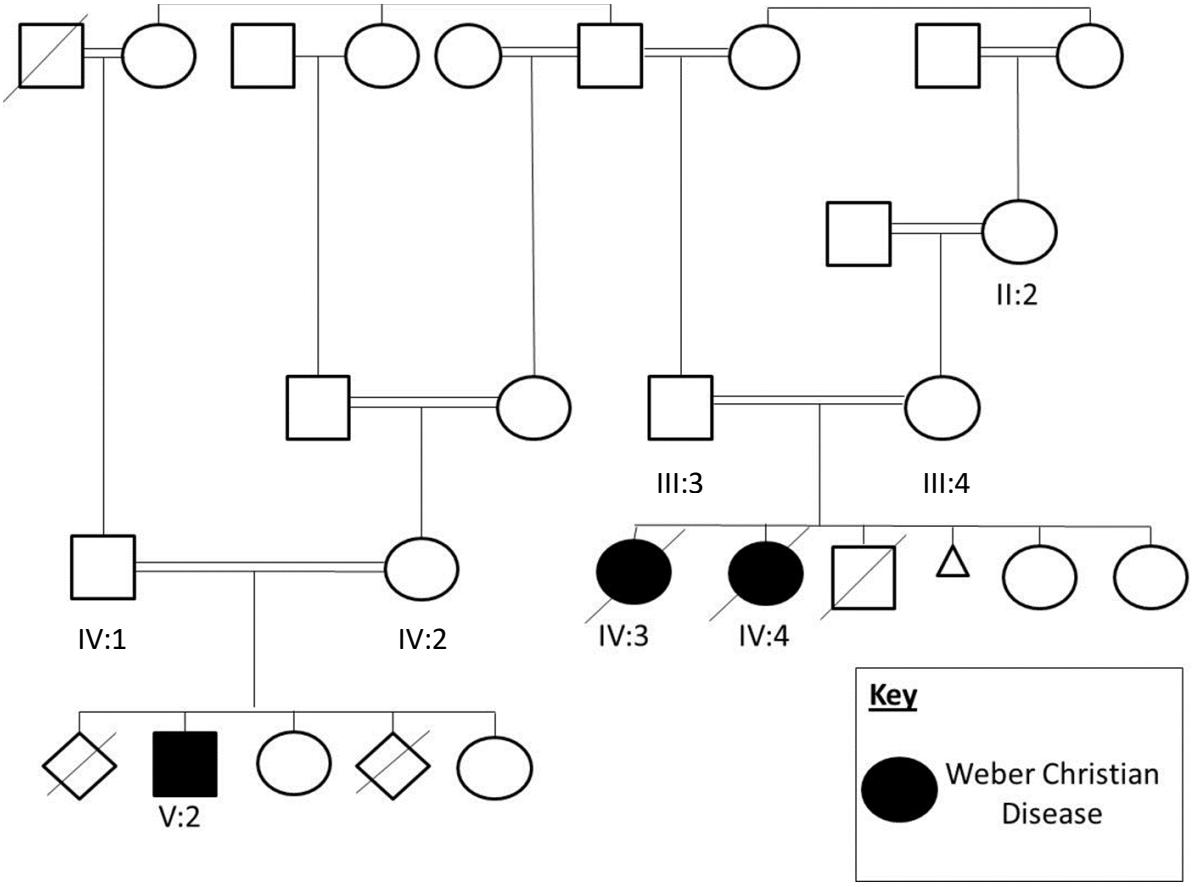
3.3.2. Clinical Description

Figure 3.1: Pedigree of Family 2



The proband, V:2, is the second child born prematurely at 28+6 weeks gestation to consanguineous parents (IV:1 and IV:2) with a birth weight of 1.23kg. He first developed relapsing nodular panniculitis at 8 weeks of age while he was on the neonatal intensive care. Prior to the appearance of his skin rash, he had had repeated episodes of possible infection, showing a rise in C-reactive protein (CRP) and white cell count (WCC), but no focus for infection had been identified. The rash was biopsied and confirmed inflammation in the dermis extending into the subcutaneous layer with a mixed inflammatory cell infiltrate, with no granulomata or vasculitis seen.

He had frequent flare-ups involving widespread painful lumps in the skin lasting 2 days to 2 weeks. During these episodes he would be systemically unwell with fever, vomiting, diarrhoea (sometimes bloody), inflamed painful joints, swollen feet, and weight loss associated with a raised CRP and raised WCC. He would lose his appetite, lose weight and become dehydrated. He had multiple admission to the high dependency unit. He seemed susceptible to frequent infections especially viral illnesses including bronchiolitis and chickenpox. These recurrent episodes had long-term consequences including poor weight gain, slow growth, developmental delay, and mild learning difficulties. Other clinical problems included congenital hydroceles, dental caries, a pathological osteoporotic tibial fracture (which may have been secondary to repeated steroid use) and juvenile cortical cataracts at 2-3 years of age requiring surgical management with bilateral lensectomy and vitrectomy. He was treated both with systemic steroids and Anakinra. Neither medication successfully prevented the recurrent episodes or additional symptoms. Infliximab (a TNF alpha blocker) was

introduced 7 years ago and has successfully controlled the disease. He has had a couple of minor exacerbations when the frequency of the infliximab was decreased or the dose of infliximab per kilogram of body weight decreased. In addition to this, he takes methotrexate, azithromycin and acyclovir prophylaxis.

On examination, his growth parameters were all less than the 0.4th centile. He had a prominent nodular rash in keeping with a flare of panniculitis. Cataracts had been noted previously in both eyes. He had coarse hair with bushy eyebrows, slight hypertelorism, broad nasal bridge, prominent nose, protruding normally formed ears and a prominent chin. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4).

He had been extensively investigated for the cause of his systemic autoinflammatory disorder. The positive findings included recurrently raised WCC (see figure 3.2), which was mainly accounted for by a neutrophilia. During one episode, he had a neutrophil count of $68x10^9$cells/l recorded with toxic granulation, vacuolation and a left shift. He also had recurrently raised CRP and Erythrocyte Sedimentation Rate (ESR), when performed (see figure 3.2). The WCC, CRP and ESR levels reflect the autoinflammatory process of the disease. These levels normalised once the condition was controlled by infliximab treatment, with levels increasing only during dose-related exacerbations described above (see figure 3.2). He had a single strongly positive anti smooth muscle antibody (1/320). The most recent skin biopsy was very similar to the

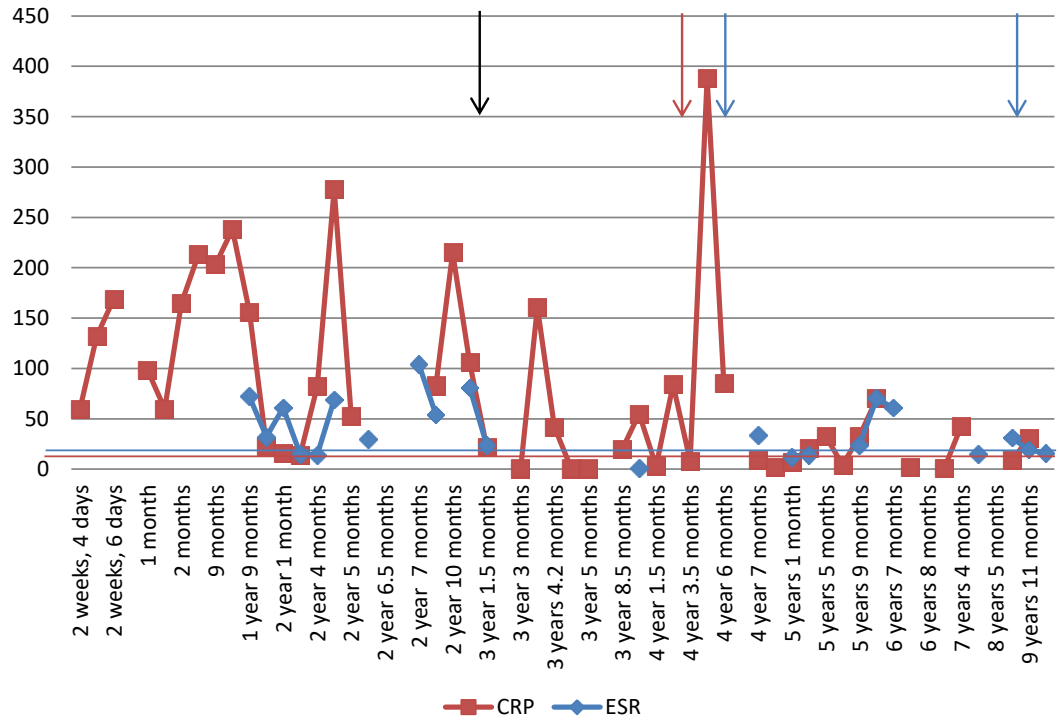
appearance of his cousin's skin biopsy (described below) showing a neutrophil-rich panniculitis with fat necrosis favouring septal distribution.

Figure 3.2: Timeline showing inflammatory markers for patient V:2 (overleaf)

Summary: Graph A demonstrates the CRP and ESR levels over the lifetime of the patient. The normal reference range is marked as seen in the key. Please note that the ESR was not recorded regularly. The recurrent spikes of inflammation are reflected in the recurrent large spikes in the CRP and ESR levels above the normal reference range. Infliximab was commenced at the age of 3 years 1.5 months. After a year of relatively good control, the prednisolone was gradually stopped. The infliximab dose is increased subsequently at 4 years 5 months of age after a marked episode of inflammation demonstrated by a massive increase in the CRP level. Subsequent minor increases in CRP level are related to attempts to space out the infliximab infusions and as the dose of infliximab per kilogram body weight reduces. CRP is an excellent marker for inflammation in this condition.

Graph B similarly demonstrates the WCC and associated neutrophil count over the lifetime of the patient. The normal reference range is marked as seen in the key. Similarly, a raised WCC and neutrophil count correlated well with the autoinflammatory disease exacerbation. These levels settle to normal with infliximab treatment, with minor exacerbations associated with altered infliximab dose or infusion interval.

A



Key

Infliximab commenced

Prednisolone stopped

Infliximab dose increase

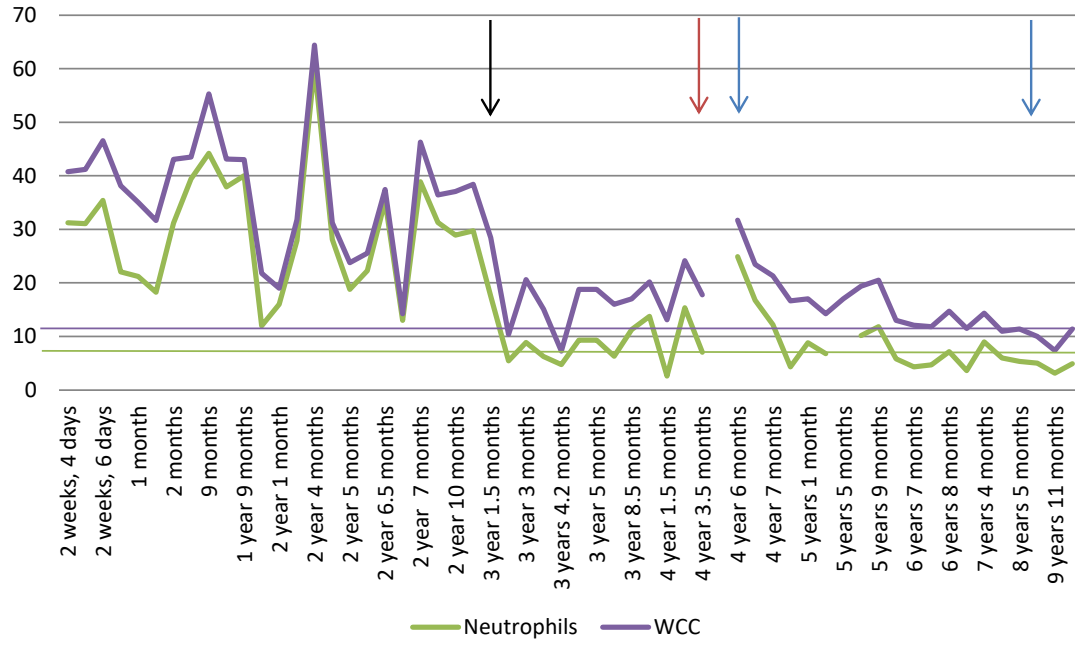
WCC normal range: 4.0 to 11.0x10^9/l

Neutrophil normal range: 2.5-7.5x10^9/l

CRP normal range 0-10mg/l

ESR normal range 0-14mm/hr

B

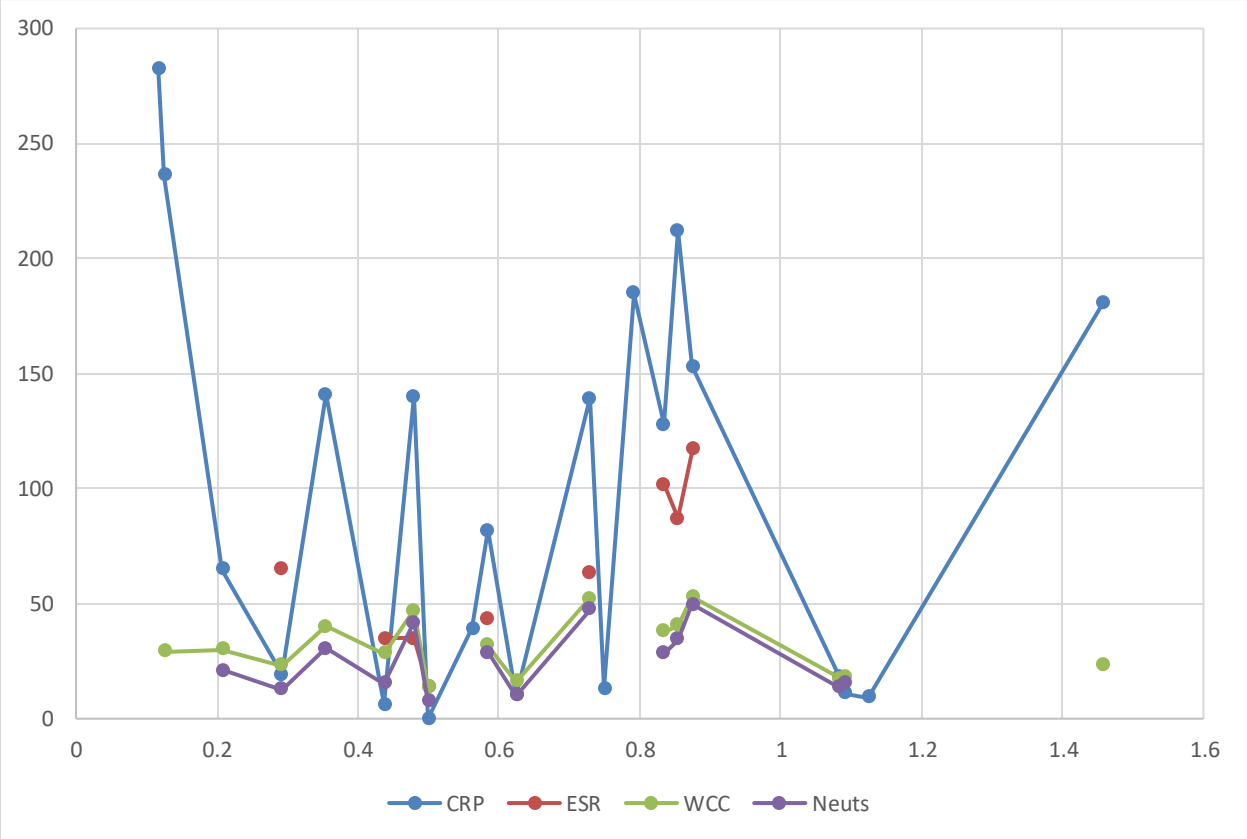


The proband had two cousins who had previously died with a similar disorder. Patient IV:3 is the first child born to consanguineous parents (III:3 and III:4) at 34 weeks gestation via normal vaginal delivery and was small for gestational age. She first developed an erythematous maculopapular rash, with poor feeding and weight gain at 6 days of age. She presented at 3 weeks of age with protracted diarrhoea, pyrexia, a widespread erythematous papulonodular rash, severely inflamed BCG scar, mild hepatomegaly and failure to thrive. At 7 months corrected gestational age all growth parameters were well below the 0.4th centiles. She required total parenteral nutrition (TPN) and later nasogastric tube feeds. During her lengthy admission she was noted to have recurrent episodes of a widespread nodular erythematous rash associated with fever, neutrophilia and an exacerbation of her diarrhoea. An initial skin biopsy, taken as the rash was resolving showed non-specific changes. Duodenal biopsies showed microvillous dystrophy. She also had mild hepatomegaly. A liver biopsy showed TPN induced micro-nodular cirrhosis. Echocardiogram identified an atrial septal defect.

Patient IV:3 was extensively investigated to identify the cause of her protracted diarrhoea and episodic skin rash including extensive immunological investigations (see Figure 3.3). The lymphocyte subsets showed all major classes were present with an increased density of polyclonal B cells and NK cells. She also had a raised IgM and IgA level, raised C3 (2.28), a positive ANCA (1:100 cytoplasmic) and SMA level. When she was treated with intravenous methylprednisolone, her fever, diarrhoea and rash would resolve, but these clinical features would return when attempts were made to change to oral prednisolone. Azathioprine and methotrexate were also trialled. She had 3

episodes of bilateral consolidation progressing to sepsis and acute respiratory collapse in the last 3 months of her life requiring ITU admission. Sadly, she died at 17 months from the final episode. These episodes were complicated by acute tubular necrosis from presumed end organ damage. Cerebral atrophy was also identified on a CT head scan during this period.

Figure 3.3: Inflammatory markers over lifetime of IV:3



For normal reference ranges, please see figure 3.2.

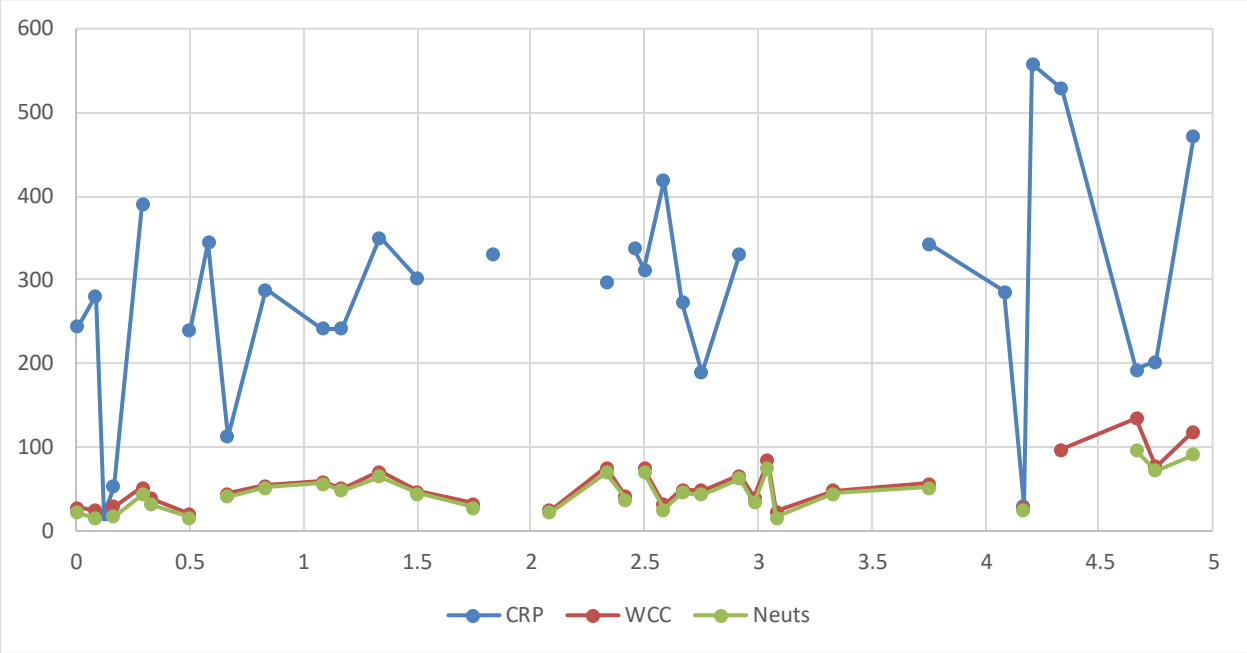
Summary: Recurrent episodic inflammation demonstrated by inflammatory marker levels.

Patient IV:4 is the second child born to consanguineous Pakistani parents (III:3 and III:4) at 36 weeks gestation via normal vaginal delivery with a birth weight of 2kg. She was diagnosed with relapsing nodular panniculitis at 3 days of age. This rash, similarly to her sister and cousin, was episodic in nature and associated with diarrhoea (bloody at presentation), vomiting, fever, painful swollen joints and difficulty sleeping. She also required nasogastric tube feeds for failure to thrive. All growth parameters were below the 0.4th centile. She had developmental delay. IV:4 had high neutrophils, and raised white cell counts, mainly reflecting a neutrophilia. The last recorded levels prior to her death demonstrated massive levels of inflammation with a CRP of 388, WCC of 95.7, and a neutrophil count of 92.3. CRP was checked at every flare up, therefore was always raised, with a maximal recorded level of 419 (See Figure 3.4). She also had bilateral cataracts diagnosed at the age of 5 months, like the proband. In addition, she had recurrent urinary tract infections with no identifiable aetiology. The cataracts were not present in the neonatal period on formal ophthalmological examination. The appearance of the cataracts was more suggestive of an inflammatory cause rather than a complication of steroid use. She was treated with prednisolone from the age of 1 month. Azathioprine and anakinra were tried unsuccessfully. At the time all known systemic autoinflammatory conditions were ruled out by single gene sequencing.

During a severe episode of inflammation at the age of 5, IV:4 demonstrated features consistent with a high cell turnover including a raised potassium, phosphate, uric acid, LDH, white cell count and a metabolic acidosis. She was admitted to paediatric intensive care with acute renal failure, pulmonary oedema and an ileus, but sadly

passed away. Treatment had included intubation and ventilation, intravenous methylprednisolone, dexamethasone, and methotrexate.

Figure 3.4: Inflammatory markers over lifetime of IV:4



For normal reference ranges, please see figure 3.2.

Summary: Recurrent episodic inflammation less well demonstrated by inflammatory marker levels in IV:4. This patient was not admitted for a sustained period of time similar to sibling and therefore normal inflammatory markers in between periods of inflammatory exacerbation are less well documented.

Table 3.3: Comparison of the affected individuals' phenotype using HPO in Family 2

(see overleaf)

Summary: Affected individuals have similar recurrent episodes with similar clinical features and long-term consequences. Infliximab treatment was life-saving for the proband.

Legend: UnK is unknown.

HPO Terms and demographics	Patient V:2	Patient IV:4	Patient IV:3
Age at presentation	1 month	3 days	1 month
Sex	male	female	female
Alive (age died)	Yes	No (5 years)	No (16 month)
Short stature HP:0004322	+	+	+
Decreased body weight HP:0004325	+	+	+
Failure to thrive HP:0001508	+	+	+
Microcephaly HP:0000252	+	+	UnK
Global developmental delay HP:0001263	+	+	+
Cataracts HP:0000518	+	+	UnK
Vomiting HP:0002013	+	-	-
Diarrhoea HP:0002014	+	+	+
Inguinal hernia HP:0000023	+	-	-
acute kidney injury HP:0001919	-	+	-
Microvillus dystrophy	UnK	UnK	+
Panniculitis HP:0012490	+	+	+
Coarse hair HP:0002208	+	+	UnK
Immunodeficiency HP:0002721	+	+	+
Neutrophilia HP:0011897	+	+	+
Hypoalbuminaemia HP:0003073	+	UnK	+
Fever HP:0001945	+	+	+
Raised C-reactive protein HP:0011227	+	+	UnK
Generalised hirsutism HP:0002230	+	+	UnK
Thick eyebrows HP:0000574	+	+	UnK
Protruding ears HP:0000411	+	+	UnK
Pointed chin HP:0000307	+	-	UnK
Arthralgia/Arthritis HP:0005059	+	+	-
Micro-nodular liver cirrhosis (TPN)	-	-	+

3.3.3. Results

WES revealed 50 rare homozygous variants, none of these were reported in to be pathogenic in ClinVar or HGMD (see Appendix 11.5.2). Among the homozygous variants, I prioritized a homozygous missense substitution within the *OTULIN* gene (c.815T>C, p.Leu272Pro). This variant was not detected on the population databases. Leu272 is located in a helix of the catalytic OTU domain which forms part of the binding pocket for M1-linked diUb. This variant is therefore predicted to disrupt ubiquitin binding domain of the *OTULIN* protein. Sanger sequencing has confirmed this result in V:2. IV:1, III:3, and III:4 were confirmed heterozygotes of this variant.

This result was consistent with previous studies in this family. Previous SNP array analysis of the 3 affected individuals identified 3 regions of extended homozygosity. The largest shared region of homozygosity was at 5p15, from 13,802,063 to 16,722,976 bp. Sequencing of the genes within this region had identified a homozygous missense variant in *FAM105B* in all affected individuals (c.815T>C; p.Leu272Pro). Little was known about the function of this gene at the time. When I subsequently reviewed this data, it was clear that we had identified the same variant. *OTULIN* was previously known as *FAM105B*.

There was no human phenotype reported for patients with variants within the *OTULIN* gene at that time. There was however, an increasing body of information regarding the biology of *OTULIN* as a key regulator of the TNF alpha pathway, discussed further below. The proband was exquisitely sensitive to TNF alpha blockade treatment, which made it highly likely that the homozygous variants in *OTULIN* were the disease-causing variants in this family. There were no other similarly affected families. We worked with David Komander's group in Cambridge, who were working with *OTULIN* mice models. The human and mouse phenotype work has subsequently been published in the peer review journal Cell (Damgaard et al., 2016). This condition has now been described as *OTULIN*-related autoinflammatory syndrome (ORAS).

3.3.4. Discussion

I have described 3 children from a multiply consanguineous family with an autoinflammatory disorder, now called ORAS, manifesting from a few days of age with recurrent episodes of relapsing nodular panniculitis, fever, myalgia, arthralgia, diarrhoea, weight loss, a raised CRP and white cell count. Additional long-term consequences included cataract, developmental delay and growth delay. The nuclear cataracts seen in two of these individuals are not consistent with those seen as a complication of steroid treatment, but rather the underlying inflammation. The affected children were miserable when this condition was poorly controlled. Treatment of affected patients with prednisolone, general immunosuppressant did seem to mildly reduce the severe inflammation seen in the affected individuals but did not reduce the

frequency of recurrence. Azathioprine, methotrexate, and Anakinra, an interleukin-1R-antagonist, made no difference to the disease course. Infliximab, TNF alpha blockade, successfully manages this condition.

The affected individuals in the family had a homozygous missense variant in the *OTULIN* gene. At the time of identification, there were no reported patients with variants in *OTULIN*. Some patients with Cri-de-Chat syndrome have a heterozygous deletion incorporating this gene, but it is unclear how this contributes to the phenotype. A knockout mouse model, called gumby, was embryologically lethal (Rivkin et al., 2013). We therefore postulated that this missense variant would be hypomorphic, and therefore some residual *OTULIN* function would remain. Blood samples from the proband confirmed the presence of *OTULIN*, although the levels were slightly reduced as compared to age-matched controls (Damgaard et al., 2016). SHARPIN was also detected in the blood sample (Damgaard et al., 2016). Interestingly, M1-linked ubiquitin chains were strongly increased in the proband, but found at very low levels in control samples, mimicked by the bone marrow chimera mice models described below (Damgaard et al., 2016). The *OTULIN* deficiency means that the M1-linked ubiquitin chains are produced by the unregulated LUBAC, leading to unregulated stimulation-independent activation of the NF-kB pathway, and thus explaining the marked systemic autoinflammation seen in Family 2 (Damgaard et al., 2016).

Detailed phenotyping demonstrated clear biological plausibility to the *OTULIN* variants being the disease-causing variants in this family. Patient V:3 was commenced on 4 weekly infusions of infliximab, a TNF alpha blocker, at 3 years of age controlling his auto-inflammatory disorder (see results 3.3.2.). Exacerbations were seen if the dose per kg body weight of infliximab was reduced to less than 5mg or the treatment interval of 4 weeks lengthened. Infliximab therefore, radically altered the disease course for the proband. His cousins did not receive infliximab treatment. They both sadly died in early childhood secondary to complications from the untreated severe inflammation seen in ORAS. We postulate that the molecular cause for the systemic autoinflammatory condition in Family 2 would lie within or interact with the TNF alpha pathway. *OTULIN* is a regulator of this pathway. I will now discuss the biological evidence explaining the causality further.

OTULIN (#615712), previously known as *FAM105B*, is part of the OTU family and works as a deubiquitinase (DUB), hence it was so named (OTU DUB with linear linkage specificity) (Keusekotten et al., 2013; “OMIM - Online Mendelian Inheritance in Man”). Ubiquitination is an important protein post-translational modification, involving the addition of ubiquitin or a ubiquitin chain. This process of ubiquitination regulates a number of cellular processes including protein degradation and activation (Keusekotten et al., 2013; Komander and Rape, 2012; Tokunaga, 2013). Polyubiquitin chains can be linked via Met-1 linked or linear polyubiquitin, by linking the C-terminal glycine at position 76 of one ubiquitin to the N-terminal methionine at position 1 on the neighbouring ubiquitin (Keusekotten et al., 2013; Lork et al., 2017; “OMIM - Online

Mendelian Inheritance in Man"; Tokunaga, 2013). There are seven other types of ubiquitin chains with the C-terminal glycine attached to one of the seven lysine residues, K6, K11, K27, K29, K33, K48 or K63, as well as mixed chain utilising more than one type of polyubiquitin link (Lork et al., 2017). The Met-1 linked polyubiquitin is assembled by the linear ubiquitin chain assembly complex (LUBAC) (Keusekotten et al., 2013). This E3 ligase is made up of 3 main components: HOIL-1L which is encoded by *RBCK1*, HOIL-1L-interacting protein (HOIP) encoded by *RNF31*, and SHARPIN encoded by *SHARPIN* (Fiil et al., 2013; Keusekotten et al., 2013; Steiner et al., 2018; Tokunaga, 2013). LUBAC is involved in the nuclear factor κB (NK-κB) activation after stimulation by inflammatory cytokines such as TNF-α (Fiil et al., 2013; Keusekotten et al., 2013; Tokunaga, 2013). The NF-κB pathway is a critical signalling pathway, which regulates both the innate and acquired immune system (Tokunaga, 2013). There are two main pathways, the canonical and alternative NF-κB pathway. The canonical pathway is triggered by the binding of pro-inflammatory cytokines, such as tumour necrosis factor (TNF) to TNF receptor 1 (TNFR1), as well as IL-1β and pathogen-associated molecular patterns (PAMPs) to their specific receptors (Steiner et al., 2018). This leads to an activation pathway cascade culminating in the release of two NF-κB subunits, p65 and p50, leading to the upregulation of proinflammatory and antiapoptotic gene transcription (Steiner et al., 2018).

OTULIN specifically degrades the met-1 linked ubiquitin chains, and therefore regulates LUBAC activity (Keusekotten et al., 2013). *OTULIN* binds to HOIP via the PUB domain and is thus able regulate LUBAC (Elliott et al., 2014; Lork et al., 2017; Schaeffer et al.,

2014). *OTULIN* is the only deubiquitinase which specifically cleaves these met-1 links (Keusekotten et al., 2013; Rivkin et al., 2013). Overexpression of *OTULIN* reduces LUBAC-mediated NK-κB activation and therefore *OTULIN* knockdown leads to increased signaling of this pathway (Keusekotten et al., 2013). Linear ubiquitination by LUBAC and deubiquitination by *OTULIN* are therefore critical regulators of the innate immune system (Tokunaga, 2013). Thus, giving substantial plausibility to the likelihood of ORAS causing the infliximab responsive autoinflammatory condition in Family 2.

The *OTULIN* knockout mice are embryonically lethal, therefore Damgaard et al created CreERT2-*OTULIN*$^{LacZ/flox}$ mice, in which *OTULIN* was systemically ablated in all cells with tamoxifen administration (Damgaard et al., 2016; Rivkin et al., 2013). This led to the mice becoming rapidly moribund (Damgaard et al., 2016). CreERT2-*OTULIN*^{flox} mixed bone marrow chimera mice were generated and treated with tamoxifen to induce *OTULIN* ablation at 6-8 weeks (Damgaard et al., 2016). This resulted rapid weight loss, increased levels of neutrophils in the bloodstream, as well as pro-inflammatory cytokines including TNF, IL-6, and G-CSF, when compared to controls (Damgaard et al., 2016). The liver, spleen, abdomen, lungs and kidneys had neutrophil infiltration (Damgaard et al., 2016). This demonstrated a very similar phenotype of systemic autoinflammatory disease to that seen in Family 2. Damgaard et al subsequently went on to see whether neutralisation of the raised inflammory cytokines, TNF, G-CSF, or IL-6, would rescue to phenotype (2016). After tamoxifen-induced *OTULIN* ablation, TNFα antibodies were administered, demonstrating a significant change in the mouse phenotype with resolution of the previously described weight loss, reduced tissue

neutrophil infiltration, and the pro-inflammatory cytokine levels were also reduced (Damgaard et al., 2016). Additionally, they identified that targeted ablation of *OTULIN* in myeloid cells led to chronic inflammation, and conversely *OTULIN* ablation in B and T lymphocytes, resulted in no specific phenotype, and interestingly HOIP and SHARPIN were simultaneously downregulated in these cells (Damgaard et al., 2016). In summary, the mice models were therefore able to recapitulate the human phenotype to demonstrate that ORAS is the cause of disease in Family 2.

Two further patients have subsequently been reported with ORAS (Zhou et al., 2016b). Both are Turkish patients, one with another homozygous missense variant and another with a homozygous frameshift variant (Zhou et al., 2016b). The first patient had recurrent episodes of panniculitis and fever from the age of four and a half months, whereas the second patient presented more similarly to Family 2 with neonatal recurrent fevers, panniculitis, arthralgia, and failure to thrive (Zhou et al., 2016b). The patient with milder symptoms of ORAS is successfully managed with anakinra and the second patient with the TNF blocker, etanercept, as well as steroids for autoinflammatory symptom flare-ups (Zhou et al., 2016b). In view of the supposition that hypomorphic variants in *OTULIN* produce ORAS and more severe variants may be lethal, this is surprisingly compatible with life, although clearly more severe than the phenotype seen in the proband of Family 2, who no longer requires steroid treatment. The missense variant reported in the first patient, p.Tyr244Cys, resulted in a milder phenotype explained by experiments showing that the *OTULIN* enzyme activity was

similar to baseline (Zhou et al., 2016b). This provides further evidence to the validity of the pathogenicity for the variant identified in Family 2.

CreERT2-*OTULIN*^{flox} mixed bone marrow chimera mice were shown to have evidence of chronic inflammation in the liver and spleen (Damgaard et al., 2016). Further unpublished work from David Komander's laboratory in mice with liver-specific deletion of *OTULIN* has shown early-onset neonatal steatosis, which developed severe liver disease with severe portal inflammation, at 3-4 weeks of age (equivalent to 12-14 years in humans) and subsequently nodules and adenomas. Worryingly, there was only mild derangement on liver function testing in these mice, which may make detection potentially difficult, unless this phenotype is specifically sought out. This is a liver-specific knockout and therefore may not be relevant to missense variants within the gene seen in Family 2.

The liver function results for the affected individuals were therefore consulted. IV:3 had known liver function derangement, which at the time was felt to be a secondary consequence of long-term total parenteral nutrition replacement. Hepatomegaly was documented in IV:4, but with no subsequent investigation, this is difficult to interpret. IV:4 also has mild liver derangement, which could have been a secondary consequence from the Anakinra. V:2 also had mild liver derangement, with an exacerbation just after 3 years which occurred soon after infliximab treatment was commenced, but this subsequently resolved. Overall, it is likely that the mild liver derangement seen in

affected individuals was potentially a secondary effect of systemic inflammation or treatment being received at the time. It is unlikely to represent primary liver involvement. It is unclear whether the phenotype seen in the liver-specific knockout mice would be relevant to the human phenotype. Equally, it is a possibility that with the TNFα blockade, this complication may be prevented. It will of course be prudent to observe the proband in Family 2 carefully for liver involvement.

Human phenotypes have been reported in patients with variants within the genes encoding the components of the ubiquitinase, LUBAC. Homozygous and compound heterozygous loss-of-function mutations in *RBCK1*, encoding the subunit of LUBAC, HOIL1, were reported in 3 individuals from 2 families (Boisson et al., 2012). This resulted in a similarly fatal autoinflammatory disorder with additional features including, increased susceptibility to pyogenic bacterial infections and muscular amylopectinosis resulting in myopathy and cardiomyopathy (Boisson et al., 2012). This results in HOIL1 and LUBAC deficiency, with no detectable formation of linear ubiquitin chains causing impaired NF-κB activation, and subsequent decreased NF-κB pathway activity in response to TNF (Boisson et al., 2012; Steiner et al., 2018). A similar phenotype was seen in a patient with a homozygous variant in *HOIP* who not only had autoinflammation, amylopectinosis, and recurrent viral and bacterial infections due to a combined immunodeficiency, but also had lymphangiectasia (Boisson et al., 2015).

Human phenotypes have also been associated with variants in other deubiquitinases. CYLD is a deubiquitinase which cleaves both the Met1-linked ubiquitin chains, as well as K63-linked ubiquitin chains from different proteins within the NF-*κ*B signaling pathway (Damgaard et al., 2016; Lork et al., 2017; Steiner et al., 2018). CYLD deficiency leads to increased NF-*κ*B pathway activity resulting in increased proinflammatory and anti-apoptotic gene transcription (Lork et al., 2017). *CYLD* also interacts with LUBAC via interaction with the PUB domain of HOIP with SPATA2 (Elliott et al., 2016; Lork et al., 2017). *CYLD* and *OTULIN* do not bind with LUBAC simultaneously, and appear to be functionally distinct (Hrdinka and Gyrd-Hansen, 2017; Lork et al., 2017). Familial cylindromatosis (genetic predisposition to multiple tumours of the skin appendages) is reported in patients with heterozygous pathogenic variants in CYLD (Bignell et al., 2000), which is unsurprising in view of the upregulation of anti-apoptotic gene transcription secondary to its deficiency, but a very different phenotype to that seen in Family 2. No specific tumour types with loss of OTULIN have been reported, but in unpublished work from David Komander's laboratory discussed earlier neoplastic changes were seen in the Otulin deficient liver model (Elliott and Komander, 2016; Steiner et al., 2018). The relevance of *OTULIN* to tumourigenesis is currently unclear and requires further study.

A systemic autoinflammatory condition, with a similar phenotype to Behcet syndrome consisting of arthritis, oral and genital ulcers, was reported in 6 families with heterozygous variants in the deubiquitinase *TNFAIP3* gene (tumor necrosis factor, alpha-induced protein 3 or A20) (Zhou et al., 2016a, 2016b). A20 expression has also

been shown to be suppressed by microRNA in diffuse large B-cell lymphoma (Kim et al., 2012; Lork et al., 2017). A20 has deubiquitinase and ubiquitinase ligase activity, which also has a regulatory role in the NF-*κ*B activation pathway in response to different proinflammatory stimuli, but its precise role is less clear (Lork et al., 2017). A20 deficient mice develop severe inflammation, cachexia, and die prematurely (Lee et al., 2000). The human A20 deficiency phenotype is a less severe, autoinflammatory disease than ORAS, but occurs in the heterozygous state.

Family 15 have an autoinflammatory phenotype, which may fit within this group of conditions. Similarly, to Family 2, they initially presented with a phenotypic diarrhoea of infancy presentation. There were 3 affected siblings born to consanguineous first cousin parents. The second sibling sadly died within the first couple of years of life. The elder sibling developed sepsis and a cerebrovascular event. This left her with a significant neurological deficit, meaning she is unable to walk or talk, she is ventilated and receives enteral feeds. The younger of the 3 siblings is developmentally normal, currently attending college. She has episodes of a neutrophilic dermatosis, which have not been seen in the older siblings. Both surviving siblings however, also have an endocrine phenotype (hypopituitarism). It is unclear how all these features tie together, and it is possible that they may represent a blended phenotype of more than one condition. I have been unable to identify the disease-causing variant explaining the phenotype in Family 15, but analysis is ongoing.

In summary, ORAS, due to homozygous missense variants in *OTULIN*, results in a fatal autoinflammatory condition. As a regulator of the TNF mediated NF-kB pathway, *OTULIN* deficiency is successfully treated with TNF alpha blockade in mice and humans. ORAS is therefore a novel human phenotype explaining the systemic autoinflammatory condition seen in Family 2.

3.4. Family 34

3.4.1. Introduction

Systemic lupus erythematosus (SLE) is a complex auto-immune multi-system disorder with multifactorial aetiology, including both genetic and environmental factors, due to hyperactive T and B cells, ANA directed against self-DNA and immune complex deposition (Carbonella et al., 2017; Costa-Reis and Sullivan, 2017). The immune complexes accumulate in the patients' vessel walls, glomeruli and joints causing a type III hypersensitivity reaction manifesting the clinical features seen in patients with SLE, namely vasculitis, glomerulonephritis, and arthritis (Napirei et al., 2000). Features suggestive of a monogenic cause include paediatric onset, consanguinity, a family history consistent with Mendelian inheritance, or disease which is not responsive to regular medication (Costa-Reis and Sullivan, 2017).

There are multiple forms of monogenic lupus and these can be grouped into several different categories. Firstly, there are the inherited complement deficiencies, such as C1q, C2 and C4 deficiencies, which result in a phenocopy of SLE due to aberrant clearance of apoptotic cells and immune complexes, resulting in exposure of self-antigen (Costa-Reis and Sullivan, 2017; Lo, 2018). In this group of SLE-like conditions, there is commonly skin involvement and a significant history of infections (Lo, 2016). Defects in nucleic acid degradation and repair results in inadequate removal of DNA, which could drive an auto-immune response similar to the clinical features seen in

individuals with SLE and patients with variants in *TREX1* with either autosomal dominant familial chilblain lupus or autosomal recessive Aicardi–Goutières syndrome (Costa-Reis and Sullivan, 2017). The type I interferonopathies also have similarities to SLE including the aforementioned Aicardi–Goutières syndrome and familial chilblain lupus, spondyloenchondrodysplasia and STING-associated vasculopathy with onset in infancy (SAVI) (described in table 3.1) (Costa-Reis and Sullivan, 2017). Affected individuals with these interferonopathies have defective interferon production and as such will have higher levels of IFNα and overexpression of IFNα-induced genes, called the interferon signature (Costa-Reis and Sullivan, 2017). These can be triggered normally with infection, particularly viral infections and endogenous nucleic acid (Costa-Reis and Sullivan, 2017). In line with this, patients with SLE may have a detectable interferon signature, and one of the first-line treatments for SLE is hydroxychloroquine, which downregulates the type I IFN signature (Costa-Reis and Sullivan, 2017; Eloranta and Rönnblom, 2016). Variants in genes associated with processing and sensing of intracellular nucleic acid have also been described in the interferonopathies (Lo, 2018).

Monogenic SLE can also occur in individuals with variants in genes important for the identification and elimination of B and T cells with receptors which recognise self-antigens (autoreactive B and T cells) (Costa-Reis and Sullivan, 2017). The resultant autoimmunity from loss of B and T cell tolerance can result in monogenic SLE, for example homozygous variants in *PRKCD* which encodes PKCδ, which is important for negative selection of B cells via apoptotic signaling cascades (Costa-Reis and Sullivan, 2017; Lo, 2018, 2016). Interestingly the RASopathies, a group of neurodevelopmental

disorders associated with short stature, congenital heart disease, and bleeding diatheses, have been implicated in monogenic SLE, due to the part Ras plays in T cell maturation (Lo, 2016). Overlapping features are also seen in the autoinflammatory conditions described above such as CANDLE (Costa-Reis and Sullivan, 2017).

3.4.2. Clinical Description

The proband is the second child born to consanguineous first cousin parents. She was diagnosed with systemic lupus erythematosus in another country at 11 years of age. She had persistent anaemia, glomerulonephritis, rash over her face, arms and trunk, and raised inflammatory markers including ESR ranging from 60 to 100. On review at the age of 13, she continued to have anaemia, haematuria secondary to glomerulonephritis, and raised inflammatory markers, as well as musculoskeletal pains, lethargy and persistent cough. She remains clinically stable managed with hydroxychloroquine, MMF and prednisolone. She was negative for ANA, ANCA, ENA, dsDNA, anti GBM and antiphospholipid antibody. She also had normal complement levels and bone marrow aspirate. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4).

The first-born child to the proband's parents, died at 7 years of age with viral pneumonitis secondary to chickenpox. She had a clinical history of rash, arthritis, and raised inflammatory markers. She was treated with prednisolone and azathioprine. I

have been unable to gather more clinical information regarding this individual. There are 3 other younger apparently unaffected siblings. There was no one else in the wider family with these clinical features. A maternal aunt did have autoimmune hepatitis.

In summary, we have a consanguineous family with 2 clinically affected siblings with a childhood-onset SLE-like condition. Due to the early age of onset and the severe phenotype seen in the sibling, a monogenic cause for the lupus-like condition was suspected. They had normal complement levels and therefore did not have an inherited complement disorder. Their normal neurological function and intellect made Aicardi–Goutières syndrome unlikely. They did not have any clinical features of the Rasopathy disorders. It was therefore possible that they had another cause of monogenic lupus, such as a biallelic variant in DNASE1L3 gene, explaining their clinical phenotype.

3.4.3. Results

WES revealed 33 rare homozygous variants (1 nonsense, 5 frameshift variants, 25 missense, 1 splicing and 1 unknown variant) in V:3 (see Appendix 11.5.34). None of these were reported to be pathogenic in ClinVar or HGMD. Among the homozygous truncating variants, I prioritised a homozygous intragenic frameshift deletion within the *DNASE1L3* gene (c.290_291delTG, p.Thr97Ilefs*2). This variant was detected in the heterozygous state in 6 out of 30972 individuals on GnomAD (0.00019), with no detected homozygotes. Another variant (c.289_290delAC, p.Thr97Ilefs*2), resulting in

the same protein alteration, has previously been reported in a family with Hypocomplementaemic urticarial vasculitis syndrome (see below discussion). The *DNASE1L3* (c.290_291delTG, p.Thr97Ilefs*2) variant was present in the homozygous state in the proband. Both parents were shown to be heterozygous for the variant. There was no available DNA from the first affected sibling for analysis. Sanger sequencing and segregation studies have confirmed the result in the proband. Her unaffected sister did not carry the variant and her unaffected brother carried the variant in the heterozygous state. Her youngest sister was also found to be a homozoygote for this variant. She is not known to have any clinical features of SLE. She is however only 9 years of age. It is therefore possible that she has not yet presented with features of this condition. This is however, likely to explain the clinical phenotype seen in this family.

3.4.4. Discussion

Family 34 have an SLE-like condition, which is likely to be a monogenic autosomal recessive disorder, because of the paediatric onset in 2 affected siblings with healthy consanguineous parents. It was therefore postulated that Family 34 may have one of the immune-related SLE-like monogenic disorders described in the introduction, or a novel condition within one of these classes of monogenic SLE.

The proband in Family 34 is homozygous for a frameshift deletion in the *DNASE1L3* gene. This monogenic form of SLE was first described in 2011 when a homozygous 1-bp deletion in DNASE1L3 (c.643delT, p.Trp215Glyfs*2) was identified in all affected members from six consanguineous families of Arab descent, which was confirmed as a founder mutation (Al-Mayouf et al., 2011). The clinical phenotype in this monogenic SLE is similar to that seen in Family 34. Affected individuals have childhood onset SLE, with an onset between 2 and 12 years of age, with a mean of around 5 years (Al-Mayouf et al., 2011). There was a predominance of lupus nephritis (Al-Mayouf et al., 2011). All of these individuals had raised anti-nuclear antibodies (ANA), low complement C3 and C4 levels, with many also having detected anti-neutrophil cytoplasmic antibodies (ANCAs) and anti-dsDNA (Al-Mayouf et al., 2011). Two of the reported affected individuals had no detectible *DNASE1L3* transcript in a lymphoblast cell line (Al-Mayouf et al., 2011).

DNASE1L3 is one of three human homologs of DNase I (Al-Mayouf et al., 2011). It functions as an endonuclease, which can cleave both single and double-stranded DNA (Al-Mayouf et al., 2011). It is important for the degradation of DNA in serum, for example, from apoptotic cells (Zhao et al., 2017). Impaired removal of endogenous DNA may induce the formation of anti-DNA antibodies, which may then attack self-DNA, which results in an auto-immune condition such as SLE (Al-Mayouf et al., 2011). Dnase1-deficient mice had classical features of SLE, including ANA, and immune complexes in the glomeruli leading to glomerulonephritis (Napirei et al., 2000).

Dnase1L3 has more recently been shown to regulates NLRP3 and NLRC4 inflammasome activation and subsequent cytokine release (Shi et al., 2017).

Hypocomplementaemic urticarial vasculitis syndrome (HUVS) is a condition which results in episodes of urticaria, which histologically looks like vasculitis, associated with low complement levels (Özçakar et al., 2013). Other systemic features, such as fever, fatigue, arthralgia, uveitis, recurrent abdominal pain, and glomerulonephritis, may also be seen with raised inflammatory markers and anaemia (Özçakar et al., 2013). More than half of patients with this condition will develop SLE (Özçakar et al., 2013). A study looking at 2 families with affected siblings suggestive of autosomal recessive disease used whole exome sequencing and autozygosity mapping to identify causative variants in *DNASE1L3* (Özçakar et al., 2013). One family had a similar frameshift deletion identified in the *DNASE1L3* gene (c.289_290delAC, p.Thr97Ilefs*2) resulting in the same protein change, as that seen in Family 34 (Özçakar et al., 2013). This segregated within the family with the disease, and at the time they were unable to identify this variant in other population variation databases (Özçakar et al., 2013). They were also able to look at the relative quantities of the cDNA levels of DNASE1L3 in this family, demonstrating marked reduction in levels for the homozygotes when compared to controls, and heterozygotes had around half the levels of those seen in controls (Özçakar et al., 2013). This suggested that the RNA was subject to nonsense-mediated decay (Özçakar et al., 2013). This 2bp deletion has also subsequently been reported in an affected proband, sibling and mother, all with an SLE-like condition, and the unaffected father was shown to be a heterozygous carrier (Carbonella et al., 2017).

The authors also wondered whether the contractures of the distal phalanges seen in the affected siblings could be part of the condition, although this was not present in Family 34 (Carbonella et al., 2017). Another patient with early onset SLE with this variant has also been described (Batu et al., 2018). This suggests that the variant seen in Family 34 is likely to be disease-causing, although it would currently be classified as a variant of uncertain significance using ACMG criteria(Richards et al., 2015).

Yang et al looked at DNASE1L3 activity in serum in 30 patients with dermatomyositis/polymyositis, 20 patients with SLE, 18 patients with rheumatoid arthritis, and 26 controls (Zhao et al., 2017). Decreased DNASE1L3 level and subsequent reduced activity was seen in patients with dermatomyositis/polymyositis and SLE (Zhao et al., 2017). The DNASE1L3 level was relatively lower in the SLE patients with higher disease activity (particularly ESR level), kidney involvement and of course those with detectable anti-dsDNA (Zhao et al., 2017). This demonstrates that DNASE1L3 activity is important in SLE and therefore provides further evidence for the pathogenicity of the variant seen in Family 34.

In summary, Family 34 have a monogenic phenocopy of SLE due to a frameshift 2bp deletion within the DNASE1L3 gene. This results in paediatric SLE-like symptoms with a predominance of lupus nephritis, similar to that described in Family 34.

CHAPTER 4: RESULTS; CONNECTIVE TISSUE DISORDERS

This chapter includes findings that have been published previously in a journal article (Meester et al., 2018; Webb et al., 2017).

4.1. Overview

Figure 4.1. Overview of Connective Tissue Disorders

Family number	Clinical Diagnosis	Molecular Diagnosis
3	Osteogenesis Imperfecta plus	*TMEM38B* variant
4	Osteogenesis Imperfecta	No variant identified
6	Connective Tissue Disorder	*COL1A2* variant
14	Odontochondrodysplasia like Disorder	No variant identified
27	Adams Oliver syndrome	*NOTCH1* variant

This results chapter looks at several consanguineous families with a rare connective tissue disorder to try to identify the disease-causing variant explaining their phenotype. There are 5 different families described with potentially novel phenotypes. I will discuss these results within this chapter.

The proband in Family 27 presented with clinical features consistent with Adams Oliver syndrome. These clinical features were: central scalp aplasia cutis congenita with wide upper parietal skull defect on skull x-ray within the region of the sagittal suture with multiple small ossified islands within the defect, short distal second, third and fourth phalanges of the right hand associated with brachyonychia and nail hypoplasia of the left index finger. In addition, she was reported to have had generalised cutis marmorata as a neonate, and she had had a normal echocardiogram. She was the only affected child within a consanguineous family.

I identified a novel likely pathogenic missense variant, c.3281G>A, p.Cys1094Tyr, in the *NOTCH1* gene from the WES results, which was not present in her father or sibling (mother's sample failed WES). Sanger sequencing confirmation studies were unfortunately not possible in this family. This gene is known to cause Adam Oliver syndrome and therefore the phenotype would fit with this variant being disease-causing. This variant is predicted to be deleterious on in silico tools, it is found within a highly conserved region of the gene and is not present in population variation databases. It was a novel variant, which was not present on clincal variant databases and was therefore published as part of a series of patients with Adams Oliver syndrome (Meester et al., 2018). This missense variant involved the replacement of cysteine within a EGF-like repeat domains, and cysteine residues within this region form essential disulfide bonds important in the tertiary structure of the protein, which means this missense variant is likely to affect the subsequent structure and therefore be pathogenic (Meester et al., 2018). It is therefore likely to explain the clinical phenotype seen in the patient.

This result highlights the importance of still considering a de novo autosomal dominant condition in a singleton affected individual in a consanguineous family. I will not discuss this result further within this chapter.

Overall, we had 5 families with connective tissue disorders. I identified the likely pathogenic disease-causing variant in 3 out of the 5 families (60%). All these variants were in known disease genes. I will discuss Family 3 and 6 in more detail in this chapter.

4.2. Introduction

Osteogenesis imperfecta (OI), or brittle bone disease, is a clinically and genetically heterogeneous condition, which manifests as bone fragility leading to recurrent fractures after minimal or no preceding trauma, which may cause secondary bone deformities and short stature (Marini and Blissett, 2013; Pagon et al., 1993). Other non-skeletal clinical features include blue or grey sclerae, hearing loss, dentinogenesis imperfecta and joint laxity (Pagon et al., 1993; Valadares et al., 2014). A classification for the different clinical and radiological presentations of OI was published in 1979, describing 4 different types of OI: type 1 is the classical non-deforming type of OI with blue sclera, type 2 is perinatally lethal OI, type 3 is a progressively deforming type of OI, and type 4 is a moderately severe type of OI with normal sclera (Marini and Blissett, 2013; Pagon et al., 1993; Sillence et al., 1979; Valadares et al., 2014). These classical types of OI

are inherited in an autosomal dominant manner, or *de novo* variants if embryonically lethal or severe OI, and are associated with heterozygous variants in the *COL1A1* or *COL1A2* genes (Marini and Blissett, 2013; Valadares et al., 2014).

COL1A1 encodes the α1 collagen 1 chain and *COL1A2* forms the α2 collagen 1 chain. Collagen 1 is the main structural protein found in the extracellular matrix of bone, skin ligaments, tendons, and cornea, which forms a triple helical structure from two α1 collagen chains and one α2 collagen chain (Nicholls et al., 2001). The variants in *COL1A1* or *COL1A2* may reduce the quantity of the resultant collagen chain or its structural quality, commonly by replacing the glycine in the chain, which is found every third amino acid and thus, affecting the overall structure of the tightly wound collagen helical structure (Marini and Blissett, 2013; Pagon et al., 1993; Valadares et al., 2014). The milder types of OI are seen in individuals with haploinsufficiency from decreased synthesis of type I procollagen, secondary to heterozygous nonsense, frameshift or splice site variants resulting in mRNA instability (Nicholls et al., 2001; Vandersteen et al., 2014). The more severe types of OI tend to occur in individuals with helical glycine substitutions or exon skipping in *COL1A1* or *COL1A2*, disrupting the helical formation of collagen type 1 (Nicholls et al., 2001; Vandersteen et al., 2014). In contrast an Ehlers Danlos syndrome (EDS) VII phenotype may be seen in individuals with variants in these genes which affect the processing of procollagen (Nicholls et al., 2001). Ehlers Danlos syndrome is a heterogeneous group of connective tissue disorders associated with a combination of clinical features, which may include generalised joint hypermobility, and skin hyperextensibility. There is a crossover in some individuals with a combined OI

and EDS phenotype. Homozygous variants in *COL1A1* are lethal (Nicholls et al., 2001; Schnieke et al., 1983). An EDS cardiac valvular phenotype has been reported in patients with biallelic variants in *COL1A2* (discussed further in Family 6 discussion 4.4.3.)

More recently, a number of other genes have been identified as the molecular cause for autosomal recessive OI including *SERPINF1, CRTAP, LEPRE1, PPIB*, and *BMP1* (Marini and Blissett, 2013; Valadares et al., 2014). The proteins produced generally interact with collagen either by affecting post-translational folding, modification or cross-linking (Marini and Blissett, 2013; Shaheen et al., 2012; Valadares et al., 2014). In 2009 the International Society of Skeletal Dysplasias recommended continuing to use the Sillence OI classification to classify the severity of OI based on clinical features with reference to the inheritance pattern (Marini and Blissett, 2013; Valadares et al., 2014; Warman et al., 2011). The additional genes associated with OI were listed separately (Warman et al, 2011). A new classification of OI based on their genetic defect in groups of 5 conditions, A to E, has also been proposed (Forlino and Marini, 2016).

4.3. Family 3

4.3.2. Clinical Description

Family 3 are a multiply consanguineous family with 4 affected individuals with an OI phenotype with other additional clinical features, which was felt to represent a novel form of OI. The proband was originally referred to the clinical genetics department at the age of four months with a fractured right femur. At the time, old healed fractures were detected on a skeletal survey in the fibula and clavicle. She is the second child born to first cousin Pakistani parents at 38 weeks via Caesarean section for pre-eclampsia with a birth weight of 2.8kg. She had recurrent fractures until the age of 11 years including bilateral femoral fractures on one occasion. She has normal hearing and no dental problems. She has normal menstruation. She also has vitamin D deficiency, hypothyroidism and a waddling gait. The waddling gait was extensively investigated including a muscle biopsy demonstrating myopathic changes, although the cause for these changes was not identified. Her height was between the 9th and 25th centiles, and her weight on the 50th centile. On examination she had a broad forehead, hypertelorism, epicanthic folds, bluish sclera, high palate, thick alveolar ridges, mild scoliosis, lumbar lordosis, and indistinct speech. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4). She therefore had clinical features consistent with OI. The myopathic changes are not seen in OI and may therefore represent a second clinical diagnosis in this patient or a new OI syndrome.

The first-born child (sibling 1) born to the same parents, was born at 36 weeks via normal vaginal delivery after induction for pre-eclampsia. She had a left femoral fracture at the age of two years. At clinical assessment of this fracture, she was also noted to have bowed tibiae. She had three subsequent fractures involving her femur and tibia. She also has normal hearing and no dental anomalies. At the age of 16 years, she developed a thrombus in the left anterior descending artery resulting in an extensive anterior myocardial infarction (MI), which was successfully stented. At the time, she had impaired glucose tolerance with acanthosis nigricans, secondary amenorrhoea, polycystic ovaries, and a fatty liver. To manage this, she had been commenced metformin and the combined ethinylestradiol/cyproterone acetate contraceptive pill upon which improved these clinical problems. The combined oral contraceptive pill was subsequently stopped after her MI. Her height was between the 25th and 50th centiles and weight over the 97th centile, with a BMI of 31.8kg/m^2. On examination she has acne, facial hirsutism, abdominal striae and acanthosis nigricans. These clinical features were consistent with a metabolic syndrome. These features are not typically seen in OI. They may represent a new OI syndrome or a second diagnosis in this multiply consanguineous family.

The fourth child (sibling 2) born to the same parents, was born at 34 weeks via Caesarean section for pre-eclampsia with a birth weight of 1.7kg. He presented with a right tibial metaphyseal fracture at the age of two. Subsequent fractures included a femoral fracture, humeral fracture, and vertebral compression fractures to T8-T10. He developed severe scoliosis requiring surgical correction with rod insertions. After

surgical correction he developed restrictive lung disease. He also had a right conductive hearing loss, but no dental anomalies. Similar to the proband, he has motor involvement with moderate hypotonia, which has meant he did not walk without support until the age of 3 years. In addition, he also has nephrocalcinosis, atrial septal defect (ASD) and ventriculo-septal (VSD), which was surgically repaired at 2 months due to clinical features of heart failure. His height is just above the 0.4th centile and weight below the 0.4th centile. On examination he had epicanthic folds, and hypertelorism. Again, sibling 2 has additional features not seen in OI, but different to his other siblings.

The fourth affected individual in this family, is the cousin of the three siblings described above. She is the first child born to consanguineous parents at 38 weeks gestation with a birth weight of 2.29kg. During the pregnancy bilateral femoral bowing and fractures were identified. A femoral fracture was evident soon after birth. She had normal hearing and no dental problems. She has not yet reached menarche. Her weight was between the 0.4th and 2nd centiles. On examination she had blue sclera. She therefore has none of the additional clinical features seen in her three cousins but has a more severe type of OI that was detectable in utero (comparison in table 4.2. below).

Table 4.2. Comparison of affected individuals' phenotype with HPO in Family 3

WES was performed in Sibling 1 (highlighted)

Proband	Sibling 1	Sibling 2	Cousin
Recurrent fractures HP:0002757	**Recurrent fractures HP:0002757**	Recurrent fractures HP:0002757	Recurrent fractures HP:0002757
Reduced bone mineral density HP:0004349	**Reduced bone mineral density HP:0004349**	Reduced bone mineral density HP:0004349	Femoral bowing HP:0002980
Blue sclerae HP:0000592	**Tibial bowing HP:0002982**	Vertebral compression fractures HP:0002953	Blue sclerae HP:0000592
Myopathy HP:0003198	**Obesity HP:0001513**	Thoracic scoliosis HP:0002943	
Prominent forehead HP:0011220	**Secondary amenorrhoea HP:0000869**	Conductive hearing impairment HP:0000405	
Hypertelorism HP:0000316	**Polycystic ovaries HP:0000149**	Hypertelorism HP:0000316	
Hypothyroidism HP:0000821	**Myocardial infarction HP:0001658**	Joint hypermobility HP:0001382	
Mandibular prognathism HP:0000303	**Acne HP:0001061**	Nephrocalcinosis HP:0000121	
Alveolar ridge overgrowth HP:0009085	**Glucose intolerant HP:0000833**	Muscular Hypotonia HP:0001252	
Epicanthus HP:0000286	**Hepatic steatosis HP:0001397**	Epicanthus HP:0000286	
High palate HP:0000218	**striae distensae HP:0001065**	Ventricular septal defect HP:0001629	
	Acanthosis nigricans HP:0000956	Atria septal defect HP:0001631	
	Facial hirsute HP:0009937	Restrictive lung disease HP:0002091	

4.3.3. Result

WES revealed 23 rare homozygous variants (3 nonsense, 2 frameshift, 3 splicing, 10 missense, 4 non frameshift insertion and 1 non frameshift deletion variant) in Sibling 1 (see Appendix 11.5.3). None of these were reported as pathogenic in ClinVar or HGMD. Among the homozygous truncating variants, I prioritized the homozygous nonsense variant c.507G>A in exon 4 (p.Trp169*) of the *TMEM38B* gene after analysis of the WES results in Sibling 1. This has been confirmed on Sanger sequencing and segregation analysis has been consistent with the expected phenotype (see table 4). *TMEM38B* had been identified during the study period as a novel cause of autosomal recessive OI. This variant is therefore likely to partially explain the clinical features seen in this family (see discussion 4.3.4.). At the time, there were only a small number of patients reported with OI secondary to variants in the *TMEM38B* gene. It is therefore possible that as further patients were identified, some of the additional clinical features observed in Family 3 may be identified. I therefore worked with another group gathering a phenotypic series of patients together with biallelic variants in the *TMEM38B* gene, in the hope that this could be better assessed in a larger group of similarly affected individuals (Webb et al., 2017).

I continued to analyse the WES results for a second and even third genetic diagnosis that may explain wider clinical phenotype in Family 3. I identified an interesting homozygous missense c.83T>C p. Leu28Pro variant in the *DYRK1B* gene, which may be relevant to the metabolic syndrome phenotype see in Sibling 1. A heterozygous

gain-of-function missense variant, Arg102Cys, was reported to segregate with disease in three Iranian families with an early-onset metabolic syndrome, including early onset coronary artery disease, type 2 diabetes mellitus, hypertension and obesity (Keramati et al., 2014). A further five unrelated patients amongst a group of 300 morbidly obese individuals with a metabolic syndrome were all identified to carry a different gain-of-function missense variant in this gene, p.His90Pro, which was shown to enhance the expression of glucose-6-phosphatase (Keramati et al., 2014). There is increased expression of DYRK1B during differentiation mesenchymal stem cells towards the formation of adipocyte cells (Keramati et al., 2014). The proband had a different missense variant in the homozygous state (c.83T>C, p.Leu28Pro) that could therefore be contributory to her metabolic phenotype. This variant was predicted to be deleterious on *in silico* analysis. It was present with a 0.00433 (1060/245034) frequency in the heterozygous state in the GnomAD population database. A higher level of frequency of this variant in the heterozygous state is acceptable, since a metabolic syndrome is relatively common in the general population. However, a subsequent report demonstrated that the p.Leu28Pro variant, seen in the proband, is a loss-of-function variant and was even shown to have a statistically significant protective effect in 42 heterozygotes against type 2 diabetes mellitus and potentially hypertension (although this did not reach statistical significance) compared to two benign variants, which had no significant differences (He et al., 2014). It is therefore difficult to conclude that the p.Leu28Pro is likely to be the cause of the metabolic syndrome seen in Sibling 1. At this stage, family segregation studies of this variant are likely to be uninformative, in view of the young age of the other family members. The metabolic traits in the

heterozygotes compared to non-carriers in the 3 reported families showed statistically significant differences between the glucose levels, BMI and blood pressure with age of onset between 34 and 58 years (Keramati et al., 2014). Biochemical results in this family did not fit with this metabolic condition, which may be age related, confirm that this variant is protective, or even benign. It was also suggested that measuring the plasma levels of the adipokines, including adiponectin, leptin, may be informative biomarkers for this metabolic syndrome (He et al., 2014). There could be further clinical phenotypic investigation to explore this variant further, but in view of the suggestion that this variant could in fact be protective in the heterozygous state, I decided to not investigate this variant in *DYRK1B* further in Family 3.

4.3.4. Discussion

We identified a homozygous nonsense variant in *TMEM38B* segregating with disease in Family 3. This explains their OI phenotype of recurrent fractures ranging in first presentation from prenatal to 2 years of age, with associated osteopaenia, scoliosis from vertebral compression fractures, blue sclera and hearing impairment. There were additional clinical features including a metabolic syndrome, myopathy, congenital heart defects and nephrocalcinosis. *TMEM38B* associated autosomal recessive OI has been classified as OI type XIV and was clinically classed as being of moderate severity most akin to type IV (Valadares et al., 2014). This does seem to fit with the phenotype of OI seen within family 3.

At the time of identification there had been three papers in the medical literature describing families with OI and biallelic variants within the *TMEM38B* gene. Three Arabian families were reported to have a homozygous deletion of exon 4 within the *TMEM38B* gene by Shaheen et al (2012). Within this group of affected individuals, there was a range of severity reported with multiple fractures and osteopaenia, commencing either prenatally or up to the age of 6 years, but no other systemic involvement (Shaheen et al., 2012). Volodarsky et al (2013) reported 3 Israeli consanguineous families with a similar homozygous deletion of exon 4, with clinical features consistent with type IV OI including 6 individuals with reported grey-blue sclera in childhood, but no other systemic involvement (Volodarsky et al., 2013). Rubinato et al (2014) reported an Albanian girl with a homozygous deletion involving exons 1 and 2 with multiple fractures including seven at birth, osteopenia and mild conductive hearing loss detected at ten years of age. The patients in family 3 presented with multiple fractures of variable onset, ranging from prenatal onset to two years of age. There were no reported dental manifestations, but one sibling had mild conductive hearing loss and two patients had bluish sclera. Therefore, the clinical features of OI only were reported in the other individuals in the medical literature. Family 3 represented the first family with OI secondary to *TMEM38B* that was not an exonic deletion. However, the additional clinical features observed in Family 3, were not described in the other reported patients.

Family 3 were subsequently included in the 8 patient case series reported by Webb et al V:3 is Sibling 1, Proband is patient 4, Sibling 2 is patient 5 and Cousin is patient 6 (Webb et al., 2017). Patient 1 in this series similarly to the cousin in Family 3, presented antenatally with a bowed femur, whereas the other patients, excluding patient 8, had all presented with their first fracture at 2 years of age or less (Webb et al., 2017). Scoliosis, like Sibling 2, was seen in patient 7 and minimally in his relatively asymptomatic sibling, patient 8 (Webb et al., 2017). All patients in the series had osteopenia (Webb et al., 2017). The three patients reported by Lv et al appear to be the same three patients described by Liu et al. They had recurrent fractures, with mild bone deformities and short stature, but did not have any dental or hearing abnormalities and were therefore phenotypically most consistent with type 4 OI (Lv et al., 2016)(Liu et al., 2017).

There were other non-skeletal phenotypes described too in this case series. Sensorineural hearing impairment was seen in patient 1 and patient 7, compared to the conductive hearing impairment in Sibling 2 (Webb et al., 2017). The scleral appearance ranged between white, grey and blue in all individuals (Webb et al., 2017). Patient 7 developed a lung condition, although it was of a different type involving lower airway obstruction at the age of 22 years rather than the restrictive lung disease see in Sibling 2 (Webb et al., 2017). Sibling 1 had an MI and Sibling 2 had an ASD and VSD. Patient 7 in this case series had tricuspid regurgitation and nonobstructive hypertrophic cardiomyopathy, although his father had concentric left ventricular hypertrophy with mitral and aortic regurgitation, suggesting this may be a separate autosomal dominant

condition (Webb et al., 2017). Blood lipid profile, echocardiogram and electrocardiogram were normal in the other patients (Webb et al., 2017). Only the Proband and Sibling 2 in Family 3 had motor involvement. Patient 1 and 2 had developmental delay that could be due to a second condition or related.

Both set of heterozygous parents appear clinically unaffected in Family 3. The heterozygous parents of patients 7 and 8 in the case series by Webb et al potentially had some minor related features that may be explainable by other mechanisms (Webb et al., 2017). The father had macrocephaly, hip laxity only, and degenerative changes in midthoracic vertebrae (Webb et al., 2017). Their mother had short stature, and mild central compressions of lower thoracic and lumbar vertebrae, and bilateral mild mid-frequency sensorineural hearing impairment (Webb et al., 2017). There are no other reports of heterozygotes being clinically affected.

TMEM38B encodes the TRIC-B protein, which is an ubiquitously expressed intracellular cation channel protein, involved in controlling the release of calcium from the endoplasmic reticulum (Caparros-Martin et al., 2017; Marini and Blissett, 2013). OI is caused by a variant in the alpha1 or 2 subunits of collagen 1, a gene encoding a post-translational modifying protein of collagen 1, a protein involved in the folding of collagen 1, or its transport (Caparros-Martin et al., 2017; Marini and Blissett, 2013). Calcium is important in many steps within the pathway for type 1 collagen synthesis, which explains the OI phenotype (Caparros-Martin et al., 2017). The main biological focus

has been to find an explanation for the OI phenotype in affected patients. However, it is conceivable that additional clinical features outside the skeleton, skin and tendons would be possible in a condition resulting in defects of the TRIC-B protein, which is ubiquitously expressed. An underlying skeletal and cardiac muscle pathology could potentially occur secondary to the altered release of calcium from the endoplasmic reticulum, directly or indirectly affecting the expression of other genes or post-translational modification of other important protein in these cells (Webb et al., 2017). Skeletal muscle and cardiac muscle cells however, actually express more TRIC-A than TRIC-B (Webb et al., 2017). Tric-A knockout mice develop hypertension and skeletal muscle dysfunction, Tric-B knockout mice have respiratory defects, and neonatal lethality, and double knockout mice are embryonically lethal secondary to cardiac arrest (Zhou et al., 2014). In addition Tric-A knockout with Tric-heterozygous mice are susceptible to stress-induced heart failure (Zhou et al., 2014). This suggests some commonality but requires much further study. Cardiovascular assessment has therefore been recommended in patients with TMEM38B related OI (Webb et al., 2017).

Interestingly a SNP just outside *TMEM38B* has been associated with age of menarche (Chen et al., 2012; Dvornyk and Waqar-ul-Haq, 2012). Personal communication with the authors of the previously published patients revealed that the female members of the reported families have so far not reached puberty and therefore it is not clear whether they may yet develop premature ovarian failure or even a metabolic syndrome in both sexes, although the corresponding author of the Volodarsky paper did not respond (Rubinato et al., 2014; Shaheen et al., 2012; Volodarsky et al., 2013). Sibling 1

in Family 3 had normal menarche, but subsequently developed secondary amenorrhoea at 11 years. The proband has had normal menarche and menses to date, and the female cousin would not have reached puberty when reviewed. Additional work is required to understand this further.

An alternative, and more likely explanation for the additional phenotypes seen in Family 3 is that there is a second diagnosis explaining the metabolic syndrome, a third diagnosis explaining the myopathy or hypotonia, and even a fourth diagnosis to explain the congenital heart defects. The only additional molecular variant identified was a homozygous variant in the *DYRK1B* gene associated with a metabolic syndrome, but this same variant was demonstrated to be protective in 42 heterozygotes for type 2 diabetes mellitus and possibly essential hypertension (He et al., 2014; Keramati et al., 2014). This variant has therefore not been explored further. Additional phenotypes were reported in other patients by Webb et al, but they are not reported elsewhere, even in patients with the same variant in TMEM38B as seen in Family 3 (Caparros-Martin et al., 2017; Essawi et al., 2018; Liu et al., 2017; Lv et al., 2016; Rubinato et al., 2014; Shaheen et al., 2012; Volodarsky et al., 2013; Webb et al., 2017). Additional features are not described in the genetic classification of OI (Forlino and Marini, 2016). In a multiply consanguineous family, it is entirely possible that other autosomal recessive disorders could be seen in the siblings resulting in a blended phenotype (discussed further in general discussion (see Chapter 8)).

The initial reported patient with OI type XIV had an exonic deletion within *TMEM38B* resulting in a subsequent predicted frameshift variant (Shaheen et al., 2012). Patients have since been reported with the same homozygous nonsense variant as that seen in Family 3, as well as other nonsense variants, frameshift, splice site variant and an intronic variant leading to the insertion of two amino acids predicted to affect an important domain of the protein (Caparros-Martin et al., 2017; Essawi et al., 2018; Liu et al., 2017; Lv et al., 2016; Webb et al., 2017). It has been demonstrated in 3 patients with variants resulting in premature stop codons within *TMEM38B* that the transcripts were subject to nonsense-mediated decay, including a patient with the same variant seen in Family 3 (p.Trp169*) (Caparros-Martin et al., 2017). This resulted in a functional null alleles demonstrated by the absence of TRIC-B protein in the fibroblasts and osteoblasts in all three affected individuals described when compared to TRIC-B protein presence in control samples (Caparros-Martin et al., 2017). They were also able to demonstrate altered Ca^{2+} flux in the cell of the proband compared to controls, particularly affecting the rate of recovery of the endoplasmic reticulum Ca^{2+} stores (Caparros-Martin et al., 2017). Importantly, the patient also had altered expression and activity of multiple proteins involved in post-translational modification of type 1 collagen, resulting in the production of misfolded collagen, which was subject to intracellular degradation, with a 50–75% reduction in collagen secretion from fibroblast and osteoblast cells compared to the control cells (Caparros-Martin et al., 2017). *TMEM38B*-related OI has been classed as a group B condition secondary to defects in collagen modification resulting in a severe OI with normal to blue sclera and no additional hearing loss or dental problems (Forlino and Marini, 2016). This therefore

demonstrates the functional impact of the homozygous variants in *TMEM38B* and confirms their role as the disease-causing variants in the OI in the affected individuals.

In summary, I describe a family with Type XIV OI secondary to a homozygous nonsense variant in the *TMEM38B* subsequently reported in other individuals. *TMEM38B* encodes the TRIC-B protein involved in control of calcium flux in the endoplasmic reticulum, which affects the activity of proteins involved in the post-translation modification of collagen 1 and therefore a misfolded protein, which is partly subject to intracellular degradation, and results in a moderate-severe form of OI. Family 3 have additional phenotypes, which might result from a blended phenotype secondary to more than one genetic diagnosis evident in a multiply consanguineous family.

4.4. Family 6

4.4.1. Clinical Description

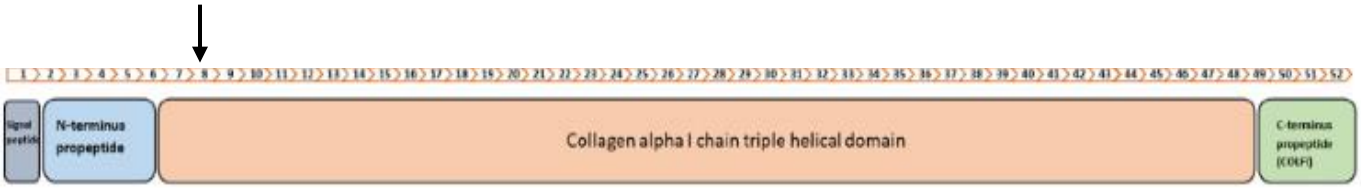
Family 6 are a multiply consanguineous family with a connective tissue disorder. The proband presented at the age of 2 with delayed gross motor development. He had a height on the 50th centile and a head circumference on the 99.8th centile consistent with macrocephaly. He came again to the genetics department in his 30s, after referral by a neurologist who had noted areflexia excluding the ankle, distal amyotrophy, and distal muscle weakness, which he thought were more likely to be secondary to a connective tissue disorder. Nerve conduction studies and electromyography were normal. He had increased susceptibility to fractures with bilateral fractured femora at the age of 4, and subsequent recurrent metacarpophalangeal fractures. In addition, he had joint hypermobility, with recurrent shoulder dislocation and tendon rupture of right index flexor tendon and right biceps. He had arthralgia, and osteoarthritis of the knee and right hip. As a child he was described as having blue sclera, and subsequently as an adult, slate grey sclera. Other clinical features included camptodactyly of the fingers and toes, convex contour of sole, pes planus, and excessive wrinkling of palmar skin. Overall the clinical phenotype has been progressive as the patient became older. It was unclear whether he had ever had an echocardiogram. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4).

The sibling of the proband also had clinical features of a connective tissue disorder as a child. It is reported by the proband that the clinical problems seen in the sibling resolved by adulthood. I was unable to contact the sibling directly to confirm this. As a child a skin biopsy was performed in the sibling, for collagen analysis, but no abnormality of collagen was identified at the time.

4.4.2. Result

I detected 45 rare homozygous variants from the WES data in the proband from Family 6 (2 frameshift, 42 missense and 1 unknown variant) (see Appendix 11.5.6). There were no reported variants in ClinVar or HGMD. Amongst these variants, I was then able to identify a homozygous missense variant in exon 8 of the *COL1A2* gene, c.370G>A p.Gly124Ser (see figure 4.1). At the time the variant was not reported on population databases including dbSNP or exome variant server. This specific variant was not reported in a disease database, but a similar variant resulting in a substitution of the same amino acid (c.371G>A p.Gly124Asp) has been previously reported as pathogenic on HGMD and ClinVar. No additional supporting data has been provided including zygosity and phenotype.

Figure 4.1: COL1A2 exons with corresponding protein domains and variant position including arrow demonstating the location of the variant in Family 6 (adapted from Figure 2 (Ho et al., 2016))



4.4.3. Discussion

The proband in Family 6 had features of a connective tissue disorder with clinical features overlapping with osteogenesis imperfecta and Ehlers-Danlos syndrome, which are progressive. He was found to have a homozygous glycine substitution, which is likely to be the disease-causing variant in this family. His brother had previously been assessed for symptoms in childhood, which we believe are largely resolved now but his genetic status is unknown. The heterozygous carrier parents are not known to be clinically affected. Biallelic mutations in genes causing autosomal dominant conditions have been described in many disorders. For example, homozygosity for achondroplasia-associated *FGFR3* mutations causes a more severe phenotype with premature death from respiratory failure, whereas individuals with biallelic Huntington expansion variants do not seem to present earlier (Migliore et al., 2019; Pagon et al., 1993; Uhlmann et al., 2015). It is difficult to clarify the range of severity seen in Family 6 without having segregation studies and detailed phenotyping, which is not possible.

There are only a few reports of patients with biallelic variants in *COL1A2*, which I will now discuss in more detail.

Individuals with biallelic variants in the *COL1A2* gene have been reported with Ehlers Danlos syndrome (EDS), cardiac valvular type (see table 4.3 for comparison of phenotypes). Three EDS patients with cardiovascular involvement were reported to have compound heterozygote splice variants or homozygous nonsense variants (Schwarze et al., 2004). The clinical features reported in patients with this type of EDS include delayed motor milestones, joint hypermobility especially of the small joints, recurrent shoulder dislocations pectus excavatum, muscle and tendon rupture, bilateral inguinal hernias, pes planus, genu recurvatum, atrophic scars over the knees and shins, easy bruising, thin or soft skin, striae, delayed wound healing, myopia and bilateral astigmatism (Hata et al., 1988; Nicholls et al., 2001; Schwarze et al., 2004). The cardiac valvular phenotypic features have included mitral prolapse, severe mitral regurgitation, aortic insufficiency, left atrial and ventricular dilatation, and ventricular hypertrophy, (Hata et al., 1988; Schwarze et al., 2004) or no cardiac phenotype yet, which may be age related (Nicholls et al., 2001). There are no known cardiac features in Family 6. On skin biopsy the fibroblasts in the affected patients have failed to make proα2(I) chains (Hata et al., 1988; Schwarze et al., 2004). Another patient with hypermobile EDS, a normal echocardiogram, and no evidence of procollagen 1α2 or collagen 1α2 chains, has been reported with a homozygous single nucleotide insertion resulting in a frameshift and a premature stop codon (Malfait et al., 2006). This patient was only 6 years of age and therefore will be observed for the cardiac valvular clinical features,

which may present in adulthood (Malfait et al., 2006). It would be helpful to perform collagen studies from fibroblasts in the proband to check the protein levels, but the patient did not want to have a skin biopsy performed. There was mild or no bone fragility reported in these patients suggesting that bone appears to function normally or almost normally in individuals with no production if the type 1 collagen α2 (Schwarze et al., 2004).

Table 4.3: Patients with biallelic variants in COL1A2

Patient	Variant effect	Skin	Bone/Joint	Cardiac	Eyes
Family 6	Homozy Glycine substitution	Excessive wrinkling of palms	hypermobility, recurrent # dislocations and tendon rupture	Nil	Blue Later slate grey
DePaepe P1	Homozy Glycine substitution	-	Severe, progressively deforming OI	-	-
Constantini P1	Homozy Glycine substitution	Not recorded	Recurrent # Osteoporosis	Not recorded	Blue sclera
Constantini P2	Homozy Glycine substitution	Not recorded	Bone fragility	Not recorded	Blue sclera
Nicholl P1	Homozy C terminal Frameshift deletion	Soft, silky, prominent veins	Multiple # Severe bony deformities Popcorn epiphyses Osteoporosis Hypermobility Motor delay	Not recorded	Not recorded
Nicholl P2	Homozy Frameshift deletion	Normal	Joint laxity Muscle hypotonia Recurrent # dislocations Pes planus	Normal	Pale blue
Schwarze P1	Comp het Splicing	Atrophic scars LL Thin skin Easy bruising Herniae	Hypermobility, pectus excavatum, muscle and tendon tears	MVP, MR, AR, AF, LVH	Myopic Astigmatism
Schwarze P2	Comp het Splicing	Soft Hyperext Atrophic scars Easy bruising	Hypermobility,	MR	Not recorded
Schwarze P3	Homozy Nonsense	Soft Hyperext Striae	Hypermobility Pes planus Talipes	sASD MVP, MR AR	Not recorded

			Genu recurvatum	LVH	
Malfait P1	Homozy Frameshift insertion	Herniae Normal skin	Joint laxity Muscle hypotonia Pes planus Genu recurvatum	Bulging mitral valve	Normal
Hata P1	Not recorded	hyperextensible skin Abnormal wound healing	Joint hypermobility	MR	Not recorded

Legend: # fractures, AR atrial insufficiency, Comp het compound heterozygous, Homozy homozygous, hyperext hyperextensibility, LL lower limbs, LVH left ventricular hypertrophy, MR mitral regurgitation, MVP mitral valve prolapse, OI osteogenesis imperfecta, sASD secundum atrial septal defect

(De Paepe et al., 1997; Hata et al., 1988; Malfait et al., 2006; Nicholls et al., 2001, 1984; Schwarze et al., 2004)

The different variants reported in patients with EDS cardiac valvular type generally seem to result in biallelic premature stop codons in the *COL1A2* gene (Nicholls et al., 2001; Schwarze et al., 2004). For the reported splice site variants in this group of patients, rather exon skipping, a cryptic splice site is utilised, resulting in premature stop codon producing unstable mRNA transcripts, which are subject to nonsense-mediated decay degradation (Schwarze et al., 2004). The variants therefore, result in an absence of procollagen 1 α2 chain synthesis, and therefore produce a similar phenotype

secondary to the same mechanism as the homozygous nonsense variants (Schwarze et al., 2004). Family 6 have a substitution variant rather than the molecular mechanism described here. An echocardiogram has however, been requested.

The second phenotype seen in patients with biallelic *COL1A2* variants is a more severe OI phenotype. A patient with severe progressive OI type 3 was reported with homozygous collagen 1 α2 deficiency (Nicholls et al., 1984). Two siblings were described with severe, progressively deforming OI with homozygous glycine to serine substitution in the *COL1A2* gene, whose heterozygous first cousin parents, and two siblings had mild clinical features of OI (De Paepe et al., 1997). Collagen studies were performed on fibroblast cells, which similar to the homozygous individuals described earlier, demonstrated unstable collagen I only (De Paepe et al., 1997). Similarly other patients with biallelic *COL1A2* glycine-to-serine substitutions have been reported with severe OI (Costantini et al., 2018). It is therefore likely that the location of the biallelic glycine-to-serine substitution will affect the resulting phenotype, hence explaining the reason for the EDS and OI overlapping phenotype seen in Family 6.

Family 6 have an overlapping EDS and OI phenotype, which has been previously described in patients with heterozygous variants in both *COL1A1* and *COL1A2* (Malfait et al., 2013). This overlapping phenotype has been reported in 3 siblings with compound heterozygous variants in *COL1A1*, including a novel frameshift variant inherited from their mother with a mild OI phenotype and a arginine missense variant,

affecting the Gly-Xaa-Yaa amino acid repeat in the triple helical domain, from their clinically unaffected father, which would be predicted to give an EDS phenotype, and had been previously described in a family with overlapping OI and EDS phenotype (Ackermann and Levine, 2017). This was the first report of biallelic pathogenic variants in *COL1A1* (Ackermann and Levine, 2017). It is therefore likely that this *COL1A2* homozygous variant is the cause of the EDS and OI overlapping phenotype seen in Family 6.

In summary, it is hypothesised that Family 6 have a OI and EDS overlapping condition, secondary to a homozygous *COL1A2* glycine substitution (c.370G>A p.Gly124Ser) in the proband, but further studies are required to confirm this.

CHAPTER 5: RESULTS; RENAL DISORDERS

5.1. Overview

Table 5.1: Renal Disorders results overview

Family number	Clinical Diagnosis	Molecular Diagnosis
7	Galloway-Mowat Syndrome - Cerebro-osteo-nephro dysplasia	Candidate homozygous variants in *COG3, ANLN*
28	Polycystic Kidney Disease	Homozygous *PKD1* variant

In this Chapter I will focus my discussions on family 28 who have polycystic kidney disease, because I believe the homozygous *PKD1* variant explains the renal disease in this family. I will discuss the clinical and genetic features of 3 patients from two consanguineous families with renal disease. Unfortunately, the affected child in family 7 passed away during the course of this project. To investigate the *COG3* candidate variant further, I could have arranged to check the affected individual's transferrin levels. This could have been done on a Guthrie card, but the family did not want any further investigations performed at this time. Further analysis of these variants has therefore been halted.

5.2. Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) affects 1/400 to 1/1000 individuals globally, making it the most common genetic cause of renal disease (Cornec-Le Gall et al., 2014). Individuals with ADPKD develop fluid filled cysts from the renal tubule epithelial cells (Cornec-Le Gall et al., 2014). They may develop hypertension, hepatic cysts, pancreatic cysts, seminiferous tubule cysts, mitral valve prolapse, abdominal wall herniae and intracranial aneurysms (Cornec-Le Gall et al., 2014; Pagon et al., 1993). 85% of individuals with ADPKD have a heterozygous pathogenic variant in *PKD1* and the remaining approximately 15% of individuals have a variant in *PKD2*. End stage renal failure (ESRD) is reported to occur with a median age at onset of 58 years for *PKD1* and 79 years for *PKD2* (Cornec-Le Gall et al., 2013). For non-truncating *PKD1* variants the median age at ESRD onset was 67.9 years, and 55.6 years for truncating *PKD1* mutations (Cornec-Le Gall et al., 2013). There is however a broad age range with a small number of patients presenting in the neonatal period (Harris and Torres, 2014). Some of this variability is due to a second hypomorphic mutation in these earlier presentations (Bergmann et al., 2011; Rossetti et al., 2009). Polycystin 1 (PC1) is a receptor like protein and polycystin 2 (PC2) is a Ca2+ cation channel protein (Harris and Torres, 2014). These 2 proteins are thought to interact and play a role in Ca2+ regulation (Harris and Torres, 2014). They are felt to be very important in later embryogenesis for differentiation of tubular epithelium as well as recovery from acute injury (Harris and Torres, 2014).

Autosomal Recessive Polycystic Kidney Disease (ARPKD) is much less common, affecting 1 in 20,000 people (Harris and Torres, 2014). Individuals typically present with very enlarged cystic kidneys, generally identified antenatally or soon after birth. However, similar to ADPKD the range can be broad, with patients presenting in later childhood or adulthood reported (Harris and Torres, 2014). Patients also have congenital hepatic fibrosis, which is generally not seen in ADPKD. Some patients have pulmonary hypoplasia secondary to oligohydramnios, with 30% of patients consequently dying within the first year of life. ARPKD is caused by mutations in *PKHD1*.

Cysts in ADPKD are lined by a single layer of epithelial cells derived from all elements of the nephron, but mainly the collecting ducts (Jiang et al., 2006). Glomerular cysts are generally not seen (Jiang et al., 2006). Biliary dysgenesis is responsible for the liver cysts seen in ADPKD (Vujic et al., 2010). Interestingly, the *Pkd1* knockout mice do not develop liver disease, although older heterozygotes do develop cysts in the liver (Vujic et al., 2010).

More recently two other genetic causes of polycystic kidney disease have been reported. Heterozygous mutations in *GANAB* cause a variable ADPKD with polycystic liver disease phenotype, which affects PKD1 protein maturation (Porath et al., 2016). Mutations in *DZIP1L* have been reported in 4 families with an autosomal recessive PKD phenotype, which was variable, including both PKD and polycystic liver disease (Lu et

al., 2017). The cilia in affected cells showed decreased levels of PKD1 and PKD2 proteins on the ciliary membrane compared to controls.

Family 28 appeared to have ARPKD by analysing their pedigree, but clinically they did much better than would be expected for this condition. Whole exome sequencing was performed to identify the molecular basis for their condition. At the time of this analysis *DZIP1L* had not been identified. A homozygous *PKD1* mutation was identified in this family.

5.3 Clinical Description of Family 28

The proband was born to first cousin consanguineous parents. During the pregnancy, antenatal ultrasound scans identified bilateral enlarged hyperechogenic kidneys, which was felt to be consistent with ARPKD. Sequencing of PKHD1 was performed, but no variant was identified. Her most recent renal function aged 5 years was normal. She is hypertensive with no evidence of left ventricular hypertrophy, which is currently treated with lisinopril. She was previously thought to have autosomal recessive polycystic kidney disease (ARPKD), but her phenotype is milder than other children with ARPKD. An MRI brain was performed due to initial concerns regarding the possibility of a ciliopathy, but this was normal. Other problems include asthma, and coeliac disease. Her neurodevelopment is normal. There is therefore unlikely to be no syndromic cause for her renal cystic disease. She is thriving with a height running along the 91-98th

centile and weight on the 50th centile. On examination, she had a single 12mm depigmented macule lower right abdomen and a few small pigmented macules right upper thigh and left lower leg near the knee with overlapping toes. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4). Both parents and her unaffected sibling have had normal renal ultrasound scans.

The first cousin of the proband was born to consanguineous first cousin parents. On renal ultrasound scan, he has large echogenic kidneys bilaterally with multiple small peripheral cortical cysts. The size of these cysts has remained stable. He has a normal appearance to his liver and spleen. He has had normal renal function tests and urinalysis. Like his affected cousin he takes medication for hypertension. The Sequencing of *HNF1β* was unable to identify any causative mutations. Both parents and her unaffected sibling have had normal ultrasound scans.

5.4. Result

WES revealed 81 rare homozygous variants (2 nonsense, 4 frameshift, 61 missense, 2 non frameshift deletion, 5 splicing and 7 unknown variants) in the proband (see Appendix 11.5.28). None of these were reported in to be pathogenic in ClinVar or HGMD. Amongst the homozygous variants, I prioritized a variant in the *PKD1* gene (c.11975C>T p.Ala3992Val) in the proband (see figure 5.1). This variant results in the substitution of a highly conserved amino acid alanine to valine. This variant is not

reported in ClinVar or the population variation databases, exome variant server, 1000 genomes, or Gnomad. This prediction tool scores are as follows: Polyphen 1, SIFT 0, GERP 0.84, mutation assessor 0.67, and phylop 0.97. The physiochemical change is however, small. It is likely to be a hypomorphic variant that would cause disease only in the homozygous state. Both parents were heterozygous for this variant. The cousin was also found to be homozygous for this variant. His parents were also heterozygous. The unaffected sibling was not homozygous for this variant.

Table 5.2: Comparison of phenotypes for patients with biallelic PKD1 variants

Patient	Age PC	Ante-natal USS	Postnatal Imaging	Clinical Course	PKD1 Variant
Both parent carriers unaffected					
IV:13	Fetus	BEHKs		HTN on lisinopril	Homozygous A3992V
IV:2	Fetus	ND	BEHKs multiple small peripheral cortical cysts	HTN on lisinopril	Homozygous A3992V
Vujic et al	Fetus at 27/40	BEHKs	ND	At birth 36/40 CPAP for RDS ACEi for HTN 8y normal growth & GFR	R3277C R2220W
	Fetus	Hecho. Oligo-hydramnios	MRI 1 year: marked BEK diffuse tubular dilation and small cysts.	At birth 34/40 pulmonary hypoplasia. HTN, normal GFR	
Vujic et al	Fetus	BEHKs	ND	ESRD at 8.5y Renal transplant	Homozygous V1045M and T1570M
I1 P192 Rossetti	ND	ND	30y: Multiple small cysts, clubbed calyces		Homozygous N3188S
II2 P192 Rossetti et al	Fetus	BEHKs	Multiple cysts scattered throughout. No liver cysts	GFR 67ml/min per 1.73m2 at 15.5 years	Homozygous N3188S
II3 P192 Rossetti et al	9 years	ND	Several cortical cysts. No liver cysts	GFR 86ml/min per 1.73m2 at 15 years	
IIV M390 Rossetti et al	11 years	ND	MSK. Bilateral renal cysts. 3 liver cysts.	Multiple UTIs	R3105W R2765C
One parent affected					

III1 P438 Rossetti et al	Fetus	BEHKs	At 17: multiple small cysts, no CMD	5 months HTN	Q2158X R3227C
III2 P117 Rossetti et al	Fetus (31/40)	BECKs	8cm kidneys at 10m.	2 years HTN. GFR 89ml/1.73m2 at 15y.	Y3819X R2765C
III1 P118 Rossetti et al	Fetus	Massively BECKs	ND	Died perinatally pulmonary hypoplasia	7915dup20 R2765C
Family E Bergmann et al	ND	ND	BECKs: multiple small cysts, 1 large cyst RK	Chronic renal insufficiency	Y2753X R2255C
Family F Bergmann et al	ND	ND	Renal Hecho loss of CMD. 1 large cyst LK. DPM.	Congenital hepatic fibrosis with complications.	R1351fs L2696R
Family G Bergmann et al	Birth	ND	BEHKs and 1 hepatic cyst	5y: normal GFR. HTN quintuple Rx	L1400fs R4138H
	Fetus	Hecho	Multiple renal cysts	HTN triple Rx. Normal RF	
II2 M34 Rossetti	ND	ND	Multiple uniform size cysts. Mild dilatation of the calyces.	ESRF at 75 years. No liver cysts	Homozygous R3277C
II3 M34 Rossetti	ND	ND	Multiple uniform size cysts. No liver cysts.	Renal transplant at 62 years.	
Audrezet et al Patient 1	Ante-natally	Hecho	UnK	Creatinine 35 at 79 months	R1602fs R3272H
Audrezet et al Patient 3	22/40	Hecho	TOP Cysts of tubules and glomeruli.	TOP	R3277C
Audrezet et al Patient 6	15/40	Hecho kidney size: +14sd	TOP	TOP	Leu727P T3945M

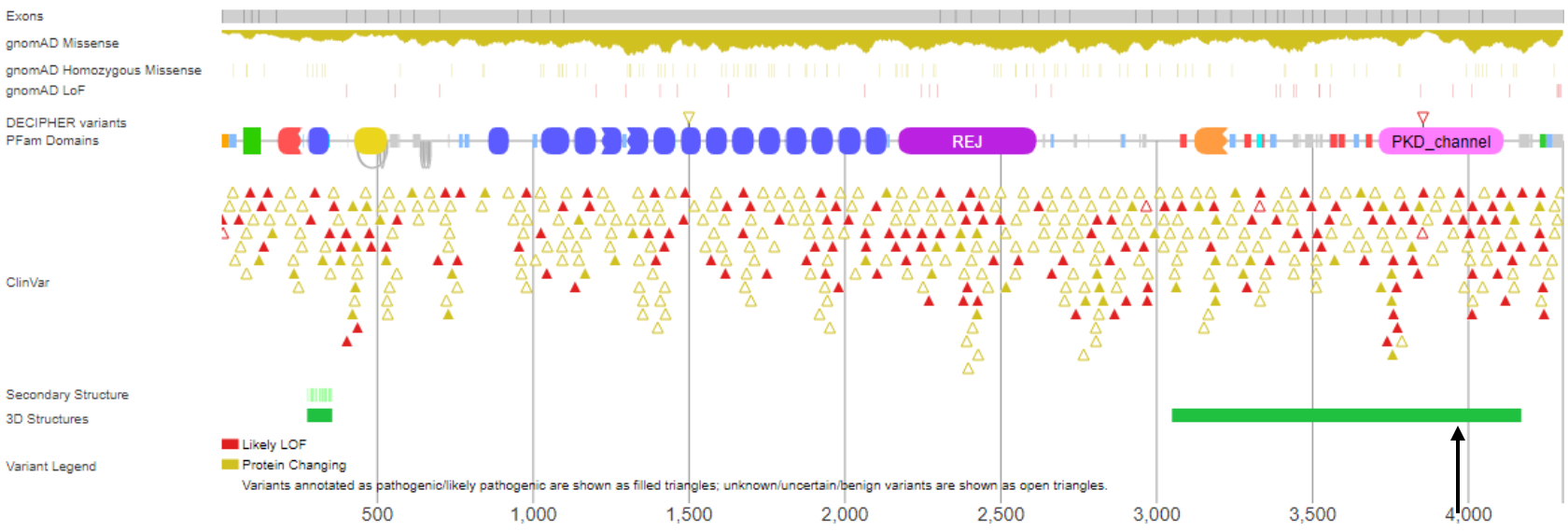
Audrezet et al Patient 8	32/40	Hecho kidney size: +3sd	UnK	Creatinine 34 at 2 months	C2370S R4154C
Audrezet et al Patient 29	22/40	Hecho kidney size: +10sd	UnK	Stage 2 CRF	Q861X E4025G
Audrezet et al Patient 31	22/40	Hecho kidney size: +3sd	UnK	Creatinine 24 at 1 month	W1958X R4154C
Audrezet et al Patient 39	22/40	Hecho	TOP: DPM Severe renal cysts of all nephronic structures.	TOP	Q2243fs R4154C

Legend: ACEi angiotensin-converting enzyme inhibitor, Aff affected, BECKs Bilateral enlarged cystic kidneys, BEHKs Bilateral enlarged hyper-echogenic kidneys, CMD corticomedullary differentiation, CPAP continuous positive airway pressure, del deletion, DPM ductal plate malformation, Hecho Hyperechogenicity, fsM frameshift mutation, HTN hypertension, LK left kidney, MSK medullary sponge kidneys, MM missense mutation, ND not documented, NS nonsense mutation, PC presentation, PDA patent ductus arteriosus, RDS respiratory distress, RF renal function, RK right kidney, Rx treatment, Unaff unaffected, USS ultrasound scan, UTI urinary tract infection, y years

Summary of the antenatal and postnatal findings in affected patients reported here and the medical literature with biallelic *PKD1* variants. Family H Bergman et al not included because the 2 variants identified in *PKD1* were in cis. 15 families are reported by Audrezet et al, but only 7 of the families are listed here with known, pathogenic, likely

pathogenic or other interesting variants (Audrézet et al., 2016; Bergmann et al., 2011; Rossetti et al., 2009; Vujic et al., 2010).

Figure 5.1: Protein structure of PKD1 with population and ClinVar variant annotation from Decipher with variant location


Exons
gnomAD Missense
gnomAD Homozygous Missense
gnomAD LoF
DECIPHER variants
PFam Domains
REJ
PKD_channel
ClinVar
Secondary Structure
3D Structures
Variant Legend
Likely LOF
Protein Changing
Variants annotated as pathogenic/likely pathogenic are shown as filled triangles; unknown/uncertain/benign variants are shown as open triangles.
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000

5.5. Discussion

Homozygous mutations in *PKD1* and *PKD2* leading to complete protein loss is embryonically lethal (Lu et al., 1997; Wu et al., 2000). Within family 28 are two individuals with biallelic hypomorphic variants in *PKD1*. All heterozygous parents were unaffected with normal renal ultrasound scans.

Another individual in the department was similarly diagnosed with a biallelic variant in *PKD1*. The proband is a 30-year-old gentleman who presented at birth with large cystic kidneys bilaterally. The phenotype was felt to be consistent with autosomal dominant polycystic renal disease, but he unusually presented at birth with renal cystic disease. At the age of 14 years he had a renal ultrasound that showed enlarged kidneys bilaterally, full of very small cysts with some cysts measuring 1 cm in size. At the age of 24 years of age, both kidneys were enlarged at 17cm in size full of cysts. He has a normal renal function. However, he takes losartan for hypertension. A renal biopsy performed at 2 months of age demonstrated that most of the renal tissue biopsied was replaced by cysts, the majority of which were hugely dilated Bowman's capsules containing normal or sometimes shrunken glomerular tufts. There was also tubular atrophy and interstitial fibrosis present, with some hyperplasia of the collecting ducts, and the other tubules were normal. This result was felt to be in keeping with the characteristics of adult onset polycystic disease. The histology did not have any

hallmarks of infantile polycystic disease. His parents have both had normal renal ultrasound scans. He has a healthy older brother, who has also had a normal renal ultrasound scan. He was found to have a homozygous c.7412C>T p.Pro2471Leu variant in exon 18 of the *PKD1* gene. This is absent in population databases and has been reported previously as pathogenic in the literature (Bouba et al., 2001).

Bilineal heterozygotes with both *PKD1* and *PKD2* variants was first reported in a large pedigree in 2001 (Pei et al., 2001). There are previous reports of individuals with biallelic ADPKD with at least 1 hypomorphic mutation in *PKD1* or *PKD2* (see table 5.2) (Audrézet et al., 2016; Bergmann et al., 2011; Losekoot et al., 2012; Rossetti et al., 2009; Vujic et al., 2010). The hypomorphic mutations appear to not cause renal cystic disease, or at most very mild disease, in the heterozygous state. Digenic inheritance and triallelic inheritance has also been reported but this is beyond the scope of this discussion.

There have been several other similar autosomal dominant genes, which have now been reported with an autosomal recessive phenotype. A good example of this is *MFN2* discussed later in chapter 7. *MFN2* is a well-known cause of autosomal dominant axonal hereditary sensory and motor neuropathy (HMSN) or Charcot Marie Tooth (CMT). Nicholson et al reported 3 patients with severe HMSN with homozygous or compound heterozygous mutations in MFN2 (Nicholson et al., 2008a). Subsequently other patients have been described with autosomal recessive HMSN (Carr et al., 2015;

Polke et al., 2011; Tan et al., 2016). Generally, a more severe phenotype is seen in these individuals with an earlier age at onset of the condition. Homozygous or compound heterozygous variants in *PKD1* and *PKD2* present with a similar phenotype to those individuals with heterozygous variants, but generally with an earlier age at onset, and more severe disease, but this is not always the case. Prenatal onset ADPKD has been reported without a second identified variant, even with a known family history of ADPKD without prenatal onset (Audrézet et al., 2016).

Mouse models of ADPKD show similar findings to those seen in humans. Knockout homozygous null *Pkd1* mutant mice are embryonically lethal with the mice dying in late gestation (Jiang et al., 2006). Functional studies using mice with wild type, p.Arg3277Cys (RC) or null mutation were assessed. The *Pkd1*$^{+/null}$ mice (haploinsufficiency with 50% reduction in mature protein levels compared to Pkd1$^{+/+}$ mice) had no abnormality, the Pkd1$^{RC/RC}$ mice (60% reduction in protein level) gradually developed cysts, and the Pkd1$^{RC/null}$ mice (80% reduction in protein levels) had early onset rapidly progressive cystic disease (Hopp et al., 2012). This suggests a threshold level for cyst formation in PKD.

The cysts formed in biallelic ADPKD are generally small and homogenous, resembling those in ARPKD, compared to the heterogeneous size of the cyst formation in ADPKD. A second-hit model for cyst development in ADPKD has been postulated, which would support this observation. Homogenous cysts are seen in biallelic ADPKD due to almost

simultaneous cyst development, whereas the heterogenous cyst formation in heterozygous ADPKD is likely due to successive cyst development after acquiring a second somatic hit (Cornec-Le Gall et al., 2014). The second hit in heterozygotes occurs later and therefore they have later onset disease, whereas patients with biallelic ADPKD have their two hits from conception and therefore have more severe early onset disease (Bergmann et al., 2011). Piontek et al showed that mice in whom *Pkd1* inactivation occurred before day 13 had severely cystic kidneys, whereas those with later inactivation had milder later onset disease (Bergmann et al., 2011; Piontek et al., 2007).

The two-hit model is unlikely to be the full explanation with other genetic and environmental influences playing a part (Bergmann et al., 2011; Jiang et al., 2006). A better explanation considers the amount of functional PC1 to be important. Renal cyst formation is therefore inversely related to PC1 function levels, over a certain threshold for cyst formation, which would be consistent with the above second-hit model and allows for additional genetic and environmental factors that may influence these functional levels (Audrézet et al., 2016; Harris and Torres, 2014). This would therefore explain why an individual with prenatal-onset ADPKD may be identified in a known ADPKD family. *PKD1, PKD2, HNF1B* and *PKHD1* were sequenced in 38 probands with prenatal onset ADPKD and a known familial mutation (36 families had a PKD1 variant, 2 families a *PKD2* variant and one family had an unknown variant) to assess the frequency of additional variants (Audrézet et al., 2016). Additional variants in *PKD1* were identified in 15 of these patients, but the remaining patients had no second

mutation identified to explain their early onset ADPKD. Other genetic and/or environmental factors may later be identified in these other families.

Patients with biallelic ADPKD generally do not have ductal plate malformations or hepatitis fibrosis, which is characteristic of ARPKD. This was reported in 2 patients listed in table 1, Family F (Bermann et al) and Patient 39 (Audrezet et al). Both patients had a frameshift mutation with a hypomorphic mutation, however other patients with a frameshift mutation did not report ductal plate malformations. The cysts in ARPKD are generally formed in the collecting ducts. Mice homozygous for hypomorphic mutations with about 20% of the polycystin 1 levels seen in wild type mice, had intact glomeruli and proximal tubules, but the distal structures did not develop normally and cysts were mainly formed from the collecting ducts postnatally (Jiang et al., 2006). It was therefore postulated that the distal parts of the nephron require greater levels of PC1 than the proximal segments to develop normally (Jiang et al., 2006). However, the patient described by Vujic et al showed cysts derived mainly from the proximal tubule (Vujic et al., 2010).

In conclusion, biallelic ADPKD involving a homozygous hypomorphic mutation, generally results in a phenotype with more homogenous bilateral polycystic disease which presents earlier than heterozygotes, often in the antenatal or newborn period. The disease course is not as severe as patients with ARPKD. Generally, individuals with biallelic hypomorphic mutations have less severe disease than those with a

nonsense or frameshift mutation. It is likely that in PKD the PC1 levels are inversely related to cystic disease, with cysts developing once a certain threshold has been reached. This may be explained by a second-hit, either constitutive or a later somatic mutation, or other genetic and environmental factors.

CHAPTER 6: RESULTS; NEUROLOGICAL DISORDERS

6.1. Introduction

This fourth result chapter looks at several consanguineous families with a rare neurological disorder to try to identify the disease-causing variant explaining their phenotype. There is no specific theme to these disorders, they range from early onset infantile epileptic encephalopathy, to congenital insensitivity to pain, and neurodegenerative disorders. This chapter will explore these diagnoses in further detail.

6.2. Overview

Table 6.1. Overview of Neurological Related Disorders

Family number	Clinical Diagnosis	Molecular Diagnosis
11	Cerebellar atrophy and Intermittent ataxic episodes	No variant identified
13	Cerebellar ataxia and peripheral neuropathy	*SETX* homozygous variant
16	Congenital insensitivity to pain and obesity	No variant identified
17	Neurodegenerative with epilepsy	No variant identified
18	Early onset infantile epileptic encephalopathy	No variant identified
19	Neurodegenerative Syndrome	No variant identified
20	Infantile spasms	No variant identified
21	Recurrent encephalopathy and ataxia	Candidate *TRIO* homozygous variant
23	Spastic diplegia	*PEX16* homozygous variant
24	Cerebellar ataxia	No variant identified
25	INAD like	No variant identified
26	Leukodystrophy	*POLR1C* homozygous variant
29	Ataxia, short, hearing loss, and neuropathy	*SLC9A1* homozygous variant
31	Infantile spasms	No variant identified
35	Progressive Cerebellar Atrophy	*LONP1* homozygous variant

Family 23 are a consanguineous family previously seen by a retired colleague. The proband presented with progressive motor impairment from the age of 2 years, which manifested as spastic paraparesis. Progressive swallowing and speech difficulties were subsequently seen. A brain MRI scan demonstrated diffuse leukodystrophy. Investigations had included normal very long chain fatty acids (VLCFAs), biotinidase levels, lactate, cerebrospinal fluid and plasma glycine levels, acylcarnitines, amino acids and lysosyme enzymes. Single gene Sanger sequencing had not identified any variants in the *GJA12* and *PLP1* genes. I was unable to identify the disease-causing variant on WES performed in this patient. The patient was subsequently enrolled in the 100,000 genomes project (described further in the general discussion 8.3.). I was asked to review the results from the 100,000 genomes project and present at the multi-disciplinary genomics meeting. A homozygous novel missense variant in the *PEX16* gene had been identified as part of this project. Biallelic variants in *PEX16* are associated with Zellweger syndrome, a peroxisomal disorder which more typically presents with hypotonia in the neonatal period though a range of clinical severity has been reported. This patient would phenotypically fit with a mild Zellweger syndrome. VLCFAs would normally demonstrate raised C26:0 and C26:1 in plasma, as well as raised ratios of C24/C22 and C26/C22 (Pagon et al., 1993). The normal VLCFAs in this patient had originally led me to discount this *PEX16* variant in the WES result. When this variant was also identified in the 100,000 Genomes project, I reviewed the medical records from the Birmingham Children's Hospital. There were two previous VLCFAs results, performed prior to the normal result recorded in the clinical genetic notes, which represented a borderline result demonstating slightly increased C24 and C24:C22 ratios

with a comment questioning whether this could be dietary related. After discussion with the clinical biochemist, it was entirely possible that these earlier borderline biochemical results could be seen in mild Zellweger syndrome. I therefore felt the *PEX16* homozygous variant was a candidate disease-causing variant in this family consistent with the patient's phenotype and subsequent updated biochemical results. This family highlight the importance of careful documentation of all results, even if a previous borderline abnormality, as this can influence the subsequent diagnosis from WES or WGS.

Family 29 includes two siblings in a consanguineous Turkish family with clinical features of ataxia, short stature, hearing loss, and neuropathy. Both siblings had a variant in the *SLC9A*1 gene, c.1273G>A p.Arg425Cys, with a high Bass summary score of 0.645. The Bass score is an internal pathogenicity summary score of the *in silico* data to aid variant interpretation. We have been unable to confirm the parents are both heterozygous for this variant. The variant was not listed in the population databases. There is one report in the medical literature of a different homozygous variant in the same gene, p.Gly305Arg, associated with a very similar clinical phenotype called Lichtenstein-Knorr syndrome, including childhood onset progressive sensorineural hearing loss and progressive cerebellar ataxia in three siblings from a Turkish consanguineous family (Guissart et al., 2015). Two siblings with cerebellar ataxia have subsequently also been reported with a homozygous truncating variant in *SLC9A1*, c.862del p.Ile288Serfs*9 (Iwama et al., 2018). The gene encodes the NHE1 protein, which is the main Na+/H+ exchanger in the plasma membrane of mammalian cells (Li et al., 2014).

The arginine at position 425 has been shown to be critical for the proteins structure and function by neutralizing the helical dipole (Li et al., 2014). This therefore suggests that this homozygous variant in SLC9A1 is likely to be the disease-causing variant in this family, but further work is required to better understand its implications.

Family 26 have 2 siblings with hypomyelinating leukodystrophy. I found a homozygous missense substitution (c.581A>C, p.His194Pro) in *POLR1C* gene on review of the WES results. There were eight other patients reported in the clinical literature with pathogenic variants within this gene. POLR1C interacts with POLR3A and B, which is a known cause of hypomyelinating leukodystrophy, hypodontia, and hypogonadotrophic hypogonadism. In the eight reported patients, all eight had hypomyelinating leukodystrophy, three had hypodontia, but no one was reported with hypogonadotrophic hypogonadism, although several were too young for this to be clinically apparent. Both siblings in family 26, had caries in their permanent teeth, but no hypodontia. This missense variant was also present in two siblings in the homozygous state amongst the DDD open access patients. I contacted the local team regarding the additional patients in DDD, to consider reviewing the family for common ancestry using a SNP array to look at the origin of this variant, but the clinicians were not interested in pursuing this further. A larger phenotype study would be very beneficial and was being carried out by a Canadian group. Family 26 decided against further collaboration with this research group. This variant in currently classed by the clinical laboratory as a variant of uncertain significance. However, based on the clinical phenotype it is likely that this will subsequently be classified as a disease-causing variant.

Family 21 have a homozygous missense variant in *TRIO*, c.199C>T p.Pro67Ser. Both parents are clinically unaffected and heterozygous for this variant. *TRIO* is highly expressed in the brain including the cerebellum, which may therefore explain the proband's ataxia (Pengelly et al., 2016). The protein is extremely important in neurodevelopment and could therefore potentially explain the epileptic encephalopathy and developmental delay seen in the proband (Pengelly et al., 2016). The *TRIO* knockout mouse is embryonically lethal late in development (O'Brien et al., 2000). It may be that these variants are hypomorphic as seen in other disorders such as PKD (see discussion). This gene is associated with autosomal dominant mental retardation syndrome 44 in heterozygotes with this disorder, which manifests as global developmental delay, microcephaly, behavioural problems, and seizures in 1 patient (Ba et al., 2016; "OMIM - Online Mendelian Inheritance in Man,"; Pengelly et al., 2016). Dental anomalies with overcrowding or delayed dentition, digital anomalies with short tapering fingers and clinodactyly, and facial asymmetry or micrognathia have been described in patients with TRIO related intellectual disability (Ba et al., 2016; "OMIM - Online Mendelian Inheritance in Man,"; Pagon et al., 1993; Pengelly et al., 2016). The proband in Family 21 does not have microcephaly (head circumference on the 11th percentile), dental or digital anomalies, although these have not been reported in all patients, and currently there are only a small number of patients reported with this condition, so the phenotypic spectrum is unclear. It is possible that an autosomal recessive form of TRIO-related intellectual disability may have a different phenotype if these variants are hypomorphic. This is reported in GnomAD with a MAF of 0.00038

(94 out of 246024). The missense constraint Z score for *TRIO* is 5.32, which is over level considered significant (>3.09). Even the loss of function variants described in TRIO could be undiagnosed in the heterozygous state in the Gnomad population in view of the borderline to mild intellectual disability seen (Ba et al., 2016). TRIO is a biologically plausible cause of the intellectual disability seen in the proband, but additional functional work is required to investigate this further.

Overall a likely or potential diagnosis has been identified in 6 out of 16 families (37.5%). I will discuss the results for Family 13 and 35 in more detail in the remainder of this chapter.

6.3. Family 13

6.3.1. Clinical Description

The proband was the first child to consanguineous first cousin Bangladeshi parents. At the age of 17 years, he first noticed that he was tripping up more easily than previously. He went onto develop cerebellar ataxia with nystagmus, lower limb weakness, abnormal movements and numbness. Consistent with this, on examination, he had an ataxic high stepping gait, reduced power in his lower limbs with normal tone, normal muscle bulk and absent reflexes, hand tremor, dysdiadochokinesis, sustained nystagmus on lateral gaze with broken pursuit and saccadic eye movements, dysarthria and reduced vibration sensation. He also reported mild memory impairment. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4). There were no other similarly affected people in his family.

Subsequent investigations identified that he had atrophy of his posterior fossa on MRI head imaging, though the pontine and midbrain structures were normal. Neurophysiology showed sensory axonal neuropathy of the lower limbs with a moderate right-sided carpal tunnel syndrome. Wilson's disease was considered because he had raised liver enzymes including an ALT of 133 U/L (normal range 5-41 U/L) initially with a low caeruloplasmin of 0.12g/L (normal range 0.2-0.45g/L) a slightly low copper of 10.2micromol/L and abnormal movements. Further evaluation revealed that he did not have Kayser-Fleischer rings, his urinary copper excretion was within normal limits on

two occasions and his liver enzymes subsequently returned to normal. He did not have any identified mutations in *SCA1, 2, 3, 6, 7, 17, Frataxin, DRPLA* or *POLG1*.

6.3.2. Result

WES revealed 81 rare homozygous variants (1 nonsense, 6 frameshift, 61 missense, 1 splicing, 1 non-frameshift deletion, 1 non-frameshift insertion, and 1 unknown variant) in the proband (see Appendix 11.5.13). None of these were reported in to be pathogenic in ClinVar or HGMD. Amongst the homozygous truncating variants, I prioritised the frameshift variant, c.5243delT p.Phe1748Serfs*3, which was identified in the *SETX* gene. Variants in *SETX* are associated with Ataxia with Oculomotor Apraxia type 2 (AOA2). This variant has not been reported in any population databases including ExAC and ESP. The variant has not been previously associated with disease, but other frameshift variants have been reported in the same exon of *SETX*. Both parents were found to be heterozygous carriers for this variant. Sanger sequencing confirmation has been performed in the proband only.

To evaluate this further, I performed alpha fetoprotein measurements, which showed raised levels of 64ng/mL (normal range 0-10). This is consistent with a diagnosis of AOA2 and gives further weight to the pathogenicity of the homozygous frameshift mutation in *SETX*.

6.3.3. Discussion

AOA2 is characterised by progressive cerebellar atrophy, axonal sensorimotor neuropathy, oculomotor apraxia with onset between 10 and 22 years (Anheim et al., 2009; Criscuolo et al., 2006; Moreira et al., 2004). The proband's clinical features would therefore be consistent with a diagnosis of AOA2, although he did not have any demonstrable oculomotor apraxia. Most individuals in the initial report by Moreira et al (2004) had this feature, but only 51% of patients have oculomotor apraxia in a large subsequent review of 90 patients (Anheim et al., 2009) and was only present in 2/10 patients reported in another smaller review paper (Criscuolo et al., 2006). The proband presented in adolescence similarly to other patients with AOA2. AOA1 by contrast tends to present earlier in childhood. The proband was reported by a neurologist to have choreiform movements, though a hand tremor only was latterly evident. Chorea has been reported in 9.5% of patients with AOA2 (Anheim et al., 2009). Some patients have also similarly reported mild cognitive impairment (Criscuolo et al., 2006).

Alpha fetoprotein (AFP) levels are raised in 99% of patient during their clinical course and generally remained at relatively stable levels (Anheim et al., 2009). A patient was reported with normal AFP after 27 years of disease progression, though his similarly affected siblings did have raised levels (Anheim et al., 2009). It is recommended that SETX is sequenced in an ataxic patient with an AFP of >7g/L (Anheim et al., 2009).

Fogel et al (2014) looked at gene expression levels in patient with *SETX* variants with both AOA2 and ALS4. *PSG4*, a pregnancy-specific glycoprotein and tumour marker, had the greatest increase in expression levels (Fogel et al., 2014). He therefore postulated that the AFP levels may be similarly raised due to alterations in gene expression levels secondary to the *SETX* homozygous variant (Fogel et al., 2014). In view of this, AFP levels were performed in the proband and found to be raised, consistent with a diagnosis of AOA2.

SETX encodes the DNA/RNA helicase, senataxin. *SETX* is responsible for recognising and repairing DNA damage. There are several other similar autosomal recessive ataxia disorders in which the causative gene is also recognising and repairing DNA damage. These include, the gene ATM and Ataxia Telangiectasia, Mre11 and A-T like disorder, as well as Aprataxin and AOA1 (Becherel et al., 2013; Yeo et al., 2014). These conditions have many phenotypic similarities. SETX is involved in the response to oxidative stress (Richard and Manley, 2014). Variants in *SETX* cause an increase in the number of R loops in the genome, which are postulated to lead to genomic instability and DNA damage (Becherel et al., 2013; Fogel et al., 2014; Richard and Manley, 2014; Yeo et al., 2014). R loops are areas of the genome with one strand of template DNA and the other complementary strand is RNA rather than a strand of DNA (Becherel et al., 2013; Fogel et al., 2014; Richard and Manley, 2014; Yeo et al., 2014). These may occur at transcription termination sites, for example. The accumulation of R loops appears to be limited to proliferating cells rather than neural tissue and is

therefore not likely to contribute to the neurodegeneration (Yeo et al., 2014). Further work to understand the pathogenesis is required.

Truncating variants in *SETX* were identified in 10 out of the first 15 patients reported with AOA2 (Moreira et al., 2004). It is postulated that loss of function mutations therefore cause AOA2 consistent with a autosomal recessive condition (Fogel et al., 2014). It is therefore highly likely that the novel homozygous truncating variant identified in the patient is disease-causing. Heterozygous missense variants in *SETX* have also been associated with autosomal dominant juvenile amyotrophic lateral sclerosis type 4. Missense variants have also been reported on AOA2. Variants causing ALS4 do not seem to cluster in different regions of the gene (Anheim et al., 2009). Heterozygous parents of patients with AOA2 do not have neurological features, similarly to the parents in family 13. It is postulated that patients with ALS4 have gain of function variants that modify the function of *SETX* consistent with autosomal dominant disease (Chen et al., 2004; Fogel et al., 2014). Consistent with this hypothesis, Fogel et al (2014) demonstrated some differences in gene expression profiles between these two conditions.

6.5. Family 35

6.4.1. Clinical Description

The proband is the first child to two healthy consanguineous parents. The parents are first cousins once removed. They have had 4 subsequent pregnancies, one molar pregnancy, 2 healthy children and 1 similarly affected child. The proband was born at term via ventouse delivery after a pregnancy complicated by renal pelvic dilatation only. He had a sternocleidomastoid tumour of the neck, which responded to physiotherapy treatment. He had swallowing difficulties at weaning but was able to gain weight normally. He had normal development until the age of 13 months. At this age he was able to crawl and cruise around the furniture.

At 13 months of age the proband developed a flu-like illness. During this illness he lost his balance. He was managed with intravenous methylprednisolone and immunoglobulins. He reportedly was able to crawl unsteadily after this episode. At the age of 3 years he was able to recognise his alphabet and count to 10. After a further flu-like illness, he seemed to loose further developmental skills. At the age of 7, he was able to crawl, stand with support, and understand two stage commands. He had no words. In addition, the proband has an intermittent convergent squint with normal vision, normal hearing, and microcephaly. On examination he has low truncal tone with increased tone in the limbs. Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4).

The proband has been fully investigated for his developmental regression, including a normal muscle biopsy with a normal histopathological appearance and respiratory chain, microarray comparative genomic hybridization test, *PLA2G6* and mitochondrial gene mutation screen, and there was no expansion mutation in *SCA1, 2, 3, 6, or 7*. An MRI brain scan has demonstrated cerebellar atrophy.

The second affected female sibling has cataracts with strabismus, and visual impairment, which were surgically removed at the age of three months. In addition, she has developmental delay, with no evidence of regression, truncal hypotonia and increased tone in both of her limbs. She also has cerebellar atrophy detectable on MRI brain scan. At the age of 3 years, she could speak in two-word sentences, and cruise, but could not walk without support. The sibling has been separately investigated for the cause of her congenital cataracts. She has had a cataract gene panel performed, which did not identify a disease-causing variant.

In summary, there are two affected siblings in Family 35 with truncal hypotonia, increased tone in the limbs and cerebellar atrophy. The proband had additional developmental regression and the sibling, additional cataracts. It is likely that they have the same autosomal recessive condition to explain their clinical problems, which is similar to Marinesco-Sjogren syndrome. The family had been included in a previous research study by Andrea Nemeth, looking into the cause of the cerebellar atrophy, but

no disease-causing variant was identified at the time, which would likely have included *SIL1* sequencing.

6.4.2. Results

WES revealed 32 rare homozygous variants (29 missense, 1 non-frameshift deletion, 1 splicing and 1 unknown variant) in the proband (see Appendix 11.5.35). None of these were reported in to be pathogenic in ClinVar or HGMD. Amongst these variants, I prioritised a homozygous missense variant in exon 15 of the *LONP1* gene (c.2282C>T, p.Pro761Leu). This has been found in the heterozygous state in 3 out of 245642 individuals, with a MAF of 0.00001 in GnomAD. The variant had a Bass summary score of 0.58. It was predicted to be damaging by PolyPhen, SIFT and MutationTaster. It is not reported in ClinVar or in the medical literature. Both parents are heterozygous for this variant. Individuals with biallelic variants in *LONP1* were originally described in a multisystemic disorder with a predominant skeletal phenotype, called CODAS syndrome. This acronym stands for **C**erebral including hypotonia and developmental delay, **O**cular including cataracts, **D**ental with delayed dentition, *A*uricular with malformed external ears and hearing loss, and *S*keletal anomalies including short stature and coronal clefts (Dikoglu et al., 2015). Cerebellar atrophy and hypoplasia have been reported (Inui et al., 2017). It is therefore possible that this represents the disease-causing variant in this family, which will be discussed further in the discussion (6.4.3.). This variant was subsequently reported in the medical literature associated with a similar neurodegenerative phenotype, which therefore makes it more likely this is the disease-causing variant in the family (Nimmo et al., 2019).

6.4.3. Discussion

Family 35 have a likely autosomal recessive disorder explaining their cerebellar atrophy, truncal hypotonia, and limb hypertonia. Visual problems are also seen in both siblings, with congenital cataracts in the second affected sibling, and an intermittent convergent squint in the proband. The proband has been found to have a homozygous variant in the *LONP1* gene, which is associated with CODAS syndrome. This is typically a multi-system disorder, rather than a neurological disorder. Both cataracts and cerebellar atrophy have been reported as clinical features within this phenotype. I will therefore explore, whether this could be the disease-causing variant in Family 35.

Homozygous and compound heterozygous missense variants in *LONP1* were first associated with CODAS syndrome in ten individuals with typical features of this condition, including a founder variant in the Pennsylvania Amish community (Strauss et al., 2015). Within the Amish community, laryngeal obstruction, was commonly reported, resulting in neonatal death in three individuals (Strauss et al., 2015). Biallelic nonsense, and in-frame deletion variants were subsequently reported in *LONP1* in seven further individuals with CODAS syndrome (Dikoglu et al., 2015). This second group of patients generally had a less severe phenotype, with all patients having early onset cataracts and an epiphyseal dysplasia, with other variable clinical features including crumpled

ears, developmental delay, short stature, congenital heart defect, though only one individual had dental anomalies (Dikoglu et al., 2015). Two individuals had cerebellar hypoplasia (Dikoglu et al., 2015).

More atypical phenotypes have since been reported. Biallelic variants in *LONP1* have been reported in patients with congenital nuclear cataracts only (Khan et al., 2015). In 2017 an atypical CODAS was reported in an individual with compound heterozygous variants in *LONP1* and imperforate anus, bilateral congenital cataracts, hypotonia, intellectual disability, regression, cerebellar atrophy and choreo-athetoid movements (Inui et al., 2017). The progressive nature of the disease with regression and cerebellar atrophy had not been previously described in CODAS syndrome (Inui et al., 2017). Subsequently, there has been a recent report of two siblings with the same homozygous missense variant in the *LONP1* gene, c.2282C>T, p.Pro761Leu (Nimmo et al., 2019). These two siblings had a very similar presentation to the proband in Family 35 with episodes of developmental regression, hypotonia, severe intellectual disability and progressive cerebellar atrophy (Nimmo et al., 2019). A muscle biopsy had shown scattered cytochrome c oxidase-negative staining and mitochondrial inclusions were observed (Nimmo et al., 2019). The proband in Family 35 had a reportedly normal muscle biopsy. The published siblings had reduced pyruvate dehydrogenase (PDH) activity on fibroblast analysis, which was shown to be secondary to increased levels of a subunit of PDH called phosphorylated E1α (Nimmo et al., 2019). This subunit inhibits PDH activity (Nimmo et al., 2019). The authors therefore suggested that this homozygous missense variant causes a functional PDH deficiency, thereby affecting

the production of energy in the cells, and subsequently causing neurologic impairment and neurodegeneration (Nimmo et al., 2019). This therefore means that this missense variant in *LONP1* is a very good candidate for the disease-causing variant in Family 35.

LONP1 is an important mitochondrial protein with multiple roles including as a protease involved in selective degradation of abnormal or damaged proteins, a chaperone protein involved in contributing to the stability of protein complexes, and a mitochondrial DNA binding protein involved in controlling expression of mitochondrial genes (Bota and Davies, 2016; Dikoglu et al., 2015; Strauss et al., 2015). *Lonp1* knockout mice were embryonically lethal by inhibiting cell proliferation (Quirós et al., 2014). LONP1 is upregulated in acute oxidative stress and is therefore most highly expressed in the most metabolically active organs such as the brain, heart, liver and skeletal muscle (Bota and Davies, 2016). LONP1 is downregulated in aging and more prolonged episodes of oxidative stress, demonstrating its role in neurodegenerative disorders (Bota and Davies, 2016). There appears to be a range of clinical features seen in individuals with *LONP1* variants, from severe CODAS, to moderate with a few clinical features including neurological, ocular and skeletal, to ocular only (Dikoglu et al., 2015; Inui et al., 2017; Khan et al., 2015; Khan and AlBakri, 2018; Nimmo et al., 2019; Strauss et al., 2015). The skeletal and structural anomalies in CODAS are not typical of mitochondrial disorders. A similar phenotype was seen in EVEN-PLUS syndrome associated with variants in *HSPA9*, which is another mitochondrial chaperone protein (Royer-Bertrand et al., 2015). A skeletal dysplasia and neurodegenerative condition have also co-existed in another mitochondrial disorder, Spondyloepimetaphyseal dysplasia associated with

AIFM1 variants (Mierzewska et al., 2017). The clinical features seen in Family 35, are similar to those seen in other mitochondrial disorders. Many of the biochemical hallmarks of mitochondrial disorders have not been seen in patients with CODAS, although a recently reported individual with a compound heterozygous missense variant had congenital lactic acidosis, recurrent apnoeas and possible seizures, and muscle weakness, had low activity of complexes I and IV on muscle biopsy with low levels of mitochondrial DNA (Peter et al., 2018). The mitochondria were also shown to be enlarged with swollen intracristal or intercristal compartments and electron-dense inclusions on electron microscopy of lymphoblast cell lines in affected individuals with CODAS syndrome, as well as demonstrable reduced LONP1 enzyme activity (Strauss et al., 2015). Mitochondrial dysfunction was also described above by Nimmo et al (2018). It is therefore clear that mitochondrial dysfunction is evident in patients with biallelic variants in *LONP1*, even if this has not been directly observed in Family 35.

In summary, Family 35 have more of a neurological phenotype associated with *LONP1* biallelic variants. Although lacking many of the hallmarks of a mitochondrial disease, there certainly are any overlapping features between these disorders and the clinical spectrum of CODAS. This family highlight the importance of remembering the range of severity of clinical phenotype that often emerges when using WES to elucidate the cause of disease in clinical genetics, although care must be taken to not make a condition fit inappropriately.

CHAPTER 7: RESULTS; MULTI-SYSTEM DISORDERS

Findings reported in this chapter have previously been published in a journal article (Rocha et al., 2017).

7.1. Introduction

This final result chapter looks at several consanguineous families with a rare multi-system disorder to try to identify the disease-causing variant explaining their phenotype. There is no specific theme to these disorders, they are each considered to be unique phenotypes, which may have a novel underlying mechanism.

7.2. Overview

Table 7.1. Overview of patients with Multi-System Disorder

Family number	Clinical Diagnosis	Molecular Diagnosis
9	Multiple Symmetrical Lipomatosis	*MFN2* homozygous variant
22	Jeune syndrome	*IFT80* homozygous variant
30	Fryns syndrome	No variant identified

I studied 3 families with multisystem disorders. I identified the likely pathogenic disease-causing variant in 2 out of the 3 families (67%). The proband in Family 22 presented with clinical features suggestive of Jeune asphyxiating thoracic dystrophy. Exome sequencing identified a homozygous missense variant in *IFT80* c.2101C>G p.Arg701Pro (rs137853116), predicted to be deleterious, found within a highly conserved region of the gene, and not present in population variation databases. This has also been reported in another consanguineous Pakistani family with Jeune asphyxiating thoracic dystrophy (Beales et al., 2007). It has also been seen in a patient with the same condition amongst the DDD open access patients. It is therefore likely to explain the clinical phenotype seen in the patient, which will therefore not be discussed further within this report. This chapter will instead focus on multi-system disorders as exemplified by Family 9 and also the process of analysis trialled in Family 30.

7.3. Family 9

7.3.1. Introduction

Lipodystrophies are a group of heterogeneous conditions, characterised by loss of body fat, which may be either inherited or acquired, generalised, affecting the whole body, or partial, affecting part of the body such as the limbs (Garg, 2011; Nolis, 2014). The commonest cause of lipodystrophy is acquired in patients with human immunodeficiency virus (HIV) treated with Highly Active Antiretroviral Therapy (HAART) (Nolis, 2014). Increased fat deposition is seen in this acquired type of lipodystrophy in the posterior neck, upper chest and abdomen, with loss of fat peripherally from the limbs and face (Cossarizza et al., 2001).

Mutations in mitofusin 2 (*MFN2*) are a very well described cause of Hereditary Sensory and Motor neuropathy (HMSN) also known as Charcot-Marie-Tooth disease (CMT), usually producing an axonal neuropathy (Nicholson et al., 2008b; Pagon et al., 1993; Polke et al., 2011; Tazir et al., 2013). Heterozygous mutations causing autosomal dominant HMSN are most common, but autosomal recessive HMSN has also been reported. HMSN is a genetically heterogeneous condition. Patients may present with a neurological phenotype of progressive distal muscle weakness, decreased or absent tendon reflexes, pes cavus, and distal sensory loss (Pagon et al., 1993; Verhoeven et al., 2006). Electrophysiological testing using nerve conduction studies (NCS) can confirm this diagnosis by demonstrating reduced motor nerve conduction velocities in

type 1 demyelinating form of HMSN and decreased amplitudes in the axonal form of HMSN in which axonal degeneration can be seen histologically (Verhoeven et al., 2006).

7.3.2. Clinical Description

This multiply consanguineous Irish traveller family presented with an interesting, and unique lipodystrophy. I have reviewed the proband, sibling and parents to undertake deep phenotyping. There are a number of other affected family members who were not accessible due to family dynamics (see discussion).

The typical lipodystrophy seen in the affected members within this family was described by the consanguineous parents illustrated by family photographs. Typically, the first clinical sign of the condition, was the development of striae over the back in childhood. After puberty, generally, mild loss of adipose tissue from the hands and arms has been seen, with fat accumulation around the neck, upper chest and upper arms. This fat forms a collar-like appearance around the neck with a buffalo hump. The face and lower limbs do not appear to be affected generally. This fat deposition continued to gradually increase in adulthood. This had caused complications including airway obstruction. The maternal uncle had reportedly died at 34 years of age from airway obstruction peri-operatively during routine hip surgery. I did not have access to his medical records and was therefore unable to substantiate this description. Also see the

HPO phenotype summary table and code (Appendix 11.3 and 11.4). There are 5 other reportedly affected family members including the paternal grandmother.

Affected family members have reportedly required de-bulking surgery of the adipose tissue in the neck and a tracheostomy to maintain a patent airway. This does not fit neatly into any of the other previously described types of lipodystrophy. The phenotype described had a similar appearance to the acquired lipodystrophy described above secondary to treatment with HAART. Additionally, there was some phenotypic similarity to multiple symmetrical lipomatosis (MSL). MSL is rare condition, more typically seen in middle aged males with excessive alcohol consumption. The clinical phenotype described is that of multiple non-encapsulated lipomas in the upper arm, upper chest and neck region, which may or may not be associated with an associated axonal neuropathy (Chong et al., 2003; Klopstock et al., 1997). Some individuals with MSL have been shown to have mitochondrial dysfunction as well as mitochondrial DNA deletions and the *MERRF* variant m.8344A>G (Berkovic et al., 1991; Klopstock et al., 1997). Both of these adipose overgrowth disorders are felt to arise from the brown fat, which is similarly distributed, and contains a greater number of mitochondria than white fat (Herbst, 2012). This family clearly had an autosomal recessive condition to explain their lipodystrophy rather than a mitochondrial genome or environmental cause, but the underlying biological process may be similar in view of the similar clinical phenotype.

The proband was 16 years of age at her follow up clinical review. She had evidence of lipodystrophy, including mild loss of adipose tissue in the hands, and adipose tissue deposition around the neck, upper back, and upper arms. The sibling was 15 years of age. At his previous initial review, he did not have any signs of lipodystrophy. Subsequently, at follow up, he was mildly with striae over his back, mild adipose tissue loss from his lower arms, a small buffalo hump and a full neck.

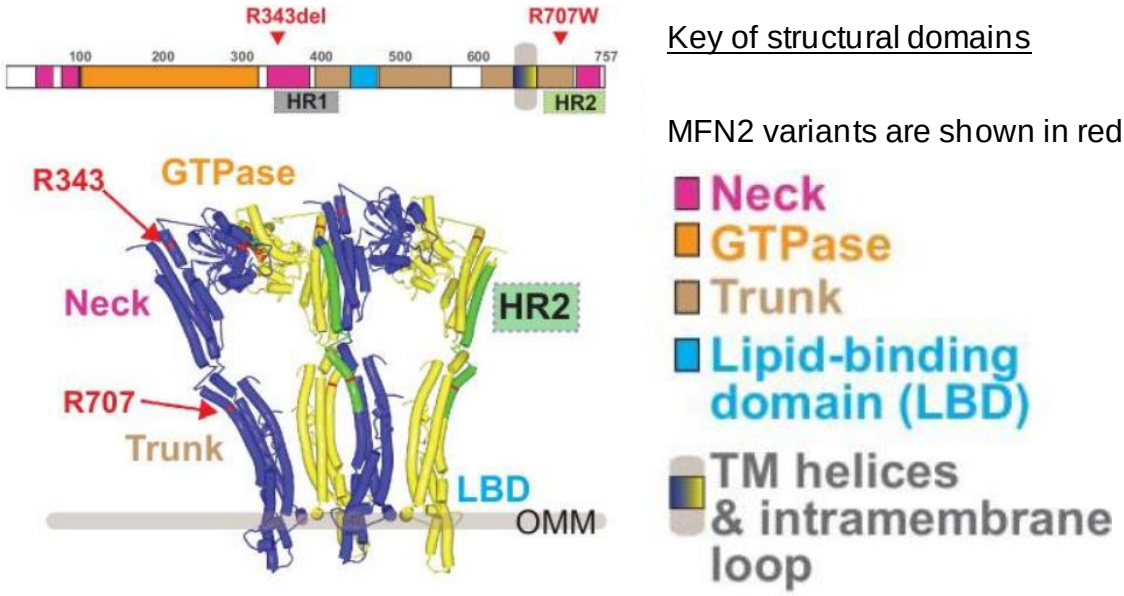
I gained consent to review the medical records only of the paternal grandmother. She had a lipodystrophy identical to the phenotypic description above. She had a long-term tracheostomy to prevent airway obstruction. An MRI of her neck has demonstrated diffuse fatty infiltrate of the tongue base causing a narrowing of the airway. Excessive adipose tissue deposition was noted. A lipomatosis lesion was also noted in the right supraclavicular fossa 4cm by 4cm.

7.3.2. Result

WES revealed 26 rare homozygous variants (1 frameshift, 12 missense, and 13 splicing variants) in the proband (see appendix 11.5.9). 1 of these were reported in to be pathogenic in ClinVar or HGMD, a homozygous mutation in *MFN2* c.2119C>T (p.Arg707Trp). This was present in both siblings. MFN2 is a ubiquitously expressed transmembrane GTPase. The variant seen in this family (p.Arg707Trp) is found in the 2nd heptad repeat (HR2) domain at C terminus of *MFN2* (see diagram 7.1) responsible

for homotypic interactions and heterotypic with MFN1. At the time of analysis, variants in *MFN2* were only known to cause the HMSN phenotype described above. This variant had been previously reported as a cause of autosomal recessive HMSN (see below). This result was confirmed on Sanger sequencing. Segregation analysis confirmed that both parents were heterozygotes for this mutation, but further family studies were not possible. In addition, at the time of initial analysis, only the elder sibling was showing signs of lipodystrophy and it was therefore not clear whether this variant simply represented an incidental finding.

Figure 7.1: Linear depiction of human MFN2 and structure of Bacterial Dynamin-Like Protein acting as a model of human MFN2a. Adapted from Figure 1 (Rocha et al., 2017)



Knockout of *Mfn2* in mice is embryonically lethal (Chen et al., 2003). At the time of initial analysis, the c.2119C>T missense variant seen in family 9 had previously been reported in 4 other individuals from 2 families with autosomal recessive HMSN (Calvo et al., 2009; Nicholson et al., 2008b). In the first family, the same homozygous c.2119C>T missense variant was described in a female individual who had had features of HMSN from the age of 2 (Nicholson et al., 2008b). She had additional features listed within a table of lipodystrophy, mild hearing loss, and kyphosis (Nicholson et al., 2008b). These additional features were not discussed further in the paper, which focused mainly on the neurological phenotype. Both parents were reportedly asymptomatic heterozygotes (Nicholson et al., 2008b). In the second family, 3 siblings aged between 19 and 25 were reported with a moderate HMSN phenotype presenting before the age of 10 (Calvo et al., 2009). They were compound heterozygotes for the p.Arg707Trp variant and another missense variant, p.Gly108Arg, which is located in the GTPase domain rather than the HR2 domain for p.Arg707Trp (Calvo et al., 2009). Both parents were also shown to be asymptomatic heterozygotes (Calvo et al., 2009). There was no lipodystrophy reported in the siblings (Calvo et al., 2009). I contacted the author to clarify if a lipodystrophy had subsequently developed, but they were unable to clarify this further. Phenotypic heterogeneity associated with different variants in the same gene is described and has been increasingly seen since the advent of NGS methods (Gilissen et al., 2011). I therefore postulated that the location of this variant in the homozygous state must cause a lipodystrophy phenotype, because Family 9 and the family reported by Nicholson et al were the only individuals known to have this homozygous variant, and both had a lipodystrophy (Nicholson et al., 2008b). I

contacted the author of the paper. He informed me that a research paper was being written about his family and another family in Canada, but they did not wish to collaborate. This meant that this was highly likely to be the disease-causing variant in this family, but we still needed to prove this biochemically.

After identifying this result, the family were reviewed again (see above). In addition to looking for signs of lipodystrophy, I assessed the patients for neurological deficit. The proband had clinical features suggestive of an early peripheral neuropathy only. She described an increased tendency to trip over. On clinical examination she had absent vibration sense in her feet and absent ankle reflexes. Nerve conduction studies were arranged to clarify whether she did indeed have features of HMSN, but this was normal. The sibling did not have any symptoms or clinical signs of a neuropathy. Nerve conduction studies will also be arranged in this patient and showed normal nerve conduction. The parents also had a normal neurological system. Review of the medical records for the paternal grandmother confirmed that her nerve conduction studies had been consistent with severe sensory motor axonal neuropathy.

Individuals with MSL may also have hyperlipidemia, fatty liver, hyperuricemia, hypothyroidism, and diabetes mellitus (Herbst, 2012). Glucose tolerance testing demonstrated that both siblings had normal glucose tolerance with elevated insulin levels suggesting insulin resistance, not yet requiring treatment.

This homozygous missense variant was biologically plausible as the cause of the patient phenotype seen in this family. MFN2 acts in the mitochondria. Both MSL and the HAART associated lipodystrophy have been linked to dysfunction of the mitochondria. This family therefore, appeared to represent a novel phenotype for a known HMSN disease gene with variants in MFN2 explaining the whole phenotype. Further biological studies were performed after collaboration with a research team in Cambridge, under the premise that the phenotype was likely to represent an abnormality of the brown adipose tissue. I therefore organised for the affected siblings in Family 9 to have an adipose tissue biopsy from affected tissue for analysis by Robert Semple's laboratory (results described in discussion 7.3.3.). In the meantime, as expected, this MSL-like phenotype was reported with the same homozygous missense variants in *MFN2* (Sawyer et al., 2015). This, therefore confirmed that the homozoygous missense variant identified in MFN2, was the disease-causing variant explaining the clinical phenotype described in Family 9 and discussed further below.

7.3.3. Discussion

The clinical features of lipodystrophy seen in this family, with mild fat loss from the upper limbs and massively excessive fat accumulation around the neck, upper truck and upper arms did not fit neatly into the other previously described types of lipodystrophy. The appearance was most like MSL and HAART related lipodystrophy, which was therefore suggestive of a mitochondrial-related pathology. The *MFN2* variant was therefore postulated to be the disease-causing variant with this representing a novel

phenotype at that time. Variants in *MFN2* are well known to cause HMSN. Patients with *MFN2* variants generally have CMT type 2 with axonal loss (Tazir et al., 2013; Verhoeven et al., 2006). The paternal grandmother in Family 9 had a severe sensory and motor axonal neuropathy on NCS. This is consistent with a diagnosis of CMT type 2 and would be compatible with a disease-causing variant in *MFN2*. The proband had absent ankle reflexes on clinical examination, although her NCS were within normal limits. Her sibling did not demonstrate any signs of HMSN. Another patient with this homozygous variant did not develop features of HMSN until the age of 53 years (Sawyer et al., 2015). Calvo et al commented that this biallelic variant including the HR2 domain of MFN2 seemed to be associated with a less severe form of axonal CMT, which is consistent with the presentation in this family (Calvo et al., 2009). It is likely that the individuals in Family 9 with homozygous variants in *MFN2* will develop a moderate neuropathy in their lifetime.

MFN2 encodes an ubiquitously expressed transmembrane GTPases found in the outer membrane of the mitochondria, which is involved in homotypic mitochondria-mitochondria fusion, tethering of mitochondria to the endoplasmic reticulum, apoptosis, and mitophagy (Pareyson et al., 2015; Rocha et al., 2017; Sawyer et al., 2015). It also makes heterotypic interactions with MFN1. In the absence of either Mfn1 or Mfn2, there will be substantially less mitochondrial fusion in cells, resulting in mitochondrial fragmentation (Chen et al., 2003). The p.Arg707Trp variant is found within the HR2 domain at the carboxy-terminal coiled coil domain of MFN2, which is thought to be important for homotypic and heterotypic interactions of MFN2 (Rocha et al., 2017;

Sawyer et al., 2015). Good mitochondrial functioning is required by the peripheral nerves (Pareyson et al., 2015). Variants in *MFN2* had only been associated with a neurological phenotype, despite its ubiquitous expression, possibly due to compensation by MFN1, another protein similarly involved in mitochondrial fusion, in most tissues except the peripheral nerves, where its expression is low (Detmer and Chan, 2007; Pareyson et al., 2015; Sawyer et al., 2015). MFN1 levels were shown to be low in brown fat, which was originally thought to be the affected tissue in affected individuals with MFN2 adipose tissue overgrowth (Sawyer et al., 2015).

Mitochondria have been implicated in one of the several mechanisms postulated to cause HIV associated lipodystrophy (Cossarizza et al, 2001). Mitochondrial dysfunction has also been seen in some patients with MSL secondary to a *MERRF* mutation, m.A8344G (Chong et al, 2003). The appearance of the adipose tissue in patients with MSL resembles brown fat, which has a high energy requirement (Chong et al, 2003). Chong et al reviewed the literature in 2003 and found that 28% of patients with MSL had a mitochondrial mutation. Sawyer et al demonstrated the mitochondria dysfunction using cells carrying the *MFN2* homozygous p.Arg707Trp variant, which showed a reduced ability to form homotypic interactions between the mitochondria (Sawyer et al., 2015). It is therefore likely that the homozygous MFN2 variant identified in Family 9 is causing both the HMSN and the lipodystrophy.

Sawyer el al were the first to report this novel second phenotype in 3 patients with MSL also harbouring the same homozygous missense variant, p.Arg707Trp, in *MFN2* (S1-3 in table 7.2) (Sawyer et al., 2015). S1 and S2 are brothers who developed MSL with lipomatosis neck, upper back and chest in their 20s, which continued to grow in size, similar to the history described in Family 9 and the family photographs visualised at clinical review (Sawyer et al., 2015). This also included the tongue hypertrophy, which gave difficulties swallowing, as described in II:2 of Family 9 (Sawyer et al., 2015). Multiple liposuction operations were required, with only a temporary benefit before regrowth occurred, which was also described in other family members in Family 9 (Sawyer et al., 2015). S2 did not actually notice any lipomatosis until the age of 45 (Sawyer et al., 2015). The parents of S1 and S2 were fifth-degree cousins of Irish descent, had no features of HMSN or MSL and were deceased at the time of publication (Sawyer et al., 2015). It is possible that the p.Arg707Trp variant is a founder variant originating in Ireland. S3, also previously reported by Nicholson et al (see 7.3.2. Results), developed lipomatosis in her late 20s, which developed to accumulate posterior to her cervical spine right down to her lumbar region, with further deposition over the anterior body and even upper thigh (Nicholson et al., 2008b; Sawyer et al., 2015). This was more widespread than the distribution reported in Family 9. Further patients with this MFN2-related MSL have subsequently been reported and are summarised in table 7.2 below. This conclusively demonstrates that the homozygous p.Arg707Trp missense variant is the disease-causing variant in Family 9. I will now focus the discussion on the phenotypic description of this newly described condition.

Table 7.2: Comparison of P1, P2, & Patients Reported with p.Arg707Trp mutation

No.	Sex	MFN2 variant (protein)	HMSN	MSL	DM or IR	Fatty liver	Other	TG	Leptin	Adiponectin	Lactate	Histology EM affected t
P1	F	R707W R707W	×	√ (12)	√	x	Irregular menses	N	N/↓	↓	NR	Thick cyto rim, ↑ Fragmented M
P2	M	R707W R707W	×	√ (15)	√	x	Nil	N	N/↓	↓	NR	Thick cyto rim, ↑ Fragmented M
S1	M	R707W R707W	√ (53)	√ (20s)	√	NR	Nil	N	↓	↓	↑	NR
S2	M	R707W R707W	√	√ (45)	NR	NR	Bowed legs infancy	NR	NR	NR	↑	NR
S3	F	R707W R707W	√ (2)	√ (20s)	NR	NR	Talipes HL	N	NR	NR	↑	NR
R1	F	R707W R343del	√ (<10)	√ (5)	√	NR	Hypog	↑	↓	↓	↑	NR
R4	F	R707W R707W	NR	√ (13)	√	NR	Scoliosis RTD	↑	↓	↓	↑	NR
Cp1	F	R707W R707W	√ (ch)	√ (30)	√	√	HL Breast Ca	↑	↓	↓	NR	Thick cyto rim, ↑ Fragmented M
Cp2	M	R707W R707W	√ (26)	√ (11)	√	√	Hypothyr Precoc Puberty	↑	↓	NR	NR	NR
Cp3	F	R707W R707W	√ (65)	√ (35)	√	√	Hypothyr	↑	↓	↓	NR	NR
Cp4	F	R707W R707W	√ (ch)	√ (25)	√	√	Thyr Ca Breast Ca	↑	↓	↓	NR	Thick cyto rim, ↑ Fragmented M
Cp5	F	R707W R707W	√ (54)	√ (6)	√	√	HL	N	↓	↓	NR	NR
Cp6	F	R707W R707W	√ (ad)	√ (2)	Fast gly	NR	Nil	↑	NR	NR	NR	NR
Ca1	M	R707W Ex7-8del	√ (24)	√	NR	NR	OA	NR	NR	NR	NR	NR

No.	Sex	MFN2 variant (protein)	HMSN	MSL	DM or IR	Fatty liver	Other	TG	Leptin	Adiponectin	Lactate	Histology EM affected t
C1	M	G108R R707W	√	×	NR	NR	NR	NR	NR	NR	NR	NR
C2	F	G108R R707W	√	×	NR	NR	NR	NR	NR	NR	NR	NR
C3	M	G108R R707W	√	×	NR	NR	NR	NR	NR	NR	NR	NR
B1	M	R707W	√ (44)	NR	NR	NR	NR	NR	NR	NR	NR	NR
Br1	F	R707W	√ (7)	NR	NR	NR	NR	NR	NR	NR	NR	NR

Legend table 7.2: () age of reported onset, ad adulthood, Ca cancer, ch childhood, DM diabetes mellitus, EM electron microscopy, Ex exon, HL hearing loss, hypog hypogonadotrophic hypogonadism, LM light microscopy, M mitochondria, N normal, NR not reported, OA optic atrophy, precoc precocious, resist resistance, RTD renal tubular dysfunction, t tissue, thyr thyroid

P1-2 Family 9, S1-3 (Sawyer et al., 2015), C1-3 (Calvo et al., 2009), B1 (Braathen et al., 2010), Br1 (Brožková et al., 2013), Ca1 (Carr et al., 2015), Cp1-6 (Capel et al., 2018)

Rocha et al subsequently reported 4 patients, including the two siblings from Family 9 as patient 2 and 3 (see table 7.2. for comparison) (Rocha et al., 2017). HMSN presented between 2 and 65 years in this group of patients with MFN2 related MSL where present, and in those in whom it was not present, it may present as they get older (Calvo et al., 2009; Capel et al., 2018; Carr et al., 2015; Rocha et al., 2017; Sawyer et al., 2015). There are no reported patients with MFN2 related MSL without at least one variant including p.Arg707Trp, in fact the majority are homozygous for this variant despite looking for other variants in MFN2 (Calvo et al., 2009; Capel et al., 2018; Carr et al., 2015; Rocha et al., 2017; Sawyer et al., 2015). It will be interesting to see whether this changes as more patients are identified. In patients with a biallelic variant in MFN2 including p.Arg707Trp, multiple symmetrical lipomatosis was apparent between 5 and 45 years, excluding the 3 siblings described by Calvo et al (Calvo et al., 2009; Capel et al., 2018; Carr et al., 2015; Rocha et al., 2017; Sawyer et al., 2015). The 3 siblings were however, reported in their mid-20s, and it is therefore possible that MSL had not yet developed (Calvo et al., 2009). For these compound heterozygous patients however, the second variant is the missense variant p.Gly108Arg, which is found within the GTPase domain (Calvo et al., 2009). The other two compound heterozygous patients reported have a deletion, for R1 this involved a single amino acid at a critical hinge region, and for Ca1 this involved exon 7 and 8, neither of which affect the HR2 domain (Carr et al., 2015; Rocha et al., 2017). At the time of publication, Sawyer et al (2015) postulated that only those with a homozygous p.Arg707Trp variant would have a MSL phenotype, which we now known not to be the case. No other biallelic variants had been reported in the HR2 domain other than those described above with MSL

(Rocha et al., 2017). It is therefore possible that a HR2 domain biallelic variant or compound heterozygous variant incorporating a variant in the HR2 domain and a deletion cause MSL (Rocha et al., 2017). Or that any patient with a biallelic variant, including at least one variant in the HR2 domain, will develop MSL in their lifetime. Further study is required to elucidate this more accurately.

Heterozygous p.Arg707Trp variants are a known cause of CMT2 (see table 7.2.). The parents of S3, and C1-3 have had normal NCS. Br1 was heterozygous for the p.Arg707Trp variant and developed CMT at 7, although a second variant could not be entirely excluded (Brožková et al., 2013). Br1's mother is said to be unaffected despite being heterozygous for this variant too, but she has not been physically examined or had NCS (Brožková et al., 2013). B1 was also reportedly heterozygous for the p.Arg707Trp variant and developed CMT2 at 44 years (Braathen et al., 2010). The parents of P1 and P2 have no symptoms of CMT. It has been suggested that heterozygotes for this variant have mild neuropathy presenting between the 2nd and 5th decade (Brožková et al., 2013). This may therefore suggest incomplete penetrance in the heterozygous state or later onset of mild disease, which may or may not be identified in an individual's lifetime. Or alternatively this variant may only be pathogenic in the biallelic state, and in the 2 heterozygotes reported, the second variant was not detected. This also requires further study.

I will now concentrate on the patients with MFN2 related MSL. Leptin levels were low in patients S1, R1, R4, Cp1-5, and the two patients from Family 9 had low to normal levels (Capel et al., 2018; Rocha et al., 2017; Sawyer et al., 2015). Leptin treatment may therefore be potentially beneficial (Rocha et al., 2017). Adiponectin levels were also low in all patients in whom it was documented, which is representative of a pattern seen usually in generalised lipodystrophy or extreme insulin resistance (Capel et al., 2018; Rocha et al., 2017). Insulin resistance and type 2 diabetes mellitus was present in most patients with associated features including acanthosis nigricans, and male hair patterning (Capel et al., 2018; Rocha et al., 2017; Sawyer et al., 2015). Adiponectin and leptin are hormones normally released by adipose tissue, and therefore in the face of overgrown adipose tissue, we would expect these hormonal levels to be increased not reduced (Rocha et al., 2017). This, together with the insulin resistance, suggests that the overgrown adipose tissue is not functioning normally (Rocha et al., 2017). Additional features suggestive of mitochondrial dysfunction included raised lactate levels, hearing loss, hypothyroidism and optic atrophy (Capel et al., 2018; Rocha et al., 2017; Sawyer et al., 2015). Cp1, Cp4, Cp5 had relatively widespread centromedullary cystic lesions previously reported in patients with congenital generalized lipodystrophy (Capel et al., 2018). Other clinical features seen included raised triglyceride levels, fatty liver, irregular periods, partial hypogonadotropic hypogonadism, scoliosis, talipes, breast and thyroid cancer (see table 7.2.) (Capel et al., 2018; Rocha et al., 2017; Sawyer et al., 2015). The significance of the breast and thyroid cancer are unclear.

Adipose tissues from the two siblings in Family 9 were described by Rocha et al and compared to 5 age-matched controls (Rocha et al., 2017). Affected tissue samples were also reviewed in Cp1 and Cp4 and compared to 3 healthy control patient (Capel et al., 2018). Histological assessment revealed the affected tissue was more compatible with white fat than brown fat, because adipocytes were unilocular and negative for the presence of uncoupling protein 1, a protein typically seen in brown fat in R2 and 3, weakly expressed in Cp1 Cp4 and controls (Capel et al., 2018; Rocha et al., 2017). Although, multilocular cells were seen occasionally in Cp1 and Cp4, but not in control samples (Capel et al., 2018). Inflammatory infiltrate and an increased number of small blood vessels were also seen in Cp1 and Cp4 when compared to controls (Capel et al., 2018). The electron microscope showed thickening of the cytoplasmic rim of the adipocytes with increased numbers of round (not tubular), enlarged mitochondria, with fragmentation compared to a thin cytoplasmic rim and ovoid mitochondria in the control samples (Capel et al., 2018; Rocha et al., 2017). RNAseq results suggested that the increased numbers of fragmented mitochondria had reduced function with reduced levels of mRNA detected from the mitochondria-encoded genes, and there was a partial compensation with increased transcription of nuclear-encoded mitochondrial genes (Rocha et al., 2017). Also, surprisingly, apoptotic signatures were reduced, and cell survival signatures were increased (Rocha et al., 2017). Leptin mRNA and protein expression, adiponectin mRNA and protein expression were severely reduced compared to controls consistent with the blood levels, whereas Mfn2 protein levels were similar to controls (Capel et al., 2018; Rocha et al., 2017). Comparison was made with histology and RNAseq in skin cells, which showed no difference from controls

demonstrating the tissue-specific phenotype seen in *MFN2* variants despite ubiquitous expression (Rocha et al., 2017). The overgrowth is therefore, the result of suppressed apoptosis and increased cell survival signalling (Rocha et al., 2017).

Rocha el al (2017) wondered whether the overgrown adipose tissue with the appearance of white fat, could indeed represent dysfunctional, 'whitened' thermogenic adipocytes. CITED1 and FGF21 are thermogenic markers which were increased in Ca1 and Ca4, which was then recapitulated in the RNAseq results, demonstrating overexpression of these two genes (Capel et al., 2018). These patients also had very high levels of FGF21 in the blood when compared to healthy controls (Capel et al., 2018). A group of other patients with a generalized lipodystrophic syndrome had been previously shown to have raised FGF21 levels in blood, but the patients with MFN2 related MSL had significantly higher FGF21 levels than even this group of individuals (Capel et al., 2018). This result is further supported by the PET-CT scan results, which demonstrated increased spontaneous glucose uptake in some patients, but lower than levels expected in brown adipose tissue. This suggests that the overgrown adipose tissue may have some thermogenic activity, and therefore some brown fat characteristics, which requires further study. It has also been suggested by Capel et al (2018) that the increased FGF21 could play a compensatory role to limit metabolic complications of this condition, including raised glucose and triglyceride levels.

Finally, there was evidence of increased mTOR activity on RNAseq in patients R2 and R3, suggesting that treatment with mTOR inibitors may be beneficial, and needs further investigation (Rocha et al., 2017). Sirolimus (mTOR inhibitor) treatment has been beneficial in mice with mitochondrial disease (Johnson et al., 2013; Rocha et al., 2017).

In conclusion, MFN2 related MSL is a multisystemic condition seen in patients with p.Arg707Trp homozygous variants and also patients with compound heterozygous variants including the p.Arg707Trp missense variant and a deletion. This results in adipose tissue overgrowth with an axonal neuropathy in most. The adipose tissue overgrowth occurs due to increased adipocyte proliferation, increased cell survival and reduced apoptosis in these cells. The overgrown adipose tissue is hypofunctional as seen by the low leptin, adiponectin levels and insulin resistance. MFN2 is important for the function of the mitochondria. Increased numbers of fragmented hypofunctional mitochondria are seen in the overgrown tissues. Mitochondrial dysfunction is further evident with raised lactate, hearing loss, and optic atrophy in some individuals. Decreased levels of leptin and adiponectin, and high levels of FGF21 in the blood may be a hallmark of patients with MFN2-associated MSL (Capel et al., 2018). Upregulation of the mTOR pathway also suggests a future potential beneficial therapy with mTOR inhibitors, such as sirolimus (Rocha et al., 2017). Further study to identify a treatment for this condition is extremely important to Family 9 and would be beneficial to all these patients described to date. A regular multi-disciplinary follow up assessment is required in these patients to detect the complications in a timely manner.

7.4. Family 30

7.4.1. Introduction

Fryns syndrome is a multi-system autosomal recessive disorder. It was first described by Fryns et al in 1979 and has subsequently been described as a condition in which the following clinical features may be seen: congenital diaphragmatic hernia, small thorax with pulmonary hypoplasia, relatively coarse face, hypertelorism, short and broad nose, micrognathia, macrostomia, low set dysmorphic ears, hypoplastic terminal phalanx and toes with hypoplastic nails (Fryns et al., 1979; Lin et al., 2005; Meinecke and Fryns, 1985; Pagon et al., 1993). Other features may include: a prenatal history of polyhydramnios, structural brain abnormalities, congenital heart defect, renal cysts, intestinal atresiae and malrotation, omphalocele, microphthalmia, cloudy cornea, cleft lip and/or cleft palate, developmental delay, and genital abnormalities (Fryns et al., 1979; Lin et al., 2005; Meinecke and Fryns, 1985; Pagon et al., 1993). This condition was initially thought to be lethal, but instead it is now known to have a high early mortality rate (Pagon et al., 1993). At the time of recruitment, no gene had been associated with Fryns syndrome.

7.4.2. Clinical Description

Family 30 are a multiply consanguineous family with four clinically affected siblings. They present with features consistent with a diagnosis of Fryns syndrome. Their clinical

features include: congenital diaphragmatic hernia, anal atresia, intestinal malrotation , omphalocele, renal cysts and/or hydronephrosis, micropenis, cryptorchidism, cleft palate, micrognathia, ear abnormality, talipes, short distal phalanx and toes with hypoplastic nails. The affected offspring have either died in utero or shortly after birth. These clinical features would meet 6 out of the 6 clinical criteria for Fryns syndrome, with 4 out of 6 meeting the narrow diagnostic criteria, including diaphragmatic hernia, significant pulmonary hypoplasia, distal digit hypoplasia, characteristic facies, affected siblings, and at least one other feature (Lin et al., 2005). Also see the HPO phenotype summary table and code (Appendix 11.3 and 11.4). There has been no post-mortem performed on the affected children or fetuses. The first cousin healthy parents have 1 healthy surviving child. A karyotype had been performed on the affected children. The condition was affecting siblings only, in a consanguineous family, with both males and females affected. We therefore assumed the inheritance is autosomal recessive.

Other diagnoses had been considered, but felt unlikely, including chromosomal abnormalities, Simpson-Golabi-Behmel syndrome, Donnai Barrow syndrome and Matthew Wood syndrome. Prior to WES analysis I organised *STRA6* sequencing in both parents, but no heterozygous variants were identified.

7.4.2. Result

There were no stored DNA samples on the affected children. I was eventually able to locate a stored fibroblast sample, but unfortunately the scientists only managed to extract a small, very fragmented DNA sample for analysis. We were therefore unable to perform WES on an affected patient's sample. I therefore performed WES on the two parental samples in the hope that we would be able to identify the disease-causing variant. I assumed this to be autosomal recessive and therefore sought a heterozygous variant, most likely the same heterozygous variant within the same gene in both parents. I utilised the method (described below) reported by Ellard et al., 2015, but without success. I also looked for variants in candidate genes with similar presentations to Fryns syndrome, including the *PIGN* gene and the other known GPI-pathway genes. I have not been able to identify the disease-causing variant within this family.

7.4.3. Discussion

Family 30 are a multiply consanguineous family with four deceased, affected individuals, who each have clinical features consistent with the diagnostic criteria for Fryns syndrome (Lin et al., 2005). There was previously no known genetic cause for Fryns syndrome. Biallelic genetic variants in the *PIGN* gene have recently been described in patients with Fryns syndrome, although this is likely to be a genetically heterogeneous condition (Alessandri et al., 2018; McInerney-Leo et al., 2016;

Thompson and Cole, 2016). *PIGN* encodes one of the proteins involved in glycosylphosphatidylinositol (GPI) biosynthesis, previously associated with Mabry syndrome, which anchors proteins to the outer layer of the cell membrane (McInerney-Leo et al., 2016). A compound heterozygous variant has subsequently been reported in the PIGV gene, another gene encoding a protein involved in GPI biosynthesis, in a family with overlapping features of Mabry syndrome and Fryns syndrome (Reynolds et al., 2017). No variants were identified in this group of genes encoding components of the GPI biosynthesis pathway in the heterozygous state in both parents. This suggests that there is further genetic heterogeneity in Fryns syndrome, or that the variant was in a non-coding region.

There was not adequate DNA available for WES in an affected individual. I therefore performed parental WES to try to identify the disease-causing variant explaining the diagnosis of Fryns syndrome in this family.

It is not unusual for those working in Clinical Genetics to be faced with a family who have recently been lost a child with a multiple congenital anomaly syndrome, whose child has either been terminated because of these lethal anomalies, died in utero or in the neonatal period. These families are often very eager to identify the disease-causing variant in the family, especially in families with multiply affected siblings, to enable them to utilise the PIGD or other prenatal testing options described in the introduction. For many families, embarking on a further pregnancy, without this information, is extremely

difficult. There may be no DNA sample from the affected child, it may be there is only a small quantity of precious DNA from the affected individual, or the sample may be of inadequate quality for NGS studies. This is a similar scenario to the presentation in Family 30.

A strategy for utilising parental WES for diagnosing lethal autosomal recessive conditions in unrelated parents was described in 2015, in families with a history of having had more than one affected child and therefore suggestive of an autosomal recessive condition (Ellard et al., 2015). In the eight unrelated couples, they identified an average of 1.0 gene with a different heterozygous variant in the same gene in which the affected child may be a compound heterozygote, after filtering, and an average of 0.75 genes with the same heterozygous variant in which the affected child may be homozygous (Ellard et al., 2015). The list of potential variants would be higher in a consanguineous family like Family 30, making the application of this method more difficult. They were able to identify the disease-causing variants in 3 families using this method (Ellard et al., 2015). I therefore tried to recapitulate this method to analyse the WES results in the parents of Family 30.

A subsequent follow up paper described this Exeter bioinformatics pipeline in more detail (Stals et al., 2018). They described the analysis of 50 families, including 11 couples with known consanguinity, and 21 families with only a single affected child (Stals et al., 2018). At the variant filtering stage, they remove variants with a MAF of

>0.001 (rare variant subset) and >0.0001 (very rare subset) in ExAC or Exome variant server, synonymous variants, intronic variants not near a conserved splice site, where the parents are homozygotes, known artefact, no annotation with gene name, variants not passing quality filters, and genes starting with MUC, HLA or LINC (Stals et al., 2018). In addition to this they also retained all variants recorded as pathogenic in ClinVar or HGMD Pro (Stals et al., 2018). They then, as before, created a list of likely autosomal recessive variants in which the parents both had a heterozygous variant in the same gene, which was either the same or a different variant (Stals et al., 2018). Subsequently each of these variants was considered by reviewing OMIM, the medical literature, in *silico* tools using Alamut, and then the variants were classified according to the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015). This pipeline is very similar to a method I had used, although my cut off MAF had been less rigorous at 0.01, I did not use the ACMG variant classification guidelines (see general discussion 8.1.1.), and I did not have access to HGMD Pro, or Alamut, although I did have results from the *in silico* analysis. I had a 17 gene shortlist (see appendix 11.5.30), but none of these genes had relevant phenotypes of interest. The success rate in the published paper was 52% (26/50 couples)(Stals et al., 2018).

The strategy employed by Stals et al (2018) remains a very useful tool despite, its lack of success in Family 30. This is particularly useful for families like Family 30, in whom WES is not possible in the affected individual either because there is no DNA stored, or the remaining DNA sample is too small for WES analysis. This remaining DNA is therefore very precious, and can be saved for subsequent confirmation of the likely

causative variant using Sanger sequencing. Especially pertinent for families contemplating a recurrence risk of 25%. This method of parental WES analysis is therefore likely to be extremely valuable to clinical geneticists utilising NGS for diagnosis of a lethal fetal condition (Stals et al., 2018).

CHAPTER 8: DISCUSSION

A key role of a clinical geneticist is to diagnose rare diseases in patients, which then enables the patient to receive information about their condition, appropriate management, reproductive genetic counselling, cascade family genetic counselling and hopefully appropriate treatment in the future. This project aimed to utilise the benefits of next generation sequencing, using WES, to identify the most efficient process for rare variant interpretation and disease gene identification, and to define the role of deep clinical phenotyping in the interpretation of comprehensive NGS analysis. The role of next generation sequencing in clinical genetics is best first considered by reviewing the overall diagnostic rate in this project.

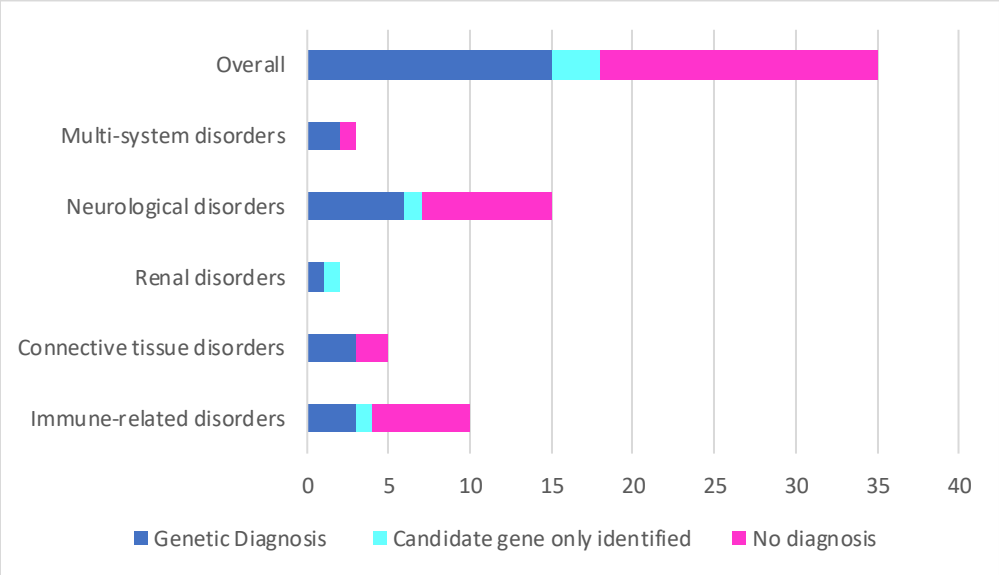
8.1. Discussion of Overall Results

Table 8.1: Summary of overall results by disorder category

Ch No.	Group of disorders	Genetic Diagnosis	Candidate gene only identified	Total no. of patients	Percentage genetic diagnosis	Percentage overall diagnosis (%)
3	Immune-related disorders	3	1	10	30	40
4	Connective tissue disorders	3	0	5	60	60
5	Renal disorders	1	1	2	50	100
6	Neurological disorders	6	1	15	40	46.7
7	Multi-system disorders	2	0	3	67	67
8	**Overall**	**15**	**3**	**35**	**42.9**	**51.4**

Key: Ch chapter, No. number

Figure 8.1: Graphical depiction of the overall results by category



The confirmed genetic diagnostic rate for this project is 42.9%. In the introduction to this project I discussed the DDD project, which commenced just prior to my own project. In the initial cohort of 1133 patients, a full or partial diagnosis was made in 454, giving an overall diagnostic rate of 40%, which is a similar rate to this project (Wright et al., 2018). This comparison is interesting, but although there are some similarities between these projects, there are also important differences. The majority of diagnoses in my project were made by the detection of homozygous variants in autosomal recessive disease genes whereas in the DDD project the highest diagnostic rate was made amongst individuals with monoallelic *de novo* variants in dominant disorders (the DDD study used trio sequencing as the default investigation). In the Saudi Arabian population, which has high rates of consanguinity, it was reported that 27% of inherited disease diagnoses were autosomal dominant or *de novo* autosomal dominant conditions compared to 71% autosomal recessive disorders (Monies et al., 2017a). In my project 14/15 (93%) diagnoses were homozygous autosomal recessive disorders. It is possible that in the remaining, undiagnosed patients, that there are other autosomal dominant disorders that I have not identified and, in particular, the diagnosis of *de novo* pathogenic variants is limited in the absence of a trio sequencing analysis strategy.

The very first study looking at the application of WES in clinical genetics had a diagnostic rate of 50% with 6 out of 12 patients reaching a diagnosis (Need et al., 2012). If we compare this result to other WES studies we see a range of diagnostic rates from 25 to 37%: a pilot study at Baylor laboratory had a molecular diagnostic rate of 25%, 28.8% for GenDx, 29% for the FORGE Canadian study, 30% at Ambry

genetics, 34% in Saudi Arabia, and 36.7% in intensive care infants in Houston, and (Eldomery et al., 2017; Farwell et al., 2015; Meng et al., 2017; Monies et al., 2017a; Retterer et al., 2016; Sawyer et al., 2016). In a study of an undiagnosed rare disease cohort from Spain there was a high diagnostic rate of 67% (20/30), which the authors attributed to having a more selective group, with stringent inclusion criteria (López-Martín et al., 2018). As one might suspect therefore the diagnostic rate seems to be very dependent on the composition of the population group studied, the degree of pre-study investigation and whether the proband only or whether the proband and their relatives are analysed. However, overall, the diagnostic rate from my project, when compared to the similar WES projects, is very good.

In my project the diagnostic rates was variable between different phenotypic groups. It is difficult to interpret these intergroup differences because some groups were small (e.g. the diagnostic rate in multisystem disorders was 67% (2 out of 3 patients) compared 30% (3/10) in patients with immunological disorders). However, it is tempting to speculate that disorders with a distinctive phenotype because of multisystem involvement may be easier to diagnose than those for which the phenotype is less specific. The immune-related disorders cohort (many of which are autoinflammatory disorders) are an evolving group, which are less well characterised from the genetic perspective. This could provide a source of novel diagnoses or it may be that some of these conditions have a multifactorial aetiology and therefore no clear Mendelian diagnosis to be made. Interestingly, the Canadian WES project found their highest diagnostic rate to be within the ciliopathy group, which would be comparable to the

multi-system disorder group here, and the lowest rate in their immunological disorder group with a rate of 11.8% (Sawyer et al., 2016). The study by GeneDx found their highest diagnostic rate was amongst their deafness group, which was not a category in this project, with a diagnostic rate of 55%, and immunological disorders was an other low rating category with a diagnostic rate of 15.8% (Retterer et al., 2016). Therefore, my findings are consistent with other similar studies.

Some patients without a diagnosis may have remained undiagnosed because the relevant variant could not be definitively classified as deleterious (e.g. previously unreported missense variants or variants in genes with no associated human or mouse phenotype, and gene products of unknown function) or went undetected (synonymous variants would have bene filtered out by my variant analysis strategy, structural variants and non-coding variants would not have been detected and some coding sequences are not well captured or sequenced). Therefore, I suggested recruitment to the 100,000 genomes project for all undiagnosed patients (discussed later).

8.2. General Discussion

Within this project I have identified several families with disease-causing autosomal recessive disorders, in well-known autosomal dominant disease genes. An autosomal dominant heterozygous variant may exert its effect as a consequence of

haploinsufficiency from the reduced amount of the affected protein, by a dominant negative effect by interfering with the normal protein (discussed in OI introduction 4.2), or gain of function, which may lead to an additional detrimental function for the encoded protein (Monies et al., 2017b). The variants causing autosomal recessive disease are more typically loss-of-function (Monies et al., 2017b). When an autosomal recessive disease is seen in a known autosomal dominant disease-gene, there are two main possibilities; the patient has the same more severe phenotype when biallelic, therefore likely consistent with haploinsufficiency, or a novel disease phenotype to the known autosomal dominant disease, likely representing a different molecular mechanism (Monies et al., 2017b).

A good example of the different phenotypes that may be seen in the same gene, is that of *BRCA2*, in its autosomal dominant heterozygous state it causes a hereditary breast and ovarian cancer, and in contrast in its autosomal recessive state it causes a developmental disorder resulting in Fanconi anaemia ("OMIM - Online Mendelian Inheritance in Man"). *MFN2*, described in this project, exemplifies both of two different scenarios described above (Monies et al., 2017b). Heterozygous *MFN2* variants have a well-established HMSN phenotype. This has also been seen with biallelic variants, possibly tending towards in an earlier age of onset, with a more severe phenotype (Nicholson et al., 2008b). However, in contrast, Family 9, and other similar families, with a specific type of biallelic variant, may also have a neuropathy, but they have a different additional more significant phenotype, resulting in an overgrowth of adipose tissue with an MSL-like phenotype (Rocha et al., 2017; Sawyer et al., 2015). I have

also described Family 28 with homozygous *PKD2* hypomorphic variants with a more severe phenotype generally than that seen in the ADPKD, but less severe than the well-known ARPKD (caused by biallelic variants in a different gene). Family 6 have a biallelic *COL1A2* variant, which results in a similar, more severe phenotype to that seen in the autosomal dominant form of the disease. I have also considered whether the variant in Family 21 could be hypomorphic in the heterozygous state, but further functional work is required to determine this further. This must be considered when reviewing NGS data in clinical genetics, particularly the potential for a different phenotype seen in the autosomal dominant and autosomal recessive conditions within the same gene.

Since the advent of NGS greater phenotypic variability for many conditions has been reported. This can sometimes influence the outcome of NGS. For example, Family 23 and the homozygous *PEX16* variant resulting in a mild Zellweger syndrome with initial inconclusive biochemical results. Even with the knowledge of the range of phenotypic severity of certain disorders, it is easy to dismiss the disease-causing variant in the face of incompatible results. However, it is also very important to not inappropriately make a genetic diagnosis fit erroneously, which can have much greater consequences for the patient and family management.

It is important to consider the possibility of *de novo* monoallelic disorders in consanguineous families. The proband in family 27 had Adams-Oliver syndrome, which

can be autosomal dominantly or autosomal recessively inherited. We found that this patient had a *de novo* variant in the *NOTCH1*. The DDD project found 6% of patients with autozygosity levels equivalent to those seen in first cousin, or closer relations, had a potentially pathogenic *de novo* disease-causing variant (DDD study, PMC, 2017). A mean prevalence of 0.34% was identified for *de novo* dominant disorders in the general population, affecting 1 in 295 births (DDD study, PMC, 2017). A strong paternal age affected was confirmed, although the parents in this family were relatively young, with a combined age-related prevalence of 0.28% in this family using the DDD data (DDD study, PMC, 2017). This demonstrates the importance of considering *de novo* dominant disorders in every patient to whom NGS analysis is being employed for diagnosis, unless incompatible with the pedigree analysis.

One of the limitations for diagnosis of a deceased proband, is the inability to perform sequencing without a DNA sample of adequate quality and quantity. This was the case for Family 30, with multiply affected deceased children with clinical features were consistent with Fryns syndrome. While I did manage to eventually locate a fibroblast sample for DNA extraction from one of the affected cases, the quality and quantity of DNA was not suitable for NGS analysis. Parental-only WES can be performed in these circumstances. This analysis has been termed “molecular autopsy by proxy” (Monies et al., 2017a). This is particularly useful in families, such as Family 30, where autosomal recessive disease is most likely. Unfortunately, I was unable to identify the disease-causing variant within this family. However, in this example, the variant may be in a gene not yet associated with a human disease since the molecular aetiology for Fryns

syndrome is not fully understood. This “molecular autopsy by proxy” will however, be invaluable to enable clinicians to utilise WES for these families who may be desperate for a molecular diagnosis (Monies et al., 2017a).

8.2.1. ACMG guidelines for variant reporting

In 2015 the American College of Medical Genetics and Genomics and the Association for Molecular Pathology published a joint consensus recommendation for the reporting of sequence variants (Richards et al., 2015). Different levels of evidence were classified separately for benign and pathogenic. Pathogenic evidence was ranked as very strong (PVS1), strong (PS1–4), moderate (PM1–6), or supporting (PP1–5) (Richards et al., 2015). Benign evidence was ranked as standalone (BA1), strong (BS1–4) or supporting (BP1–6) (Richards et al., 2015). This encompasses evidence described in the introduction, such as the presence in population and disease variant databases, medical literature review, familial segregation studies, genotype-phenotype correlation, computational predictive tools and functional studies. A framework to classify the variants based on this evidence was drawn up (see diagram 8.2.)

Figure 8.2. Variant Classification

1. Benign
2. Likely benign (90% confidence the variant is benign)
3. Variant of Uncertain Significance
4. Likely pathogenic (90% confidence the variant is pathogenic)
5. Pathogenic

These guidelines are quite stringent, resulting in more variants being classified as variants of uncertain significance. This does however, give clinicians more confidence in the results that are classified as likely pathogenic or pathogenic. Since the conclusion of this project, this variant classification system has now been adopted by the UK genetic laboratories for the classification of all variants reported. One of its limitations in application is to variants in novel disease genes, such as Family 2 with the *OTULIN* variant or novel phenotypes for known disease phenotypes, such as Family 9 and the *MFN2* variant. Examples of using this classification can be seen below in table 8.2. Based on the body of evidence now in the medical literature however, these variants in *MFN2* and *OTULIN* are classed as pathogenic and likely pathogenic respectively. In view of this, I have not used this variant classification system throughout this project.

Table 8.2: ACMG classification of variants detailed in the results chapters

Family number	Variant	Original ACMG classification	Current ACMG Classification
2	*OTULIN*: c.815T>C, p.Leu272Pro	Uncertain significance PM2, PP1, PP3	Likely Pathogenic PS3, PM2, PP1, PP3, PP4
34	*DNASE1L3:* c.290_291delTG, p.Thr97Ilefs*2	N/A	Pathogenic PVS1, PS1, PM1, PP4
3	*TMEM38B:* c.507G>A p.Trp169*	Uncertain significance PM2, PP1, PP3	Pathogenic PVS1, PS3, PM2, PP1
6	*COL1A2:* c.370G>A, p.Gly124Ser	N/A	Likely Pathogenic PM1_STR, PM2, PM5, PP4
28	*PKD1:* c.11975C>T p.Ala3992Val	N/A	Uncertain Significance PM2, PP1, PP3, PP4
13	*SETX* c.5243delT p.Phe1748Serfs*3	N/A	Pathogenic PVS1, PM2, PP4
35	*LONP1:* c.2282C>T, p.Pro761Leu	Uncertain significance PM2, PP3	Likely Pathogenic PS1, PS3, PM2
9	*MFN2:* c.2119C>T p.Arg707Trp	Uncertain significance PM2, PP3, PP5	Pathogenic PS1, PS3, PM1, PM2, PP3, PP4, PP5

8.2.2. Bioinformatics Programmes

As discussed above, there are a number of bioinformatics tools available to aid interpretation of exome sequencing data, including POLYPHEN and SIFT (Ng et al, 2001; Adzhubei et al, 2010). More recently bioinformatics programs have tried to incorporate phenotypic analysis, family-based analysis, and/or variant analysis into a single instrument. I considered using several tools to aid analysis. PhenIX ranks WES variants based on pathogenicity and similarity of the patients phenotype to that

described for the Mendelian disease (Zemojtel et al, 2014); Agile suite of programs have several programs to assist in data filtering and analysis, including a program which can use WES data to identify autozygous regions to aid identification of the causal mutation in consanguineous families (Carr et al, 2012; Carr et al, 2013a; Carr et al, 2013b); and SPRING identifies whether a single nucleotide variant is likely to be pathogenic by combining several functional effect scores with the likelihood that a given variant is disease-causing using sources such as the gene ontology and pathway information (Wu and Jiang, 2014). Phenotype based analysis normally utilises HPO terms, but this is limited by the specificity of the HPO terms available in the ontology. There is a drive to improve the phenotypic terminology, which will improve the output of all phenotype-based analytical tools. By the end of the project I had not utilised any of these bioinformatic tools either due to security concerns with patient data upload, poor performance in a feasibility project performed by an MSc student, unsuitable for current project or genome build incompatibility.

Table 8.3 Comparison Table of the WES variant identification bioinformatics tools

Legend: Com command, Web internet based.

Tool	Web based	Patient File uploaded	Phenotype analysis	Family analysis	Variant analysis	Problems
Exomiser + phenix	Web/ Com line	Yes, deleted straightaway	Yes	No	Yes	Best for known genes Performed poorly in MSc project
Exomiser + phive	Web/ Com line	Yes, deleted straightaway	Yes	No	Yes	Best for known genes Performed poorly in MSc project
PhenGen	Web/ Com line	Yes, for several days	Yes	Yes	Yes	Patient file upload to web page required
Phevor	Web based only	Yes, for several days	Yes	Yes	Yes	Need to pre-filter VCF
Agile	Download program	N/A	No	Auto -zygosity mapping	Possible	Need BAM file ideally. Data file too large
SPRING	Web/ Com line	No	Query disease only	No	Yes	List of candidate SNVs only
Galaxy	Web only	Yes	No	Yes	Yes	Patient file upload to web page required
Sapientia	Web only	Yes, securely	Yes	Yes	Yes	Not compatible with GRCh38 currently

Homozygosity mapping within a family can be very powerful to aid identification of the disease-causing variant from next generation sequencing results. This can be done

using a Single Nucleotide Polymorphism (SNP) array analysis or specific tools aimed at utilising next generation sequencing results to generate this data. In Family 2 the homozygous disease-causing variant was identified within one of two areas of shared homozygosity amongst the affected individuals only, which provided further evidence that the *OTULIN* variant was likely to be causative. Performing additional SNP arrays is possible but incurs additional cost and analysis.

Wakeling et al demonstrated that 79% of pathogenic variants were found in one of the largest region of homozygosity using SavvyHomozygosity and SavvyVcfHomozygosity (Wakeling et al., 2018). Woods el al had previously found that the disease-causing homozygous variant was found in the longest region of homozygosity in 8 out of 48 individuals (17%) studied (Woods et al., 2006). The premise behind searching in the larger regions of homozygosity, is that these larger regions are most likely to have occurred from a more recent consanguineous union, whereas smaller regions of homozygosity are less likely to contain the disease-causing variant because they are more likely to be ancestral and as such have greater longevity in the population, thus have a greater likelihood of already being subjected to natural selection (Pemberton et al., 2012; Woods et al., 2006). The Savvy suite of tools were not available for use during this project. It would be interesting to apply these tools to the families within this project. I did however, use an in-house bioinformatic tool to look at variants within a large homozygous run, but I did not find that the disease-causing variants identified in these families correlated with a high scoring region of homozygosity. WES is not, however, ideal for identification of homozygous runs. Wakeling et al found that the

disease-causing variant was more likely to be within one of the 10 largest regions of homozygosity if the patients had a <8% homozygosity in their genomes and the variants were more than 3Mb from the telomere. Most of the consanguineous unions in this project were first cousin marriages, and therefore the offspring would be expected to have 1/16 (6.25%) of their genome homozygously inherited by descent, which would therefore be conducive to this analysis. Woods et al actually found that on average the rate of homozygosity was around 11% within the Pakastani or Arab group in unions of first cousins, which is not surprising given the multiple consanguinity seen within the pedigree (Woods et al., 2006). This may therefore be a limitation to this analysis. Where present, however, this provides supportive information.

8.1.3. Deep Phenotyping Critical to Diagnosis

Phenotypic analysis is a very important part of NGS analysis. I have found in this project, that filtering the list of potential variants according to the patient's phenotype has been very effective for identification of the disease-causing variant. When performing this analysis, it is important to remember that a novel allele-disease phenotype association may be overlooked. Family 9 with the lipodystrophy and *MFN2* variant exemplify this well. At the time of first analysis, no lipodystrophy phenotype had been clearly linked to this gene. When considering the mitochondrial underlying aetiology and other similar phenocopies of their condition, it because clear that this *MFN2* homozygous missense variant could be a novel phenotype for this gene. Further evidence for this supposition subsequently came from deeper phenotyping with

biochemical analysis, transcriptomics, and histological assessment, as well as additional case reports.

The phenotyping in Family 2 was crucial to the subsequent molecular diagnosis. When considering why the proband was thriving and his cousins were deceased, it became clear that a TNF-related pathogenicity was key to the underlying molecular mechanism in view of the successful treatment of the disease with TNF-alpha blockade in the proband. This enabled me to be more confident that homozygous missense variant in *OTULIN* was the disease-causing variant in this family despite the lack of human disease phenotype reported for this gene at that time. This ensured that the phenotype helped successfully identify the genotype when analysing NGS results.

There are many people who advocate a genome first approach. This can be advantageous, but there are of course limitations to this. For example, WES may not actually be required because the clinical geneticist may recognise the clinical phenotype resulting in single gene sequencing or single gene panel sequencing. With the increased potential for incidental finding and variants of uncertain clinical significance with more extensive sequencing, performing a genome first in these circumstances would be inappropriate. It is also important to not make the phenotype fit with the genotype result, thus remaining objective when reviewing the patient. Otherwise, this can lead to erroneously making a diagnosis in the patient, who may then receive incorrect management, and therefore missing the correct diagnosis in the patient.

Additional corroborative evidence however, in the knowledge of the phenotype can be beneficial. For example, for Family 13, I was able to demonstrate a raised AFP level, which was additional evidence to confirm this novel variant was disease-causing.

Blended phenotypes can be difficult to unpick where more than one genetic diagnosis in a patient may produce a combined phenotype that can be difficult to unravel (Wright et al., 2018). This is particularly likely in multiply consanguineous family. For example, I have discussed the various phenotypes seen in Family 3 with OI and a homozygous nonsense variant in *TMEM38B*. The proband has a metabolic syndrome, two siblings have myopathic features and one sibling has congenital heart defects. It is difficult to be certain how many different diagnoses are present within this family and how much can be attributed to phenotypic variation within a relatively recently described rare disease. In contrast, Family 9, discussed above, had a family history of peripheral neuropathy and a lipodystrophy, which had the same underlying molecular genetic aetiology once fully explored. Dual diagnoses only represent a small number of individuals. Even amongst the consanguineous population of Saudi Arabia, only 1.5% had a dual diagnosis (Monies et al., 2017a). Dual diagnoses and blended phenotypes, however, need to be considered in the analysis of NGS in clinical genetics.

8.1.4. Family analysis

Extended family segregation studies associated with deep phenotyping of every family member can be extremely useful in interpretation of next generation sequencing results. This type of assessment is the cornerstone of clinical genetics. One of the main limitations of this study has been the ability to do this. For example, within Family 9, with the homozygous *MFN2* missense variant, there are a number of affected individuals in three generations of Family 9 with a wide range of ages. It would have been extremely beneficial to deeply phenotype all these individuals. However, the dynamics within this consanguineous traveler family limited phenotyping to the single nuclear family unit only. Family dynamics are common limitations to variant interpretation in clinical genetics.

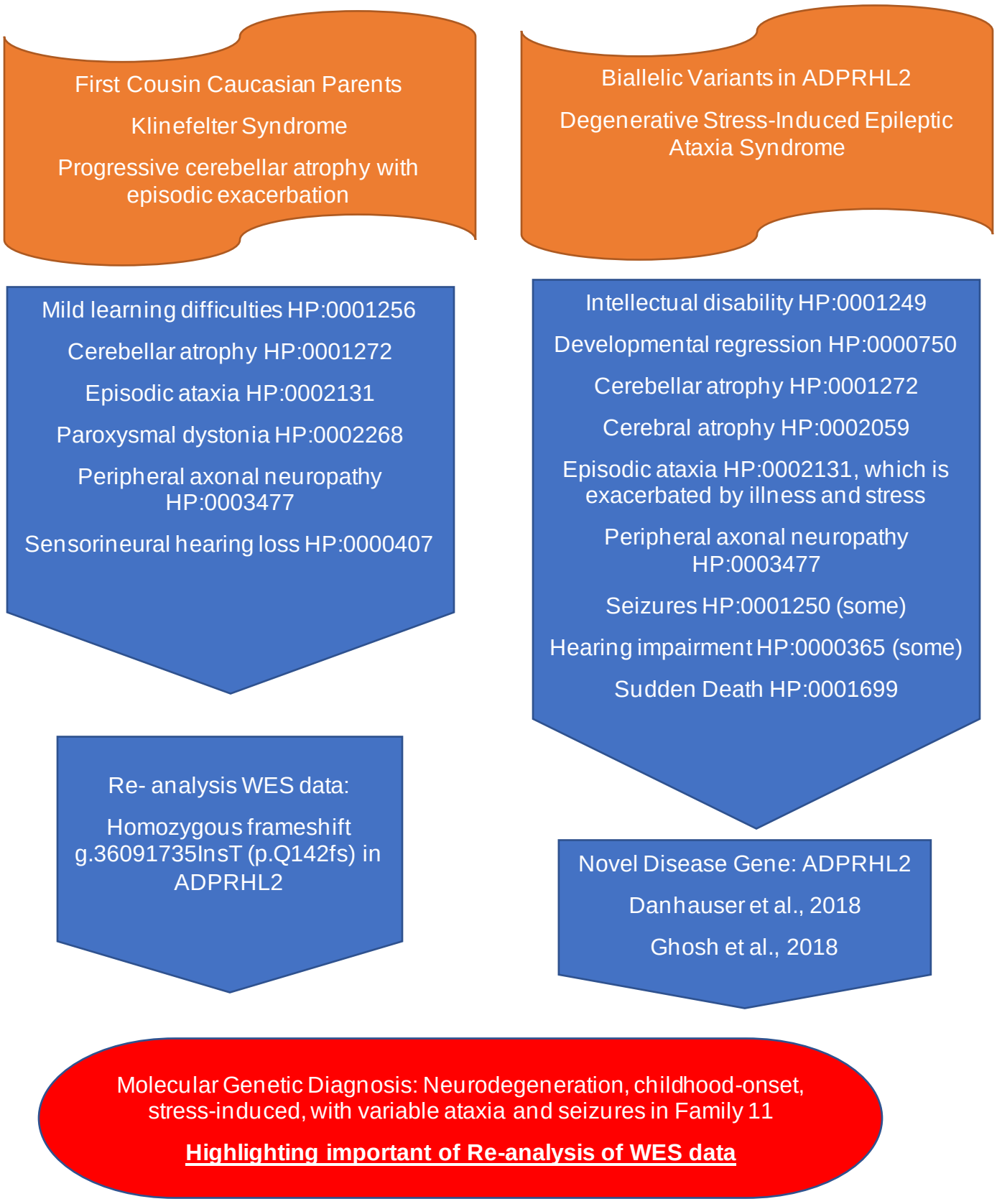
Another limitation to further analysis of the patient and family phenotype in this study has been the difficulty of re-contacting patients once time has elapsed from the initial consent process. For some families, they remember well the initial consent to the project, they are extremely pleased to hear that a potential molecular genetic diagnosis has been made and are very keen to help with further assessment and phenotyping. Family 2, both the family of the proband and the cousins' parents, have been extremely pleased to help and understand the ORAS condition in their family. For other families, having a genetic diagnosis can be very difficult to deal with. They may not want, therefore, to have the confirmatory Sanger sequencing to confirm that both parents are carriers, similar to the parents in Family 13. Another example can be seen with Family

6. We have found a candidate variant in *COG3*, which could be worth exploring further. Since consenting to the project and identification of this variant the proband has died and the family did not want to pursue the diagnosis further because this was no longer important to them.

In the introduction I discussed the explosion of new genetic diagnoses that has occurred secondary to NGS methods. In the DDD project alone their initial diagnostic rate in the first 1133 patients improved from 27% to 40% more recently (Wright et al., 2018, 2015). This is in part due to the new diagnoses that have been made in the three-year time interval. A Baylor genetics research project, reviewing 74 undiagnosed families with WES performed in a diagnostic laboratory, were able to identify a diagnosis in 27 families (36%) secondary to novel disease gene identification, newly described conditions since the original analysis, dual diagnosis, copy number variant analysis, uniparental disomy identification and additional family analysis (Eldomery et al., 2017). 10% of individuals in another study, looking at 40 individuals with a previously uninformative WES result, were diagnosed purely due to newly described genes or phenotypes (Wenger et al., 2017). They demonstrate that there are about 250 new gene–disease associations each year, therefore it is not surprising that re-analysis of sequencing data is beneficial (Wenger et al., 2017). In Family 35 with the *LONP1* and Family 29 with the *SLC9A1* variant, the corrobatory evidence for pathogenicity has increased during the write-up phase of this project. This highlights the importance of re-analysis of WES data. In addition, during the thesis awaited stage of the project, a

genetic diagnosis was made in Family 11 from re-analysis of the WES due to a novel gene publication (see Figure 8.3.).

Figure 8.3. Genetic Diagnosis in Family 11 (Danhauser et al., 2018; Ghosh et al., 2018)



It is important to consider the impact of re-analysis of results for the patients, families and clinicians involved. This means that a negative result is never truly negative, which can be difficult for families to deal with, when they may wish to move on with their lives. Equally, problems can arise if a diagnosis is subsequently withdrawn (Wright et al., 2018). To try to prevent the latter problem, DDD has produced a DDG2P gene list, which details only the genes considered to be definite or probable developmental disorder genes, dependent upon adequate evidence in peer-reviewed journal (Wright et al., 2018, 2015). This list is then used to analyse the WES data. This has been extremely useful for the development of developmental disorder gene panels and analysis of NGS results in clinical genetics within the National Health Service (NHS). Re-contacting families is facilitated practically by the NHS national database to enable access to the relevant patient's most up to date address, especially if several years has elapsed. However, patients may be difficult to engage after a prolonged period. In addition to this, the psychological impact of re-contacting the families can be difficult to gauge, when we hope to do no harm at the very least. We need to consider how to counsel the patients for this uncertainly and how to manage re-communication of new NGS diagnoses. These difficulties are likely to be ongoing problems that need further consideration in this new genomic era.

A systemic review considering re-contacting patients with results tried to address this consideration in more detail. They concluded that the legal precedent and guidelines for re-contacting patients was lacking, and it is not currently considered part of standard care. Often the arguments against re-contacting families focused on the practical

difficulties including, contact details, staffing, time consideration and funding. Where patient experience was considered, the outcome was generally felt to be positive. They therefore felt that clinical genetics should now be considering when we should be re-contacting families, and establishing international guidelines detailing these circumstances. I certainly agree that in this genomic era, where a new diagnosis can be made on data that may only be a couple of years old, we should have clear pathways for re-contacting patients with updated results. This must be part of the conversation when consenting patients for NGS results in clinical genetics (in this research study patients had the option of deciding whether or not they wanted to be re-contacted with relevant results).

8.1.5. Data sharing

When identifying a novel disease or disease phenotype, identification of further patients with similar phenotypes can be very important to aid further interpretation. Previously this has been very dependent on chance. Patients with novel disease phenotypes would be presented at large conferences in the hope that a clinician would recognise similar phenotypes in their own patients. This was very much dependent on the chance of the right clinician seeing the specific poster at the right time in the specific conference and remembering their patient with that phenotype. For example, I presented Family 2 at the 2014 Manchester Dysmorphology meeting and the 2015 European Society of Human Genetics without success (see section 11.7.1. and 11.7.2.). However, at the later meeting I was able to see a poster confirming the same MSL like phenotype in 3

patients with the same homozygous *MFN2* variants I had found in Family 9, which was later published (Sawyer et al., 2015). At the time I was aware of a group working on this disorder after contacting the lead author from the paper by Nicholson et al (2008), who informed me that the lead researcher did not want to collaborate at this stage. I was then able to contact this group directly, but they still did not want to collaborate because they were near publication.

A much better way of identifying clinicians who are interested in the same gene is through larger, well established data sharing sites. Gene Matcher is one such site used by a large number of researchers now, which is likely to make a significant impact in identification of novel disorders in the coming years (www.genematcher.org) (Sobreira et al., 2015). This was useful for identification of additional affected patients with disease-causing variants in novel disease genes identified from the research based re-analysis described above by the Baylor genetic laboratory (Eldomery et al., 2017). Unfortunately, I have used this site to look for other researchers interested in *OTULIN*, *COG3* and *NLRC3*, without success so far.

Decipher (https://decipher.sanger.ac.uk/) is another useful site for the identification of similar affected patients. This site was very beneficial in enabling me to be confident in the molecular diagnosis for Family 26 who had homozygous missense variant in *POLR1C*. I was able to identify two additional patients through decipher with the exact same variant in *POLR1C* and a similar phenotype to Family 26 and that also described

in the literature (Thiffault et al., 2015). Data sharing with other clinicians and researchers is therefore extremely beneficial to aid interpretation of NGS data in clinical genetics.

8.1.6. Incidental findings

In one family an incidental *SDHB* was variant (c.725G>A p.Arg242His) was detected. This has been found in 2 out of 246208 individuals on GnomAD with a MAF of 0.00001 and has been reported on ClinVar as pathogenic. This is associated with the cancer-predisposing syndrome, Hereditary Paraganglioma-Phaeochromocytoma Syndromes. The patient did not have any clinical features of this condition and so it was not permissible to feedback this result to the patient as the ethical approval and consent form, specify that only results associated with the patient's clinical phenotype are returned.

The *SDHB* gene is one of the medically actionable genes detailed in the ACMG guidelines for reporting of secondary findings from genomic sequencing. This guideline was published, and used more widely, since the inception of this project. We are however, bound by our original ethically approved consent, and unable to report this variant. Similarly, the DDD project, which was set up at a similar time to this project, will only feedback results that are relevant to the patient's phenotype, and will not feedback other findings including medically actionable results or non-paternity.

What is the potential scale of these incidental or secondary looked for findings? It is important first to clarify the difference between an incidental and secondary finding. An incidental finding is a result identified through the interpretation of NGS data, which is not the primary cause for genetic testing. A secondary finding involves actively looking for variants in certain genes considered clinically actionable. They are incidental findings (not related to the disease being investigated), but rather than being purely incidentally found, they are actively sought out with appropriate consent. I have identified an incidental finding in 1 out of 36 families, representing 2.7% of this patient group. The same rate of secondary findings were reported in a Dutch cohort, with 1 in 38 (2.7%) healthy individuals having a likely pathogenic variant in the ACMG medically actionable genes (Haer-Wigman et al., 2019). A lower rate was found amongst the Saudi population, with 1.2% of patients undergoing WES in Saudi Arabia having an ACMG secondary finding identified (Monies et al., 2017a).

In clinical genetics, the potential for incidental findings also occurred prior to the advent of NGS. For example, the genome wide array CGH, performed in a child with developmental delay, which identifies a deletion involving the *BRCA1* or *BRCA2* gene. This would still typically be reported back to the patient. The 100,000 Genomes Project has specific consent to detail whether the patient and parents wish to receive additional secondary findings (described above). Reporting of secondary and incidental findings has now been incorporated into the consent process in clinical genetics, with a separate section to consent to receive, or not receive these additional findings. Whether it is

possible to truly consent to receiving these additional or incidental findings, is beyond the scope of this project. Currently, since the ACMG guidelines were produced, it is standard practice in clinical genetics, when consenting a patient to perform genetic testing, to discuss the possibility of identifying an incidental, or secondary finding, and to ask the patient to decide whether they would like to receive this type of result or not.

8.2. 100,000 Genomes Project

WGS is the next option to try to identify the disease-causing variant in the remaining 20 families without a firm diagnosis. This was available through recruitment to the 100,000 genomes project, which was offered to patients through Genomics England. Prior to the closure of this project, I reviewed every undiagnosed family to ensure that, where appropriate, they had been informed of this project and given the opportunity to participate.

The potential for additional diagnoses from the 100,000 genomes project is unclear. Clearly sequencing the whole of the genome to include the non-coding region, versus only up to 3% of the genome through WES, enables clinicians to be hopeful for additional diagnoses that may not have been identified through WES, particularly structural variants and non-coding sequence variants. However, the vast number of benign variants increases when performing WGS rather than WES, and our bioinformatic understanding to enable accurate interpretation of non-coding disease-causing variant is very limited. In fact, the 100,000 genomes project has mainly limited their analysis to large groups of gene panels dictated by the phenotype recorded, which will limit the project's ability to identify additional diagnoses. It also highlights the critical importance of accurately recording the patient's phenotype. The vast scale of the 100,000 genomes project, will potentially enable the project to make novel diagnoses possible. Identification of novel disease genes will be through the research groups, called Genomics England Clinical Interpretation Partnerships (GeCIPs). These groups

will perform complimentary research projects to aid interpretation of the results from this project.

What is the likely potential to make additional diagnoses in the 100,000 genomes project, in patients who have already had WES? This is yet unclear, since the results are still being analysed. Even in the coding regions, there may be potential for diagnosis, if there was low coverage of certain regions of the genome or structural variants, which may be better detected by WGS. The DDD project so far know of only 6 'missed' diagnoses in their cohort (Wright et al., 2018). I have already discussed Family 23 with the homozygous *PEX16* variant, which I had previously discounted in view of the documented normal VLCFA results. After this result came back from the 100,000 genomes project, I further reviewed the biochemical laboratory results, which revealed two previous borderline results prior to the final normal result documented. Rather than showing the power of WGS to make additional diagnoses, this emphasises the importance of clear documentation in the patient's notes of normal, borderline and abnormal results.

We can also consider the WGS500 study. This was a preparatory project, performing WGS in 500 patients to understand the potential benefit for employing this sequencing method in clinical practice (Taylor et al., 2015). In a subset of 156 patient with a severe likely genetic disease, the group identified a likely pathogenic variant in 33 of these patients (21% of the total) (Taylor et al., 2015). The majority of the variants identified

were in genes and therefore within the capability of detection from WES, but may have been outside the target region for WES, or in a region of low coverage (Taylor et al., 2015). Other variants identified included non-coding variants, large deletions and uniparental disomy (Taylor et al., 2015).

It is therefore possible that the 100,000 genomes project may be able to help make additional diagnoses in this cohort of undiagnosed patients. This may be through newly described conditions since the closure of this project, through the sheer power of the project compared to this much smaller project, through sequencing of regions poorly covered by WES in coding regions of the genomes, by identifying variants in non-coding variants, or copy number variants, which are better identified by WGS. This is the future direction for many of the undiagnosed patients from this project to receive diagnoses either from the 100,000 genomes project directly, or subsequent research analysis through the GeCIPs.

8.3. Functional Work

The majority of the affected patients within this project are expected to have autosomal recessive disease. Many of these families have relatively unique phenotypes, which can make it difficult to make a definite diagnosis in the family, even if the disease-causing variant has been correctly identified. Identifying patients with the same novel condition can be enhanced by data sharing as previously discussed. Another method for doing this, is to increase the power of the study, which was employed by the much larger DDD project using statistical genotype and phenotype analysis amongst a cohort of 4,125 patients to identify four novel autosomal recessive disorders (Akawi et al., 2015). This is a small project and therefore this level of analysis is not possible, thus limiting the potential for novel disease identification. Collaboration is therefore the key to this. I have therefore collaborated with other research groups to provide additional evidence for pathogenicity of disease-causing variants within this project.

The variants causing autosomal recessive disease are typically loss-of-function, which means that knockout mouse models are potentially helpful to provide further evidence for causality of a variant (Monies et al., 2017b). Family 2 had a novel human disease, that I felt was caused by the homozygous *OTULIN* variant identified on WES and previously from autozygosity mapping studies. Collaboration with David Komander's group was extremely beneficial because the mouse model recapitulated the human phenotype, and thus enabled us to describe this novel human disease (Damgaard et al., 2016). This collaboration continues to be beneficial as we consider the liver phenotype

that has been identified in the mouse and try to clarify whether this could be relevant to Family 2 and other patients with ORAS. Biological treatments such as infliximab, can be extremely beneficial, but there is always the potential for the patient to develop antibodies to the treatment, and thus making the treatment ineffectual in the patient. Working with mouse models and the pharmaceutical company in this case, may potentially identify novel treatments in ORAS families, if the biological agent no longer treats the patient disease.

Additional functional studies were also performed in Family 9 (Rocha et al., 2017). This provided wider additional insights into the biology of adipose tissue. It also identified potential areas to pursue for treatment of this adipose tissue overgrowth. I have advised the clinicians involved in the care of Family 9 to observe for the metabolic consequences of this condition, including the possibility of leptin deficiency, which may require treatment in the future. There is also the possibility that mTOR blockade may influence the course of the disease, which needs to be considered further. The main focus for diagnosis in Family 9, was to identify a potential treatment for these children before the disease becomes more severe. The future consideration for study of this disease is to make a mouse model to recapitulate the disease and identify potentially beneficial treatments. Therefore, as well as proving pathogenicity of a variant, functional studies, can provide additional hopes for therapy of the genetic disease.

CHAPTER 9: CONCLUSION

This project has demonstrated the utility of NGS in diagnosis of rare inherited diseases, which will hopefully reduce the diagnostic odyssey in this group of patients. Identifying the disease-causing variants is important for the patient with the rare disease and their families to enable the provision of more accurate genetic counselling, prognostic information, more tailored clinical management, reproductive options, and ultimately direct gene or biological therapies.

I have identified known disease variants, a novel gene (*OTULIN*), a novel disease phenotype for a known gene (*MFN2*), phenotypic expansion of disease severity (*PEX16*), autosomal recessive disease genes in previously known autosomal dominant genes (*PKD1*), and blended phenotypes (Family 3). Phenotype based analysis has been crucial in this project to identify the disease-causing variant. I have also demonstrated the importance of collaborative work to prove pathogenicity for causative gene variants with additional functional work or identifying a case series to provide additional information in the medical literature. Re-analysis of NGS data is also important with the rapid identification of new disease genes, although the practically of doing this within Clinical Genetics needs further consideration. For those families without a diagnosis, Whole Genome Sequencing is likely to be the next step in their diagnostic odyssey.

CHAPTER 10: LIST OF REFERENCES

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CHAPTER 11: APPENDICIES

11.1 Figure 11.1 Patient Information Leaflet

INVITATION TO TAKE PART IN A PROJECT
TO INCREASE RESEARCH INTO THE CLINICAL AND GENETIC FEATURES OF:
(INSERT CONDITION HERE)

You are invited to take part in a research project. Before you decide whether or not you wish to take part it is important for you to understand why the project is being done and what it will involve if you take part. Please read the following information carefully. Discuss it with your friends and relatives if you wish. Ask us if there is anything you don't understand or if you would like more information. You will be given as much time as you want to make a decision.

WHAT ARE WE AIMING TO DO?

We would like to study the gene(s)/genetic factors involved in (INSERT CONDITION HERE), and may be the cause the condition in you and/or your family.

WHY ARE WE ASKING FOR YOUR HELP?

We are seeking your help as we need to investigate the genes/genetic factors of many individuals and/or families with exactly the same condition. The cells in your blood carry a complete set of your genetic material (genes), which we can study. We would like to store some of the genetic material in the laboratory because it may take some years and many experiments to understand the genetic factors that cause the condition in you and/or your family.

WHAT WILL YOU HAVE TO DO?

We will ask you and other members of your family to consider giving us a small blood sample **(e.g. 10mls/2 teaspoons)** taken through a needle from a vein in your arm. Usually this only causes brief discomfort and occasionally a small bruise. The sample will be used to obtain the genetic material from the white cells. Sometimes we may use alternative samples to obtain genetic material (e.g. saliva, mouth swab, tissue samples from biopsies, surgery or stored pathology specimens). We will ask some questions about your medical and family history. We may want to ask for your permission to look at your medical records. We will ask for your permission to put information about your family onto a computer database to store the information. We will remove all personal details, such as names and addresses, so your family cannot be recognized from it.

WILL THIS PROJECT BENEFIT YOU OR YOUR FAMILY?

We cannot guarantee to discover anything that will directly benefit you or your family. However, we hope to find the gene(s)/genetic factors causing the condition in you or your family. If this happens then genetic tests may be available for your relatives to find out if they are at risk of developing the condition. In this case we will inform you (unless you indicate you would not wish to be contacted). We hope that in the long term future, this research could lead to improved treatments for the condition.

DO YOU HAVE TO TAKE PART?

Your taking part in this project is voluntary. If you would prefer not to take part you do not have to give a reason. You may also withdraw from the project at any time. This will not affect your or your family's medical care. If this research leads to the development of a new treatment or medical test, you will NOT benefit financially from this. If in the future you lose the ability to understand the research or die, you may still want us to carry on the research (unless your family states otherwise).

WHO WILL KNOW ABOUT YOU TAKING PART?

The information collected about you during the course of the research project will be kept strictly confidential and you will not be identifiable from it. Any results arising from this research work will be kept strictly confidential. If any research results are published in medical articles as a result of this project all personal details will be removed so that your family cannot be recognized from it. With your permission your G.P. will be told that you have agreed to be involved in the project.

WHAT OTHER INFORMATION MAY BE PRODUCED BY THE RESEARCH?

You will NOT be told about any genetic alterations which are identified as a by-product of this research that are not relevant to you or your family's illness. In some cases the genetic information produced by studying your genetic material may be placed in an electronic archive with no connection to your name or other personal identifier. This archive will only be accessible to appropriate doctors and researchers who have been approved by a committee set up to ensure the results are only used to advance scientific and medical understanding. Although there is a theoretical possibility that you could be identified by the deposited information (e.g. if you are entered into another independent genetic study), this is extremely unlikely.

WHO DO YOU CONTACT WITH ANY CONCERNS?

If you have any questions or concerns about this project, please contact Prof. Eamonn Maher on [redacted] **or the Patient Advise & Liaison Service (PALS) on 0121 627 2747.** Alternatively you can write to the following contact addresses:

Prof. Eamonn Maher
Medical and Molecular Genetics
(University of Birmingham)
Norton Court
Birmingham Women's Hospital
Edgbaston
Birmingham
B15 2TG

11.2 Figure 11.2 Consent form

Birmingham Women's NHS
NHS Foundation Trust

UNIVERSITY OF BIRMINGHAM

PRINCIPAL INVESTIGATOR:
PROFESSOR EAMONN MAHER

Eamonn Maher
Professor of Medical Genetics
BSc MBChB MRCP FRCP MD MA FMed Sci
Department of Medical and Molecular Genetics
(University of Birmingham)
Norton Court,
Birmingham Women's Hospital,
Edgbaston, Birmingham, B15 2TG
Tel: [redacted] Fax: [redacted]
Research Nurse
Gail Kirby Tel: [redacted]

Family no:
Patient Identification Number:

CONSENT FORM

Title of Project: Molecular Pathology of Human Genetic Disease

A PROJECT TO STUDY THE CLINICAL AND GENETIC FEATURES OF:
(INSERT CONDITION HERE)

Name of Researcher: Prof. Eamonn Maher

Please initial boxes

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

2. (a) I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

   (b) I give you permission to continue to use the samples and information collected as part of this research in the event of my losing capacity or dying unless my next of kin requests otherwise. ☐

3. I understand that relevant sections of any of my/ my child's medical notes and data collected during the study, may be reviewed by individuals from the project team, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. (a) I agree to allow medical information about my family to be entered on a confidential computer database. ☐

   (b) In addition, in some cases the genetic information produced by studying my/ my child's DNA may be placed in an electronic archive with no connection to my/ my child's name or other personal identifier. I understand that this archive will only be accessible to appropriate doctors and researchers who have been approved by a committee set up to ensure the results are only used to advance scientific and medical understanding. Although there is a theoretical possibility that I/ my child could be identified by the deposited information (e.g. if I/ my child is entered into another independent genetic study), this is extremely unlikely. ☐

   (c) If further medical information is requested by members of the project team, I agree to be contacted again for this purpose. ☐

5. (a) I agree to provide a sample, or for..... (my child) to provide a sample, which will be stored and may be used for genetic research studies appropriate to my/ my family's condition. I understand that any results arising from this research work will be kept strictly confidential. ☐

(b) I understand that the techniques used are NOT suitable for diagnostic testing for known genetic defects, and that I will NOT be told about any genetic alterations which are identified as a by-product of this research that are not relevant to my/ my family's illness. This will not affect my/ my child's access to clinically approved genetic advice and testing through other doctors caring for me in any way. ☐

(c) If a genetic test becomes available as a result of medical research on my/ my family's sample(s) I would like to have the opportunity to discuss the implications of these findings with appropriate medical experts. ☐

6. I understand that I/ my child will not benefit financially if this research leads to the development of a new treatment or medical test. ☐

_____	_____	_____
Name of Participant	Date	Signature (If signing on behalf of Participant state your name & relationship to them)

_____	_____	_____
Name of Person taking consent (If different from researcher)	Date	Signature

_____	_____	_____
Researcher	Date	Signature

(1 for participant; 1 for researcher; 1 for hospital notes)

11.3. Phenotype Summary Table

Table 11.1 Coding key for HPO terms listed in Table 11.2

System	HPO terms	HPO terms
All	HP:0011421	Death in adolescence
	HP:0003819	Death in childhood
	HP:0001522	Death in infancy
	HP:0003677	Slow progression
Growth	HP:0008915	Childhood-onset truncal obesity
	HP:0004325	Decreased body weight
	HP:0000823	Delayed puberty
	HP:0001508	Failure to thrive
	HP:0001531	Failure to thrive in infancy
	HP:0004324	Increased body weight
	HP:0000256	Macrocephaly
	HP:0008845	Mesomelic short stature
	HP:0000252	Microcephaly
	HP:0001513	Obesity
	HP:0003510	Severe short stature
	HP:0004322	Short stature
	HP:0001824	Weight loss
Craniofacial	HP:0011071	Abnormality of permanent molar morphology
	HP:0000164	Abnormality of teeth
	HP:0000463	Anteverted nares
	HP:0000248	Brachycephaly
	HP:0000670	Carious teeth
	HP:0000175	Cleft palate
	HP:0000490	Deeply set eye
	HP:0000684	Delayed eruption of teeth
	HP:0000678	Dental crowding
	HP:0000703	Dentinogenesis imperfecta
	HP:0005280	Depressed nasal bridge
	HP:0000268	Dolichocephaly
	HP:0002307	Drooling
	HP:0000286	Epicanthus
	HP:0040199	Flat midface
	HP:0000218	High palate
	HP:0000685	Hypoplasia of the teeth
	HP:0000527	Long eyelashes

	HP:0000276	Long face
	HP:0000343	Long philtrum
	HP:0000303	Mandibular prognathia
	HP:0000347	Micrognathia
	HP:0000665	Narrow forehead
	HP:0000160	Narrow mouth
	HP:0000194	Open mouth
	HP:0011327	Posterior plagiocephaly
	HP:0011107	Recurrent aphthous stomatitis
	HP:0005989	Redundant neck skin
	HP:0000664	Synophrys
	HP:0010804	Tented upper lip vermilion
	HP:0000574	Thick eyebrows
	HP:0000687	Widely spaced teeth
	HP:0002645	Wormian bones
Cognitive Behavioural and developmental	HP:0001344	Absent speech
	HP:0002381	Aphasia
	HP:0000717	Autism
	HP:0002194	Delayed gross motor development
	HP:0000750	Delayed speech and language development
	HP:0000750	Developmental regression
	HP:0001260	Dysarthria
	HP:0001263	Global developmental delay
	HP:0001249	Intellectual disability
	HP:0010864	Intellectual disability, severe
	HP:0001256	Mild learning difficulties
	HP:0002167	Neurological speech impairment
	HP:0007301	Oromotor apraxia
	HP:0011098	Speech Apraxia
Neurological	HP:0006846	Acute encephalopathy
	HP:0001274	Agenesis of the corpus callosum
	HP:0000718	Aggressive behaviour
	HP:0001284	Areflexia
	HP:0001251	Ataxia
	HP:0011445	Athetoid cerebral palsy
	HP:0002136	Broad-based gait
	HP:0001272	Cerebellar atrophy
	HP:0002059	Cerebral atrophy

	HP:0007305	CNS demyelination
	HP:0003429	CNS Hypomyelination
	HP:0100543	Cognitive impairment
	HP:0012185	Constrictive median neuropathy
	HP:0003693	Distal amyotrophy
	HP:0002460	Distal muscle weakness
	HP:0001332	Dystonia
	HP:0011203	EEG with abnormally slow frequencies
	HP:0010851	EEG with burst suppression
	HP:0011185	EEG with focal epileptiform discharges
	HP:0010883	EEG with focal slow waves
	HP:0010845	EEG with generalised slow activity
	HP:0012000	EEG with generalised spikes
	HP:0200134	Epileptic encephalopathy
	HP:0002131	Episodic ataxia
	HP:0002373	Febrile seizures
	HP:0007359	Focal seizures
	HP:0002066	Gait ataxia
	HP:0001290	Generalised hypotonia
	HP:0002123	Generalised myoclonic seizures
	HP:0002069	Generalised tonic clonic seizures
	HP:0002171	Gliosis
	HP:0001301	Hemiplegia
	HP:0012302	Herpes simplex encephalitis
	HP:0002079	Hypoplasia of the corpus callosum
	HP:0002521	Hypsarrythmia
	HP:0012469	Infantile spasms
	HP:0002140	Ischaemic stroke
	HP:0002415	Leukodystrophy
	HP:0011181	Low voltage EEG
	HP:0002354	Memory impairment
	HP:0001324	Muscle weakness
	HP:0001253	Muscular hypotonia
	HP:0008936	Muscular hypotonia of the trunk
	HP:0001336	Myoclonus
	HP:0003458	Myopathic abnormalities

	HP:0002058	Myopathic facies
	HP:0007021	Pain insensitivity
	HP:0002268	Paroxysmal dystonia
	HP:0003477	Peripheral axonal neuropathy
	HP:0002073	Progressive Cerebellar ataxia
	HP:0007024	Pseudobulbar paralysis
	HP:0007335	Recurrent encephalopathy
	HP:0007274	Recurrent meningitis
	HP:0001315	Reduced tendon reflexes
	HP:0002322	Resting tremor
	HP:0001250	Seizures
	HP:0010871	Sensory Ataxia
	HP:0003390	Sensory axonal neuropathy
	HP:0000763	Sensory neuropathy
	HP:0002360	Sleep disturbance
	HP:0001264	Spastic diplegia
	HP:0002510	Spastic tetraplegia
	HP:0002273	Tetraparesis
	HP:0001727	Thromboembolic stroke
	HP:0030051	Tip-toe gait
Eye Defects	HP:0008323	Abnormal rod and cone electroretinogram
	HP:0000591	Abnormality of the sclera
	HP:0000498	Blepharitis
	HP:0000592	Blue sclera
	HP:0000518	Cataracts
	HP:0006934	Congenital nystagmus
	HP:0000524	Conjunctival telangiectasia
	HP:0100704	Cortical visual impairment
	HP:0020045	Esodeviation
	HP:0000565	Esotropia
	HP:0000666	Horizontal nystagmus
	HP:0007817	Horizontal supranuclear gaze palsy
	HP:0000540	Hypermetropia
	HP:0000545	Myopia
	HP:0000639	Nystagmus
	HP:0000648	Optic atrophy
	HP:0000543	Optic disc pallor
	HP:0001138	Optic neuropathy
	HP:0000508	Ptosis
	HP:0000556	Retinal dystrophy

	HP:0000554	Uveitis
	HP:0000505	Visual impairment
Ear defect	HP:0000377	Abnormaility of the pinna
	HP:0030025	Auricular pits
	HP:0000405	Conductive hearing loss
	HP:0000365	Hearing impairment
	HP:0400004	Long ear
	HP:0000369	Low-set ears
	HP:0000396	Overfolded helix
	HP:0000358	Posteriorly rotated ears
	HP:0000411	Protruding ear
	HP:0000407	Sensori-neural hearing loss
Respiratory	HP:0000776	Congenital diaphragmatic hernia
	HP:0012735	Cough
	HP:0006528	Cystic lung disease
	HP:0002875	Exertional dyspnea
	HP:0001601	Laryngomalacia
	HP:0002643	Neonatal respiratory distress
	HP:0000768	Pectus Carinatum
	HP:0002204	Pulmonary embolism
	HP:0002206	Pulmonary fibrosis
	HP:0002205	Recurrent respiratory infections
Cardiovascular	HP:0000822	Hypertension
	HP:0001712	Left ventricular hypertrophy
	HP:0002564	Malformation of the heart and great vessels
	HP:0001658	Myocardial infarction
Gastro-intestinal	HP:0002027	Abdominal pain
	HP:0002867	Abnormality of the ileum
	HP:0002023	Anal atresia
	HP:0002608	Celiac disease
	HP:0001081	Cholelithiasis
	HP:0002611	Cholestatic liver disease
	HP:0100281	Chronic colitis
	HP:0002091	Constipation
	HP:0002014	Diarrhoea
	HP:0002015	Dysphagia
	HP:0002910	Elevated liver transaminases
	HP:0100594	Esophageal web
	HP:0011968	Feeding difficulties
	HP:0002020	Gastro-esophageal reflux

	HP:0011471	Gastrostomy tube feeding in infancy
	HP:0001397	Hepatic steatosis
	HP:0002240	Hepatomegaly
	HP:0001433	Hepatosplenomegaly
	HP:0000023	Inguinal hernia
	HP:0002566	Intestinal malrotation
	HP:0005235	Jejunal atresia
	HP:0004385	Malabsorption
	HP:0001539	Omphalocele
	HP:0004385	Protracted diarrhoea
	HP:0011473	Villous atrophy
	HP:0002580	Volvulus
	HP:0002013	Vomiting
Genito-urinary	HP:0001919	Acute kidney injury
	HP:0000028	Cryptorchidism
	HP:0010945	Fetal pyelectasis
	HP:0000097	Focal segmental glomerulosclerosis
	HP:0000099	Glomerulonephritis
	HP:0000790	Hematuria
	HP:0000126	Hydronephrosis
	HP:0000054	Micropenis
	HP:0005562	Multiple renal cysts
	HP:0000113	Polycystic kidney dysplasia
	HP:0000149	Polycystic ovaries
	HP:0000103	Polyuria
	HP:0000093	Proteinuria
	HP:0000010	Recurrent UTIs
	HP:0001917	Renal amyloidosis
	HP:0000803	Renal cortical cysts
	HP:0000869	Secondary amenorrhoea
	HP:0003774	Stage five chronic kidney disease
	HP:0000029	Testicular atrophy
Cutaneous	HP:0000956	Acanthosis nigricans
	HP:0001061	Acne
	HP:0001057	Aplasia cutis congenita
	HP:0008386	Aplasia/ hypoplasia of the nails
	HP:0007598	Bilateral single transverse palmar creases
	HP:0000957	Café au lait spot

	HP:0000965	Cutis marmorata
	HP:0000964	Eczema
	HP:0007605	Excessive wrinkling of palmar skin
	HP:0009937	Facial hirsutism
	HP:0002230	Generalised hirsutism
	HP:0000953	Hyperpigmentation of the skin
	HP:0001010	Hypopigmentation of the skin
	HP:0001053	Hypopigmented skin patches HP
	HP:0007432	Intermittent generalised erythematous papular rash
	HP:0002164	Nail dysplasia
	HP:0012322	Perifolliculitis
	HP:0000988	Skin rash
	HP:0001065	Striae distensae
	HP:0001025	Urticaria
Connective tissues	HP:0009125	Lipodystrophy
	HP:0012490	Panniculitis
Immunological	HP:0003453	Antineutrophil antibody positivity
	HP:0003493	Antinuclear antibody positivity
	HP:0004431	Complement deficiency
	HP:0002721	Immunodeficiency
	HP:0002716	Lymphadenopathy
	HP:0011107	Recurrent aphthous stomatitis
	HP:0002783	Recurrent lower respiratory tract infections
	HP:0003262	Smooth muscle antibody positivity
	HP:0011110	Tonsillitis
Blood	HP:0001070	Abnormal immunoglobulin level
	HP:0001903	Anaemia
	HP:0004315	IgG deficiency
	HP:0001875	Neutropenia
	HP:0011897	Neutrophilia
	HP:0004841	Reduced factor XII activity
	HP:0004406	Spontaneous, recurrent epistaxis
Endocrine	HP:0040075	Hypopituitarism HP
	HP:0000821	Hypothyroidism
	HP:0100651	Type I diabetes mellitus
Metabolism	HP:0011034	Amyloidosis

	HP:0010837	Decreased serum ceruloplasmin
	HP:0011227	Elevated C-reactive protein level
	HP:0003565	Elevated erythrocyte sedimentation rate
	HP:0001954	Episodic fever
	HP:0003073	Hypoalbuminaemia
	HP:0001955	Unexplained fevers
Musculoskeletal	HP:0003312	Abnormal form of the vertebral bodies
	HP:0000924	Abnormality of the skeletal system
	HP:0005616	Accelerated skeletal maturation
	HP:0001226	Acral ulceration and osteomyelitis leading to autoamputation of digits
	HP:0000705	Amelogenesis imperfecta
	HP:0006390	Anterior tibial bowing
	HP:0010185	Aplasia/hypoplasia of the distal terminal phalanges of the toes
	HP:0003365	Arthalgia of the hip
	HP:0005059	Arthralgia/Arthritis
	HP:0012453	Bilateral wrist flexion contracture
	HP:0003865	Bowed humerus
	HP:0100490	Camptodactyly of the finger
	HP:0001836	Camptodactyly of the toe
	HP:0100749	Chest pain
	HP:0001217	Clubbing
	HP:0011303	Convex contour of sole
	HP:0003417	Coronal cleft vertebrae
	HP:0002812	Coxa vara
	HP:0002750	Delayed skeletal maturation
	HP:0003042	Elbow dislocation
	HP:0002987	Elbow flexion contracture
	HP:0002980	Femoral bowing
	HP:0002359	Frequent falls
	HP:0003273	Hip contracture
	HP:0030043	Hip Subluxation
	HP:0005639	Hyperextensible hand joints
	HP:0003307	Hyperlordosis
	HP:0002659	Increased susceptibility to fractures
	HP:0003796	Irregular iliac crest
	HP:0001382	Joint hypermobility

	HP:0006380	Knee flexion contracture
	HP:0002751	Kyphoscoliosis
	HP:0001377	Limited extension of elbow
	HP:0100807	Long fingers
	HP:0003394	Muscle cramps
	HP:0003326	Myalgia
	HP:0010557	Overlapping fingers
	HP:0001845	Overlapping toe
	HP:0001763	Pes planus
	HP:0000926	Platyspondyly
	HP:0002757	Recurrent fractures
	HP:0004349	Reduced bone mineral density
	HP:0001838	Rocker bottom foot
	HP:0002651	Scoliosis
	HP:0010034	Short first metacarpal
	HP:0009882	Short distal phalanx of finger
	HP:0100864	Short femoral neck
	HP:0003834	Shoulder dislocation
	HP:0000246	Sinusitis
	HP:0001762	Talipes equinovarus
	HP:0001883	Talipes
	HP:0100550	Tendon rupture
	HP:0002944	Thoracolumbar scoliosis
	HP:0002982	Tibial bowing
	HP:0002953	Vertebral compression fractures

11.2 Phenotype Summary table of recruited Patients using HPO terms

Family	Family member	All	Growth	Craniofacial	Cognitive Behavioural, developmental
1	1		HP:0000252 HP:0004322 HP:0001508		HP:0001263 HP:0001249
2	1		HP:0004322 HP:0004325		HP:0001263 HP:0001249 HP:0000750
2	2		HP:0000252 HP:0004322 HP:0001508		HP:0001263 HP:0001249
3	1		HP:0001513		
4	1		HP:0004322		
5	1		HP:0003510 HP:0004325		HP:0001263 HP:0010864
5	2		HP:0003510		HP:0001263 HP:0001249
6	1		HP:0000256	HP:0000164	HP:0002194
7	1		HP:0000252 HP:0004322 HP:0004325	HP:0005280	HP:0001263 HP:0001249 HP:0000750
8	1				
9	1		HP:0008915		
9	2		HP:0000256		
10	1		HP:0000252 HP:0004322 HP:0001508 HP:0000823	HP:0000678	HP:0001263 HP:0001256
11	1		HP:0000256	HP:0000276	HP:0001256 HP:0000750 HP:0011098
12	1				
12	2				
13	1	HP:0003677			HP:0001260

14	1		HP:0000252 HP:0008845 HP:0003510 HP:0004325	HP:0000684 HP:0000703 HP:0000194 HP:0000248 HP:0011327 HP:0002645	HP:0012736 HP:0002187 HP:0001344
15	1		HP:0004322 HP:0001531 HP:0000823		HP:0012736 HP:0002187 HP:0001344 HP:0000750
15	2		HP:0004322 HP:0001531 HP:0000823	HP:0000760	
16	1		HP:0004324		
17	1		HP:0000252		HP:0001263 HP:0001249 HP:0001260
17	2		HP:0001513	HP:0000303	HP:0001263 HP:0001249
18	1		HP:0000252 HP:0004325	HP:0000490 HP:0000463 HP:0000343 HP:0000218 HP:0010804 HP:0000347 HP:0000527 HP:0005989 HP:0000268	HP:0001263
19	1			HP:0000687 HP:0002307	HP:0002381 HP:0000750 HP:0007301
20	1		HP:0000252		HP:0010864
21	1			HP:0000286 HP:0000160 HP:0040199 HP:0000248	HP:0001263 HP:0010864
22	1				
23	1				HP:0002167
24	1				HP:0001249
24	2				HP:0001249 HP:0001260

25	1	HP:0011421	HP:0000252 HP:0001508		HP:0010864 HP:0007301
25	2	HP:0003819	HP:0000252 HP:0001508		HP:0010864 HP:0007301
26	1				
27	1				
28	1		HP:0001531	HP:0000665	
28	2				
29	1		HP:0000252 HP:0004322 HP:0004325	HP:0000670	HP:0001263 HP:0001249 HP:0000750 HP:0000717
29	2		HP:0000253 HP:0004323 HP:0004326	HP:0000671 HP:0011071 HP:0000664	HP:0001264 HP:0001249 HP:0000751 HP:0001260
30	1	HP:0001522		HP:0000175 HP:0000347	
31	1				
31	1				
32	1		HP:0001824	HP:0000685 HP:0011107	
33	1		HP:0001513	HP:0000574	HP:0001263
34	1				
35	1				HP:0001263 HP:0000750

Family	Family member	Neurological	Eye Defect	Ear Defect	Respiratory
1	1	HP:0001250 HP:0001290 HP:0001251			
2	1		HP:0000518		HP:0002643
2	2		HP:0000518 HP:0000591		
3	1		HP:0000591		
4	1				

5	1	HP:0001264 HP:0007274 HP:0001274			HP:0002206 HP:0006528
5	2				
6	1	HP:0001284 HP:0003693 HP:0002460	HP:0000591		
7	1	HP:0002079	HP:0000543 HP:0000498		
8	1		HP:0008323 HP:0001138		
9	1				
9	2				
10	1	HP:0001301			
11	1	HP:0001251 HP:0002131 HP:0002268 HP:0003477 HP:0001272	HP:0000639	HP:0000407 HP:0000369 HP:0000411	HP:0000768
12	1	HP:0011203 HP:0006846 HP:0012302 HP:0007305			
12	2	HP:0011203 HP:0006846 HP:0012302 HP:0007305			
13	1	HP:0002354 HP:0002322 HP:0001251 HP:0001284 HP:0001324 HP:0001284 HP:0002066 HP:0003390 HP:0012185 HP:0001272			
14	1	HP:0001290	HP:0000540 HP:0006934	HP:0000405 HP:0000396	
15	1	HP:0100543 HP:0001250 HP:0010845 HP:0008936 HP:0002140 HP:0002510	HP:0100704	HP:0400004	HP:0002204 HP:0002205

		HP:0002510 HP:0002510			
15	2	HP:0003458 HP:0000763			
16	1	HP:0002373 HP:0007021	HP:0000545 HP:0000592		
17	1	HP:0002373 HP:0002069 HP:0000718 HP:0003477			
17	2	HP:0002373 HP:0002070 HP:0003477			
18	1	HP:0002123 HP:0200134 HP:0012469 HP:0010851 HP:0200134 HP:0001290	HP:0000648	HP:0000358	HP:0001601
19	1	HP:0002354 HP:0002123 HP:0010883 HP:0001336 HP:0010871 HP:0002273 HP:0001301 HP:0007024 HP:0002360 HP:0003458 HP:0002059	HP:0000524 HP:0007817		
20	1	HP:0002123 HP:0200134 HP:0012469 HP:0002521 HP:0012000 HP:0011181 HP:0008936 HP:0002509 HP:0001272	HP:0100704 HP:0000648		

21	1	HP:0200134 HP:0007335 HP:0007359 HP:0011185 HP:0008936 HP:0001251 HP:0002058 HP:0030051 HP:0001727	HP:0000508		
22	1	HP:0012469			
23	1	HP:0001264 HP:0001348 HP:0002360 HP:0002415	HP:0000639		
24	1	HP:0001252 HP:0002073 HP:0002136			
24	2	HP:0001253 HP:0002074 HP:0001264 HP:0001348 HP:0002136 HP:0007305			
25	1	HP:0011445 HP:0003429 HP:0001272	HP:0000505 HP:0000565 HP:0000556		HP:0000768
25	2	HP:0011445 HP:0003430 HP:0001272			
26	1	HP:0100543 HP:0001336 HP:0001251 HP:0001332 HP:0002415 HP:0003429 HP:0001273			
27	1				
28	1			HP:0000369	
28	2				
29	1	HP:0001290 HP:0001251 HP:0001315 HP:0002136 HP:0003390	HP:0000666	HP:0000365	

29	2	HP:0001291 HP:0001252 HP:0001316 HP:0002137			
30	1			HP:0000377	HP:0000776
31	1	HP:0012469			
31	1				
32	1		HP:0000554		
33	1			HP:0030025	
34	1	HP:0002360			HP:0012735 HP:0002875
35	1	HP:0006846 HP:0001290 HP:0001251 HP:0001272 HP:0002171	HP:0000639 HP:0020045		

Family	Family member	Cardiovascular	Gastro-intestinal	Genito-urinary	Cutaneous
1	1		HP:0002014 HP:0002240 HP:0002910 HP:0002910		HP:0001053 HP:0001010
2	1		HP:000023		HP:0000953 HP:0009654
2	2			HP:0001919 HP:0000010	
3	1	HP:0001658	HP:0001397	HP:0000869 HP:0000149	HP:0000956 HP:0001065 HP:0001061 HP:0009937
4	1				
5	1		HP:0002910	HP:0000028 HP:0000029	HP:0001010 HP:0001061
5	2				HP:0001010
6	1				HP:0007605
7	1		HP:0002014	HP:0000097 HP:0003774 HP:0000010	HP:0000953

8	1				
9	1		HP:0002240		HP:0000965
9	2				HP:0002230
10	1		HP:0002013 HP:0002014 HP:0002240 HP:0100594 HP:0011473 HP:0002867		
11	1				HP:0000965
12	1				
12	2				
13	1		HP:0002910		
14	1		HP:0011968 HP:0011471 HP:0002020		
15	1		HP:0004385 HP:0004385 HP:0002091 HP:0011968 HP:0011473 HP:0100281		HP:0009937
15	2		HP:0004385 HP:0004385 HP:0011968 HP:0001433 HP:0001081 HP:0011473	HP:0000103	HP:0007432
16	1		HP:0002091 HP:0002580 HP:0005235	HP:0000010	HP:0000964 HP:0002164
17	1				
17	2				
18	1		HP:0002611	HP:0010945	HP:0007598
19	1				
20	1		HP:0011968 HP:0011471		
21	1				
22	1				
23	1		HP:0002015		
24	1				

24	2				HP:0007598
25	1		HP:0011968		
25	2		HP:0011968		
26	1				
27	1				HP:0000965 HP:0001057 HP:0008386
28	1	HP:0000822	HP:0002608	HP:0000803 HP:0000113 HP:0000010	HP:0001053 HP:0000957
28	2			HP:0000803 HP:0000114	
29	1				
29	2				
30	1		HP:0002023 HP:0002566 HP:0001539	HP:0005562 HP:0000028 HP:0000054 HP:0000126	HP:0008386
31	1				
31	1	HP:0002564			
32	1	HP:0001712		HP:0001917 HP:0000099 HP:0000093 HP:0000790	HP:0001025
33	1				HP:0012322
34	1		HP:0002013	HP:0000099 HP:0000093 HP:0000790	HP:0000988
35	1				

Family	Family member	Connective tissues	Immunological	Blood	Endocrine
1	1		HP:0002721	HP:0001903	
2	1	HP:0012490	HP:0003453 HP:0003262 HP:0002721	HP:0011897	
2	2	HP:0012490		HP:0011897	
3	1				
4	1				
5	1		HP:0002721	HP:0001903	
5	2		HP:0002721		
6	1				
7	1			HP:0004315	
8	1		HP:0002721		
9	1	HP:0009125			
9	2				
10	1			HP:0001903	
11	1				
12	1				
12	2				
13	1				
14	1				
15	1		HP:0002783	HP:0001875 HP:0001903	HP:0040075 HP:0000821 HP:0100651
15	2		HP:0011107	HP:0001070 HP:0001875 HP:0004841	HP:0040075
16	1				
17	1				
17	2				
18	1				
19	1		HP:0003493		
20	1				
21	1				
22	1				
23	1				
24	1				
24	2				
25	1				
25	2				

26	1				
27	1				
28	1				
28	2				
29	1				
29	2				
30	1				
31	1				
31	1				
32	1	HP:0009125	HP:0004431 HP:0002716	HP:0004406 HP:0001903	
33	1		HP:0011110 HP:0002716		
34	1			HP:0001903	
35	1				

Family	Family member	Metabolism	Musculoskeletal
1	1		
2	1	HP:0003073 HP:0011227 HP:0003565	
2	2	HP:0011227 HP:0003565	HP:0005059
3	1		HP:0004349 HP:0002659 HP:0002982
4	1		HP:0004349 HP:0002659 HP:0002757 HP:0002980 HP:0003865 HP:0002953
5	1		HP:0100490
5	2		

6	1		HP:0005059 HP:0002659 HP:0002757 HP:0001382 HP:0100550 HP:0003834 HP:0003312 HP:0100864 HP:0100490 HP:0011303 HP:0001763 HP:0001836
7	1	HP:0003073	HP:0005616
8	1		
9	1		
9	2		
10	1	HP:0003073	HP:0002750 HP:0001217
11	1		HP:0002359 HP:0003042 HP:0001377
12	1		
12	2		
13	1	HP:0010837	HP:0002359 HP:0003394 HP:0002943 HP:0000246
14	1		HP:0004349 HP:0002659 HP:0002757 HP:0005639 HP:0000926 HP:0003417 HP:0002944 HP:0003796 HP:0006390 HP:0002812 HP:0010034 HP:0006380 HP:0003273
15	1	HP:0100651 HP:0011227 HP:0003565 HP:0001954	HP:0002812

15	2	HP:0011227 HP:0003565 HP:0001954	HP:0005059
16	1		HP:0001226
17	1		
17	2		HP:0001762
18	1		HP:0010557 HP:0001838 HP:0002987 HP:0006380
19	1		
20	1		HP:0030043 HP:0002944 HP:0003307 HP:0012453
21	1		
22	1		
23	1		
24	1		HP:0001763
24	2		HP:0001763
25	1		HP:0002751
25	2		HP:0002651
26	1		
27	1		HP:0009882
28	1		HP:0100807 HP:0001845 HP:0001883
28	2		HP:0001883
29	1		HP:0000705
29	2		
30	1		HP:0009882 HP:0010185 HP:0001883
31	1		
31	1		HP:0000924
32	1	HP:0011227 HP:0003565 HP:0011034 HP:0001955	HP:0003326 HP:0005059

33	1	HP:0011227 HP:0003565 HP:0001955	HP:0100749 HP:0003365
34	1	HP:0003565	HP:0003326
35	1		

11.4 Tables of filtered variants for each individual family

Table 11.4.1 Family 1 Filtered Variants

Position	Change	GT	Gene	Effect	DbSNP	Clin Var
12:70747693	T>TA	HOM	CNOT2	Frame-shift	rs35192504	
19:16855411	T>TG	HOM	NWD1	Frame-shift		
10:3208567	T>TGCACGCTAGGGAAGAGAGA	HOM	PITRM1	Frame-shift	rs4266975	
5:139931628	A>AGT	HOM	SRA1	Frame-shift	rs3085220	
19:17397485	GTGTGTGTGTGTGTGTT>G	HOM	ANKLE1	Frame-shift		
7:133886253	CA>C	HOM	LRGUK	Frame-shift		
7:142143722	CCT>C	HOM	TRBV6-7	Frame-shift		
17:30469470	CCCGCCGCCG>C	HOM	ENSG00000214708	Deletion		
19:7504973	A>ACATGGG	HOM	ARHGEF18	Insertion		
12:7045891	A>ACAGCAGCAG	HOM	ATN1	Insertion	rs60216939	
1:154842199	G>GGCTGCTGCTGCTGCT	HOM	KCNN3	Insertion	rs58327065	
15:89386715	T>C	HOM	ACAN	Missense		
10:99019316	A>T	HOM	ARHGAP19	Missense		
3:112358380	C>G	HOM	CCDC80	Missense		
8:65527771	A>G	HOM	CYP7B1	Missense		

19:11314765	C>T	HOM	DOCK6	Missense		
10:99019316	A>T	HOM	ENSG0000 0269891	Missense		
2:239040104	G>A	HOM	ESPNL	Missense	rs140616772	
10:85993888	C>A	HOM	LRIT1	Missense		
19:50763907	G>A	HOM	MYH14	Missense		
11:4566878	G>A	HOM	OR52M1	Missense	rs138834789	
3:110796077	C>T	HOM	PVRL3	Missense		
2:241517259	G>A	HOM	RNPEPL1	Missense		
8:73921242	T>C	HOM	TERF1	Missense		
17:73824966	G>T	HOM	UNC13D	Missense		
19:11917594	T>C	HOM	ZNF491	Missense	rs150689659	
7:143085695	C>A	HOM	ZYX	Missense	rs146818692	
19:16268207	TA>T	HOM	HSH2D	Splicing	rs5827321	
13:47469577	CACATG CTCTTTA TTACCAG TGCGAAT ATAGCTG GGAAACT AATGCCA CTCACCA T>C	HOM	HTR2A	Splicing		
4:55592215	A>G	HOM	KIT	Splicing		
11:20668480	C>T	HOM	SLC6A5	Splicing	rs144357826	
4:41945831	C>T	HOM	TMEM33	splicing		

Table 11.4.2 Family 2 Filtered Variants

See published paper 11.6: Table S2. Homozygous variants in ORAS patients

Damgaard et al, 2016)

Chr	Start	Ref	Alt	GT	Gene	Effect
3	15876057	T	-	HOM	ANKRD28	Splicing
1	20313291	T	C	HOM	PLA2G2D	Missense
1	153108866	-	CTGCTGCT GCTGCTG	HOM	KCNN3	Insertion
1	218345897	C	T	HOM	IARS2	Missense
1	220869301	G	C	HOM	MIA3	Missense
1	223673599	C	A	HOM	LBR	Missense

1	246789053	-	TA	HOM	OR2T29	Frameshift
2	168812070	-	GGGCCG	HOM	STK39	Insertion
2	219786639	C	T	HOM	ABCB6	Splicing
3	33619497	G	C	HOM	CLASP2	Missense
3	45242305	A	-	HOM	TMEM158	Frameshift
3	54888407	TC	-	HOM	CACNA2D3	Splicing
3	135452128	-	G	HOM	RYK	Frameshift
3	196991227	AGA	-	HOM	MUC4	Deletion
4	3046460	-	GCA	HOM	HTT	Insertion
4	14613991	-	GCCGCC	HOM	CPEB2	Insertion
4	14614036	-	GCC	HOM	CPEB2	Insertion
5	14422479	A	T	HOM	TRIO	Splicing
5	14743368	T	C	HOM	OTULIN	Missense
5	79067816	T	C	HOM	CMYA5	Missense
5	79068878	G	A	HOM	CMYA5	Missense
5	98157103	G	T	HOM	RGMB	Missense
6	1557040	-	CGG	HOM	FOXC1	Insertion
7	44630434	GTA	-	HOM	OGDH	Splicing
8	22022655	G	-	HOM	NUDT18	Frameshift
8	120289959	G	-	HOM	MAL2	Frameshift
9	6855	-	GTGGTGCTG	HOM	WASH1	Insertion
10	15769982	C	G	HOM	ITGA8	Missense
11	6388805	A	T	HOM	APBB1	Missense
11	7803299	T	A	HOM	OR5P3	Missense
11	11305255	G	A	HOM	GALNTL4	Missense
11	35154767	G	C	HOM	CD44	Missense
11	35284347	T	C	HOM	SLC1A2	Missense
12	110521161	CTG	-	HOM	ATXN2	Deletion
13	20460500	-	TGGGCG	HOM	LATS2	Insertion
13	45022414	G	T	HOM	FAM194B	Missense
13	71338684	CCGCCG	-	HOM	DACH1	Deletion
14	76563540	CTGCTG	-	HOM	C14orf4	Deletion
15	40089634	-	CGCC	HOM	PLA2G4E	Frameshift
17	3605158	-	TCAGGTGGC CCCGCCCTCA	HOM	ITGAE	Splicing
17	36600147	-	AGCTGTGGGT CCAGCTGCTG CCAGCCT	HOM	KRTAP9-1	Insertion
17	43470073	-	G	HOM	COPZ2	Frameshift
17	43470084	-	GG	HOM	COPZ2	Frameshift
19	411668	C	G	HOM	SHC2	Missense
19	54847198	CGCTCC	-	HOM	SCAF1	Deletion

19	55523964	T	C	HOM	KCNC3	Missense
19	58477893	G	A	HOM	LOC646508	Missense
20	1540047	GGT	-	HOM	SIRPB1	Deletion
20	61794611	G	C	HOM	RTEL1;RTEL1	Missense
20	61799116	T	G	HOM	TNFRSF6B	Splicing
21	33811230	A	C	HOM	GART	Missense
21	37300741	C	T	HOM	DSCR6	Splicing
22	21318945	G	T	HOM	GGTLC2	Missense

Table 11.4.3 Family 3 Filtered Variants

Position	Change	GT	Gene	Effect	DbSNP	Clin Var
10:3208567	T>TGCACGCT AGGGAAGAG AGAGGAA	HOM	PITRM1	Frameshift	rs4266975	
19:56125165	G>GC	HOM	ZNF865	Frameshift		
12:7045891	ACAGCAGCAG CAGCAG>A	HOM	ATN1	Deletion	rs377147612	
13:72440538	T>TGCCGCC	HOM	DACH1	Insertion		
1:154842199	G>GGCTGCTG CTGCTGCTG CT	HOM	KCNN3	Insertion	rs58327065	
19:55494559	G>GCTA	HOM	NLRP2	Insertion		
3:40503520	A>ACTGCTGC TG	HOM	RPL14	Insertion	rs147295890	
9:990963	G>T	HOM	DMRT3	Missense	rs199600890	
19:40321404	A>G	HOM	DYRK1B	Missense	rs34587974	
4:57179403	C>T	HOM	KIAA1211	Missense		
7:42977102	C>T	HOM	MRPL32	Missense		
19:45898883	G>A	HOM	PPP1R13L	Missense		
9:13188785	G>T	HOM	MPDZ	Missense	rs111794040	
19:45864824	G>A	HOM	ERCC2	Missense		
8:38880766	A>G	HOM	ADAM9	Missense		
4:36085023	C>T	HOM	ARAP2	Missense	rs199670262	
20:3660162	G>A	HOM	ADAM33	Missense		
12:11420454	T>C	HOM	PRB3	Splicing	rs11054202	
11:46342259	A>AG	HOM	CREB3L1	Splicing	rs67904785	
10:50534969	A>AACACACA CACACAC	HOM	C10orf71	Splicing	rs66701434	
17:72540796	G>A	HOM	CD300C	Stopgain	rs144695162	

9:108484867	G>A	HOM	TMEM38B	Stopgain		
22:19865895	A>C	HOM	TXNRD2	Stopgain	rs202059967	

Table 11.4.4 Family 4 Filtered Variants

Chr	Position	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
16	20770164	ACSM3	Missense	rs145080272	G	C	HOM	
1	114677968	AMPD1	missense		T	A	HOM	
1	179393979	AXDND1	missense	rs139341288	G	C	HOM	
17	80183913	CARD14	missense		G	A	HOM	
16	19542695	CCP110	missense	rs570527798	A	G	HOM	
1	27382554	CD164L2	missense	rs2474297	C	T	HOM	
1	27382530	CD164L2	missense	rs2504779	C	T	HOM	
19	44513190	CEACAM20	missense	rs1465723	C	T	HOM	
1	114725326	CSDE1	missense		A	T	HOM	
7	142865498	EPHB6	missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	missense		C	T	HOM	
19	39906242	FCGBP	missense		T	C	HOM	
1	159813554	FCRL6	missense	rs148328479	G	A	HOM	
8	143213308	GPIHBP1	missense	rs11538389	T	G	HOM	
7	74797697	GTF2IRD2	missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	missense		G	T	HOM	
16	28989581	LAT	missense	rs138166678	C	T	HOM	
1	150308112	MRPS21	missense	rs10480	T	C	HOM	
1	146069630	NBPF10	missense		G	C	HOM	
1	148126839	NBPF11	missense	rs2064455	C	G	HOM	
1	146974762	NBPF12	missense		C	A	HOM	
1	149071659	NBPF9	missense		C	T	HOM	
1	149071644	NBPF9	missense		T	C	HOM	
1	149063750	NBPF9	missense		C	A	HOM	
16	81141373	PKD1L2	unknown	rs117006360	G	A	HOM	
17	50168409	SGCA	missense	rs35130237	C	A	HOM	Uncertain
20	46350521	SLC35C2	missense	rs143795303	T	C	HOM	
8	144415811	SLC39A4	missense		A	G	HOM	
7	75501512	SPDYE5	missense	rs62477724	G	C	HOM	
19	54074853	TARM1	missense		T	C	HOM	
10	49945053	TIMM23B	missense		G	C	HOM	
21	10592359	TPTE	missense	rs212146	A	G	HOM	
7	142929454	TRPV5	missense		T	C	HOM	
1	173873492	ZBTB37	missense	rs143211330	C	A	HOM	

19	44497294	ZNF180	missense	rs2571108	A	G	HOM	
3	75741362	ZNF717	missense	rs149568659	T	C	HOM	

Table 11.4.5 Family 5: Homozyogus rare variants present in both affected siblings

Position	Change	GT	gene	Effect	DbSNP	Clin Var
5:150513996	C>T	HOM	ANXA6	Missense		
8:145541635	T>C	HOM	DGAT1	Missense	rs146196839	
8:144942804	TG>T	HOM	EPPK1	Frameshift		
9:128025962	ACAGCACTC CATCTGTAGG TATGTCTGT>A	HOM	GAPVD1	Splicing	rs143312600	
2:136598443	A>G	HOM	MCM6	Missense	rs55660827	
10:3208567	T>TGCACGCTA GGGAAGAGAG AGGA	HOM	PITRM1	Frameshift	rs4266975	
8:144688309	C>T	HOM	PYCRL	Missense	rs144848854	
1:169454907	G>A	HOM	SLC19A2	Missense		

Table 11.4.6 Family 6 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
8	66444705	ADHFE1	Frameshift	rs567577128	T	-	HOM	
9	136677540	AGPAT2	Missense	rs563539429	C	T	HOM	
8	67277415	ARFGEF1	Missense		T	C	HOM	
2	9368479	ASAP2	Missense		C	T	HOM	
15	83018162	BTBD1	Missense	rs781192572	T	G	HOM	
2	47090289	C2orf61	Missense	rs541454105	C	T	HOM	
1	207126743	C4BPA	Missense	rs775885891	A	G	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
19	44513190	CEACAM20	Missense	rs1465723	C	T	HOM	
7	94404738	COL1A2	Missense		G	A	HOM	
8	67193537	CSPP1	Missense	rs546683385	T	G	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
1	1243474	FAM132A	Missense	rs202178204	T	C	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
19	39902274	FCGBP	Missense		G	C	HOM	
19	39902287	FCGBP	Missense		A	G	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
19	54158305	LENG1	Missense		T	C	HOM	
12	85098493	LRRIQ1	Missense	rs747103001	T	C	HOM	
4	140482408	MGAT4D	Missense	rs111869533	T	C	HOM	
1	66984736	MIER1	Missense	rs371342631	C	T	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146135471	NBPF10	Missense	rs3926769	T	C	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
9	136472074	SEC16A	Missense	rs374240602	T	C	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
5	35793322	SPEF2	Missense		C	A	HOM	
2	45413238	SRBD1	Missense	rs556291003	C	G	HOM	
9	133360384	SURF2	Missense		G	A	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
5	110667280	TMEM232	Missense	rs554845418	G	C	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
17	81647906	TSPAN10	Frameshift		-	TAAC	HOM	
1	2645487	TTC34	Missense	rs768127990	G	C	HOM	
5	113515301	YTHDC2	Missense	rs185928501	A	G	HOM	

19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	

Table 11.4.7 Family 7 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
15	63716328	HERC1	Missense	rs541583356	C	T	HOM	
12	120304012	SIRT4	Missense		A	G	HOM	
7	36439210	ANLN	Missense	rs199806594	T	C	HOM	
12	121420246	RNF34	Frameshift		AA	-	HOM	
3	75741293	ZNF717	Missense	rs141084845	G	A	HOM	
15	75682381	CSPG4	Missense	rs143855050	C	T	HOM	
1	149071659	NBPF9	Missense		C	T	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
10	79712351	NUTM2B	Missense	rs451438	T	C	HOM	
7	72728896	TYW1B	Stopgain	rs3015858	C	T	HOM	
1	149071644	NBPF9	Missense		T	C	HOM	
1	148972321	PDE4DIP	Missense	rs1628172	C	T	HOM	
13	45516144	COG3	Frameshift		CT	-	HOM	
1	156384556	RHBG	Frameshift	rs71591938	-	C	HOM	
11	124250452	OR8G1	Stopgain	rs4268525	C	G	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
19	44513190	CEACAM20	Missense	rs1465723	C	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39902287	FCGBP	Missense		A	G	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
19	54158305	LENG1	Missense		T	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	149063750	NBPF9	Missense		C	A	HOM	
7	74779322	NCF1	Missense		A	G	HOM	
11	124225570	OR8G2	Missense	rs2512268	C	T	HOM	
11	124225572	OR8G2	Missense	rs2466612	A	G	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
9	133402955	STKLD1	Missense		G	A	HOM	
19	54074853	TARM1	Missense		T	C	HOM	

12	11186063	TAS2R42	Missense		T	C	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
7	72744551	TYW1B	Stopgain		-	A	HOM	
7	72728899	TYW1B	Missense		A	C	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	

Table 11.4.8 Family 8 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
16	20559434	ACSM2B	Missense	rs74479331	A	G	HOM	
8	144379425	ADCK5	Missense	rs533374578	C	A	HOM	
2	201492473	ALS2CR11	Missense	rs184129762	A	G	HOM	
2	197078765	ANKRD44	Missense	rs61752172	T	G	HOM	
2	200605559	AOX1	Missense	rs373426863	G	A	HOM	
12	123744700	ATP6V0A2	Missense	rs532258057	A	G	HOM	VUS
7	99967247	AZGP1	Missense	rs143279151	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
15	42728706	CDAN1	Missense	rs529452785	A	C	HOM	
19	44513190	CEACAM20	Missense	rs1465723	C	T	HOM	
16	21136440	DNAH3	Missense	rs760379497	C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
1	15360494	FHAD1	Missense	rs137894128	G	A	HOM	
11	72195749	FOLR1	Splicing	rs144637717	T	C	HOM	Conflicting
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
11	94104605	HEPHL1	Missense	rs148114445	C	A	HOM	
5	52915467	ITGA1	Missense	rs576217087	A	G	HOM	
19	54158305	LENG1	Missense		T	C	HOM	
1	11045485	MASP2	Missense	rs41307788	C	T	HOM	Conflicting
2	201680936	MPP4	Missense	rs759225136	C	G	HOM	
11	68891350	MRPL21	Missense		A	G	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
11	47338586	MYBPC3	Missense	rs730880563	C	T	HOM	VUS
16	28752539	NPIPB9	Missense	rs750279492	G	A	HOM	

11	124250452	OR8G1	Stopgain	rs4268525	C	G	HOM	
11	124225570	OR8G2	Missense	rs2512268	C	T	HOM	
16	21687618	OTOA	Missense	rs142850013	G	A	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
2	131263430	POTEE	Missense	rs775831296	A	G	HOM	
22	30532806	SEC14L6	Missense	rs556277502	C	G	HOM	
11	63143377	SLC22A24	Splicing	rs1939749	C	T	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
19	54160472	TMC4	Missense		C	G	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
7	72728899	TYW1B	Missense		A	C	HOM	

Table 11.4.9 Family 9 Filtered Variants

Position	Change	GT	Gene	Effect	DbSNP	ClinVar
1:12726036	C>G	HOM	AADACL4	Missense	rs142659885	
10:46321904	C>T	HOM	AGAP4	Missense	rs202104169	
2:219081983	T>TTGGG	HOM	ARPC2	Splicing		
3:182620350	CTT>C	HOM	ATP11B	Frameshift	.	
1:179335264	A>G	HOM	AXDND1	Splicing		
1:179335265	T>G	HOM	AXDND1	Splicing		
9:72000704	A>T	HOM	FAM189A2	Splicing		
7:112724949	G>GA	HOM	GPR85	Splicing		
1:152185790	T>C	HOM	HRNR	Missense	rs76102381	
1:152186178	C>T	HOM	HRNR	Missense	rs4845748	
1:152187562	A>C	HOM	HRNR	Missense	rs61814936	
1:152190720	C>T	HOM	HRNR	Missense	rs7514457	
1:12069698	C>T	HOM	MFN2	Missense	rs119103267	Yes
19:17516586	C>CT	HOM	MVB12A	Splicing		
1:146465262	A>G	HOM	NBPF12	Splicing		
1:148004795	C>G	HOM	NBPF14	Splicing		
10:81603929	C>T	HOM	NUTM2E	Missense		
11:7716918	C>G	HOM	OVCH2	Splicing		
11:7717216	A>C	HOM	OVCH2	Splicing		
2:108479487	A>T	HOM	RGPD4	Missense		
2:108479487	A>T	HOM	RGPD4	Missense	rs199689341	
2:113145814	C>T	HOM	RGPD8	Missense	rs200737546	

6:108243119	GAAAA>G	HOM	SEC63	Splicing		
1:185137464	T>TAA	HOM	SWT1	Splicing		
20:43355998	C>G	HOM	WISP2	Missense	rs142405609	
5:124080872	AG>A	HOM	ZNF608	Splicing		

Table 11.4.10 Family 10 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	Clin Var
3	128908237	ACAD9	Missense	rs549861940	C	T	HOM	
18	46253736	C18orf25	Deletion		CTG	-	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
3	97874168	CRYBG3	Missense	rs764284373	A	G	HOM	
17	37624364	DDX52	Missense		C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39902287	FCGBP	Missense		A	G	HOM	
10	48180858	FRMPD2	Missense	rs61840030	C	T	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
14	31483548	GPR33	Stopgain	rs17097921	G	A	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
8	2101041	MYOM2	Missense		G	A	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
1	149071659	NBPF9	Missense		C	T	HOM	
11	54603136	OR4C46	Missense	rs11246609	T	C	HOM	
11	124225570	OR8G2	Missense	rs2512268	C	T	HOM	
11	124225572	OR8G2	Missense	rs2466612	A	G	HOM	
22	15708029	POTEH	Missense	rs2845206	T	C	HOM	
1	12920302	PRAMEF7	Missense		C	G	HOM	
1	156384556	RHBG	Frameshift	rs71591938	-	C	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	
9	133402955	STKLD1	Missense		G	A	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
12	11186063	TAS2R42	Missense		T	C	HOM	

10	49945053	TIMM23B	Missense		G	C	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
7	72728896	TYW1B	Stopgain	rs3015858	C	T	HOM	
7	72744551	TYW1B	Stopgain		-	A	HOM	
7	72728899	TYW1B	Missense		A	C	HOM	
4	9245168	USP17L17	Missense	rs758453564	G	C	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	

Table 11.4.11 Family 11 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
16	21403569	NPIPB3	Unknown		T	C	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
11	66560624	ACTN3	Stopgain	rs1815739	C	T	HOM	Confli -cting
1	171208951	FMO2	Stopgain	rs6661174	C	T	HOM	
22	26027319	MYO18B	Missense	rs149103381	C	T	HOM	
1	20722874	SH2D5	Missense	rs763650474	G	A	HOM	
1	33725350	CSMD2	Missense	rs61735686	C	G	HOM	
1	20353827	VWA5B1	Missense		T	G	HOM	
1	27879739	THEMIS2	Missense	rs41284294	T	C	HOM	
1	201210246	IGFN1	Missense	rs74891027	G	A	HOM	
4	9211890	USP17L10	Missense	rs753269470	C	T	HOM	
4	9210859	USP17L10	Missense	rs181084641	C	T	HOM	
4	9211468	USP17L10	Missense	rs368919398	A	C	HOM	
1	12725527	AADACL3	Missense	rs7513079	T	G	HOM	
1	12719616	AADACL3	Missense	rs3010877	C	T	HOM	
1	12760937	C1orf158	Missense	rs1132185	C	T	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
9	41996327	CNTNAP3B	Missense	rs62554986	A	T	HOM	
9	41894095	CNTNAP3B	Missense		T	A	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39902558	FCGBP	Missense		A	G	HOM	
19	39902287	FCGBP	Missense		A	G	HOM	
20	35434589	GDF5	Missense	rs224331	C	A	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46550427	GPRIN2	Missense	rs3127679	T	C	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	

7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
7	130733862	KLF14	Missense	rs111400400	G	A	HOM	
7	130733894	KLF14	Missense		G	T	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146066541	NBPF10	Missense		T	G	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141137592	PCDHB5	Missense	rs400562	C	T	HOM	
7	142773417	PRSS2	Missense	rs201787957	G	A	HOM	
14	21523492	SALL2	Missense	rs1263810	G	C	HOM	
7	102285204	SH2B2	Missense	rs803074	C	G	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
19	44477666	ZNF180	Missense	rs1897820	G	C	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
1	36091735	ADPRHL2	Frameshift		-	T	HOM	
16	88533296	ZFPM1	Frameshift		-	CC	HOM	
1	16759590	MST1L	Frameshift	rs200532237	-	C	HOM	
22	36191798	APOL4	Frameshift		CT	-	HOM	
11	72139111	FOLR3	Frameshift		TA	-	HOM	
12	121626866	ORAI1	Splicing		GC CCC	-	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
6	148514458	SASH1	Splicing		-	A	HOM	

Table 11.4.12 Family 12

Chr	Start	Gene	Effect	avsnp144	Ref	Alt	ClinVar
17	69035739	ABCA9	Missense	rs61744902	A	G	
16	20543260	ACSM2B	Missense	rs142632912	T	G	
2	215349546	ATIC	Missense	rs76436141	C	T	
10	50068136	FAM21A	Missense		C	T	
19	39906242	FCGBP	Missense		T	C	
22	37135335	IL2RB	Missense	rs116250343	C	T	
6	17850345	KIF13A	Missense	rs140337156	G	A	
1	146135471	NBPF10	Missense	rs3926769	T	C	
9	137216285	NDOR1	Missense	rs75647712	A	G	

2	151583715	NEB	Missense		G	A	
13	52143915	NEK3	Frameshift		T	-	
11	124250452	OR8G1	Stopgain	rs4268525	C	G	
12	54588434	PPP1R1A	Missense	rs182798718	G	A	
12	11267400	PRB3	Frameshift		-	G	
18	63712604	SERPINB11	Stopgain	rs4940595	G	T	
5	151317022	SLC36A2	Missense	rs61572410	G	T	
9	39358957	SPATA31A1	Missense	rs10125162	C	T	
9	39360952	SPATA31A1	Missense	rs62550833	G	A	
20	63543478	SRMS	Missense	rs116061089	G	A	
9	69248154	TJP2	Missense	rs28556975	T	C	
3	75741293	ZNF717	Missense	rs141084845	G	A	
3	75741362	ZNF717	Missense	rs149568659	T	C	

Table 11.4.13 Family 13 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	Clin Var
10	47503470	AGAP9	Missense	rs200283865	G	A	HOM	
10	47502991	AGAP9	Missense		C	T	HOM	
10	47502343	AGAP9	Missense		T	C	HOM	
10	47502604	AGAP9	Missense		A	G	HOM	
10	47502650	AGAP9	Missense		G	C	HOM	
2	73448098	ALMS1	Deletion		CTC	-	HOM	
10	47474375	ANXA8	Missense		C	A	HOM	
22	36191798	APOL4	Frameshift		CT	-	HOM	
22	38087153	BAIAP2L2	Insertion		-	GGTG TCATG	HOM	
22	38087150	BAIAP2L2	Insertion		-	ATGG GTGTC	HOM	
9	68256920	CBWD3	Splicing		G	C	HOM	
17	36013244	CCL23	Missense		T	C	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
5	176584197	CDHR2	Missense	rs115353627	A	G	HOM	
19	44524136	CEACAM20	Missense	rs7260180	T	C	HOM	
19	41686928	CEACAM7	Missense	rs8102488	T	A	HOM	
9	41996327	CNTNAP3B	Missense	rs62554986	A	T	HOM	
11	57815409	CTNND1	Missense		C	A	HOM	
19	55358535	FAM71E2	Frameshift		-	G	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	

19	39902274	FCGBP	Missense		G	C	HOM	
19	39902287	FCGBP	Missense		A	G	HOM	
19	39902558	FCGBP	Missense		A	G	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
1	171208951	FMO2	Stopgain	rs6661174	C	T	HOM	
11	72139111	FOLR3	Frameshift		TA	-	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
9	136338656	GPSM1	Missense		G	A	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
4	6084967	JAKMIP1	Splicing	rs56874913	-	AA	HOM	
7	130733862	KLF14	Missense	rs111400400	G	A	HOM	
7	130733894	KLF14	Missense		G	T	HOM	
19	7032419	MBD3L5	Missense		G	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	155208991	MTX1	Missense	rs760077	T	A	HOM	
8	2101041	MYOM2	Missense		G	A	HOM	
1	146994509	NBPF12	Missense	rs202167770	C	G	HOM	
10	46027444	NCOA4	Missense	rs10761581	A	C	HOM	
16	21404696	NPIPB3	Unknown		G	A	HOM	
16	21404704	NPIPB3	Unknown		G	C	HOM	
16	21404714	NPIPB3	Unknown		T	G	HOM	
16	21404724	NPIPB3	Unknown		T	C	HOM	
16	22534808	NPIPB5	Missense		T	C	HOM	
12	121626866	ORAI1	Splicing		GCCCC	-	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141180333	PCDHB8	Missense	rs2740583	C	T	HOM	
1	149021033	PDE4DIP	Missense		C	A	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
12	11393837	PRB2	Missense	rs745957159	G	C	HOM	
12	11393206	PRB2	Missense	rs781281667	C	T	HOM	
7	102496501	RASA4B	Missense	rs757444689	T	C	HOM	
10	120355038	RPL21	Missense	rs12781587	T	A	HOM	
14	21523492	SALL2	Missense	rs1263810	G	C	HOM	
6	148514458	SASH1	Splicing	-9	-	A	HOM	
9	132326355	SETX	Frameshift	-9	A	-	HOM	
7	102285204	SH2B2	Missense	rs803074	C	G	HOM	
13	77698132	SLAIN1	frameshift insertion	rs201380414	-	GG	HOM	
2	32171369	SLC30A6	Missense	rs534453447	G	A	HOM	

8	144415811	SLC39A4	Missense	-9	A	G	HOM	
8	144414297	SLC39A4	Missense	-9	C	G	HOM	
5	1213527	SLC6A19	Missense	rs202220597	C	T	HOM	
5	491913	SLC9A3	Missense	rs566685003	C	T	HOM	
9	42187569	SPATA31A6	Missense	rs11537002	C	G	HOM	
9	42186134	SPATA31A6	Missense	rs12552679	T	G	HOM	
7	102348423	SPDYE6	Missense	-9	T	C	HOM	
9	133360384	SURF2	Missense	-9	G	A	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
21	10592359	TPTE	Missense	rs212146	A	G	HOM	
2	32787127	TTC27	Missense	rs552911750	G	A	HOM	
4	9211212	USP17L10	Missense	-9	C	T	HOM	
7	75073650	WBSCR16	Missense	rs6955671	C	T	HOM	
16	88533296	ZFPM1	Frameshift	-9	-	CC	HOM	
16	88533297	ZFPM1	Ins	-9	-	CCC	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
19	44477666	ZNF180	Missense	rs1897820	G	C	HOM	
3	75738575	ZNF717	Missense	rs1962893	G	A	HOM	

Table 11.4.14 Family 14 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	Clin var
7	21773913	DNAH11	Missense	rs566490774	C	A	HOM	
21	32515159	EVA1C	Missense	rs199918768	C	T	HOM	
10	17849701	MRC1	Missense	rs606231248	G	A	HOM	Other
22	40419116	MKL1	Missense	rs200792263	A	C	HOM	
7	143719852	TCAF2	Missense	rs62486260	C	T	HOM	
7	143719852	TCAF2	Missense	rs62486260	C	T	HOM	
7	143719852	TCAF2	Missense	rs62486260	C	T	HOM	
17	21702902	KCNJ18	Missense		G	A	HOM	
21	33353770	IFNAR1	Missense	rs369713150	C	T	HOM	
4	150850786	LRBA	Missense	rs761765555	C	T	HOM	
2	240042532	PRR21	Missense		G	A	HOM	
2	240042488	PRR21	Missense		G	C	HOM	
17	21702905	KCNJ18	Missense		G	A	HOM	
15	101652279	TM2D3	Missense	rs181135440	G	A	HOM	

15	101652279	TM2D3	Missense	rs181135440	G	A	HOM	
2	240042504	PRR21	Missense		G	A	HOM	
9	133071387	CEL	Missense		A	C	HOM	
16	22534154	NPIPB5	Missense	rs28561499	C	T	HOM	
9	77357708	VPS13A	Missense		C	T	HOM	
7	5370382	TNRC18	Missense	rs200279443	C	G	HOM	
2	240042475	PRR21	Missense		C	T	HOM	
18	31134130	DSC1	Missense	rs199512546	C	T	HOM	
6	16327684	ATXN1	Missense	rs11969612	A	C	HOM	
14	105144964	JAG2	Missense	rs767092332	G	A	HOM	
16	22534185	NPIPB5	Missense		C	T	HOM	
14	96237073	BDKRB2	Splicing	rs746820715	G	A	HOM	
1	146126409	NBPF10	Splicing	rs61816394	G	C	HOM	
12	121626866	ORAI1	Splicing		GC CCC	-	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
1	153261531	LOR	Insertion		-	GGC GGC GGC TCT	HOM	
22	29489604	NEFH	Insertion		-	TGA GAA GGC CAA GTC CCC	HOM	
16	22534730	NPIPB5	Insertion		-	TAT	HOM	
1	145872994	ANKRD35	Missense	rs41315701	T	C	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
9	41996327	CNTNAP3B	Missense	rs62554986	A	T	HOM	
10	94842866	CYP2C19	Missense	rs3758581	A	G	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46550013	GPRIN2	Missense	rs3127683	T	C	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
10	46550427	GPRIN2	Missense	rs3127679	T	C	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
17	21702953	KCNJ18	Missense		C	A	HOM	

1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146135471	NBPF10	Missense	rs3926769	T	C	HOM	
1	149071644	NBPF9	Missense		T	C	HOM	
10	46027444	NCOA4	Missense	rs10761581	A	C	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141180333	PCDHB8	Missense	rs2740583	C	T	HOM	
10	120355038	RPL21	Missense	rs12781587	T	A	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
7	75073650	WBSCR16	Missense	rs6955671	C	T	HOM	
19	44386639	ZNF285	Missense	rs12610859	C	T	HOM	
4	131416	ZNF718	Missense		G	C	HOM	
1	171208951	FMO2	Stopgain	rs6661174	C	T	HOM	
7	149818100	SSPO	Stopgain		-	A	HOM	
16	21405078	NPIPB3	Unknown		G	A	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.4.15 Family 15: Homozygous rare variants present in both affected siblings

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
18	13731854	RNMT	Missense	rs769888601	C	G	HOM	
19	54735274	KIR3DL3	Missense		T	C	HOM	
19	23145949	ZNF730	Frameshift	rs529180941	-	T	HOM	
11	54603136	OR4C46	Missense	rs11246609	T	C	HOM	
12	11186063	TAS2R42	Missense		T	C	HOM	
19	15682425	CYP4F12	Missense	rs2285888	C	T	HOM	
19	54158305	LENG1	Missense		T	C	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
22	42141208	CYP2D6	Unknown		C	T	HOM	
22	42141217	CYP2D6	Unknown		C	T	HOM	
22	42141261	CYP2D6	Unknown		C	T	HOM	
22	42141587	CYP2D6	Unknown		G	A	HOM	
22	42141231	CYP2D6	Unknown		T	C	HOM	
22	42127941	CYP2D6	Missense	rs16947	G	A	HOM	Drug Response
7	74798013	GTF2IRD2	Missense		G	A	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	

Table 11.4.16 Family 16 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
16	21405078	NPIPB3	Unknown		G	A	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
20	35176805	PROCR	Stopgain	rs541848028	C	T	HOM	
1	171208951	FMO2	Stopgain	rs6661174	C	T	HOM	
8	66457095	ADHFE1	Missense	rs376912450	C	T	HOM	
8	68446397	C8orf34	Missense	rs533895950	A	G	HOM	
19	40617195	LTBP4	Missense	rs541226628	G	A	HOM	
19	45698873	QPCTL	Missense	rs145016874	C	T	HOM	
19	36819278	ZNF790	Missense		G	A	HOM	
19	38305437	YIF1B	Missense		T	C	HOM	
10	68827772	STOX1	Missense		A	C	HOM	
8	28716532	EXTL3	Missense	rs573052861	G	A	HOM	
20	31908595	TTLL9	Missense	rs184016363	G	A	HOM	
19	7032419	MBD3L5	Missense		G	C	HOM	
8	65622312	ARMC1	Missense	rs771008579	T	A	HOM	
9	137199456	TPRN	Missense	rs149753507	G	A	HOM	
16	22534292	NPIPB5	Missense		C	G	HOM	
1	201210394	IGFN1	Missense	rs202092854	C	A	HOM	
1	201210502	IGFN1	Missense		C	A	HOM	
1	201210546	IGFN1	Missense		A	G	HOM	
1	201210525	IGFN1	Missense	rs202174330	A	G	HOM	
1	201210477	IGFN1	Missense		A	G	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
19	44477666	ZNF180	Missense	rs1897820	G	C	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
9	41996327	CNTNAP3B	Missense	rs62554986	A	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
10	46550427	GPRIN2	Missense	rs3127679	T	C	HOM	
10	46550040	GPRIN2	Missense	rs11204659	C	A	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	

1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146069569	NBPF10	Missense		C	T	HOM	
1	149063750	NBPF9	Missense		C	A	HOM	
5	141184136	PCDHB16	Missense		C	A	HOM	
5	141184133	PCDHB16	Missense	rs17844651	A	G	HOM	
5	141184153	PCDHB16	Missense	rs2697532	G	A	HOM	
5	141184188	PCDHB16	Missense		A	C	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141180333	PCDHB8	Missense	rs2740583	C	T	HOM	
5	141188034	PCDHB9	Missense	rs11167743	T	C	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
7	102348423	SPDYE6	Missense		T	C	HOM	
9	133360384	SURF2	Missense		G	A	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
7	75073650	WBSCR16	Missense	rs6955671	C	T	HOM	
9	66919319	ZNF658	Missense		T	C	HOM	
9	66919305	ZNF658	Missense		G	A	HOM	
9	66919316	ZNF658	Missense		A	G	HOM	
9	66919310	ZNF658	Missense		T	A	HOM	
9	66919296	ZNF658	Missense		A	G	HOM	
19	4511713	PLIN4	Frameshift	rs747124466	T	-	HOM	
12	121626866	ORAI1	Splicing		GCCCC	-	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
6	148514458	SASH1	Splicing		-	A	HOM	

Table 11.4.17 Family 17 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	ClinVar
17	36013244	CCL23	Missense		T	C	
1	27382554	CD164L2	Missense	rs2474297	C	T	
7	142865498	EPHB6	Missense	rs8177146	G	T	
8	144355665	FBXL6	Missense		C	T	
19	39906242	FCGBP	Missense		T	C	
17	74866471	FDXR	Missense	rs690514	T	C	
17	74866908	FDXR	Missense	rs1688149	C	T	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	
2	171976274	HAT1	Missense	rs73976541	T	C	
10	17849701	MRC1	Missense	rs606231248	G	A	
1	150308112	MRPS21	Missense	rs10480	T	C	

8	2101041	MYOM2	Missense		G	A	
1	12920302	PRAMEF7	Missense		C	G	
18	63712604	SERPINB11	Stopgain	rs4940595	G	T	
8	144414297	SLC39A4	Missense		C	G	
9	133402955	STKLD1	Missense		G	A	
19	54074853	TARM1	Missense		T	C	
10	49945053	TIMM23B	Missense		G	C	

Table 11.4.18 Family 18 Filtered Variants

Chr	Start	Gene	Effect	avsnp144	Ref	Alt	GT	ClinVar
13	77698132	SLAIN1	Frameshift	rs201380414	-	GG	HOM	
1	248638310	OR2T35	Missense	rs143981271	C	T	HOM	
1	248638309	OR2T35	Missense	rs150878651	G	A	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
10	48180858	FRMPD2	Missense	rs61840030	C	T	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
1	146069630	NBPF10	Missense		G	C	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
11	124225570	OR8G2	Missense	rs2512268	C	T	HOM	
11	124225572	OR8G2	Missense	rs2466612	A	G	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
12	11186063	TAS2R42	Missense		T	C	HOM	
19	54173068	TMC4	Missense		T	C	HOM	

Table 11.4.19 Family 19 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
19	40667979	NUMBL	Deletion	rs758624756	TGC TGC TGC TGC TGT	-	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
11	124225179	OR8G2	Missense	rs2466614	G	A	HOM	
11	124225570	OR8G2	Missense	rs2512268	C	T	HOM	
11	124225572	OR8G2	Missense	rs2466612	A	G	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
1	205918866	SLC26A9	Missense	rs3811428	C	T	HOM	
19	54173068	TMC4	Missense		T	C	HOM	
7	142912583	TRPV5	Missense		T	C	HOM	
19	44386639	ZNF285	Missense	rs12610859	C	T	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
3	75738859	ZNF717	Missense	rs151311432	A	C	HOM	
11	124250452	OR8G1	Stopgain	rs4268525	C	G	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.4.20 Family 20 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
8	141494938	MROH5	Splicing	rs6578193	C	T	HOM	
19	57492212	ZNF419	Splicing	rs2074071	G	A	HOM	
7	100773854	ZAN	Frameshift		G	-	HOM	
10	117210250	KCNK18	Frameshift	rs765485428	T	-	HOM	
12	76031158	PHLDA1	Deletion	rs755808437	TGC TGC	-	CHET	
12	76031161	PHLDA1	Deletion		TGC	-	CHET	
5	1221252	SLC6A19	Deletion	rs760474536	TCT	-	HOM	
14	77027442	IRF2BPL	Insertion	rs778015822	-	TGT	HOM	
10	99831784	ABCC2	Missense	rs144521346	G	C	HOM	
10	93404215	MYOF	Missense	rs778168720	A	G	HOM	
3	49125008	LAMB2	Missense	rs764009381	G	A	HOM	
2	37260260	PRKD3	Missense	rs200951892	C	T	HOM	
4	185415857	UFSP2	Missense	rs142500730	A	T	HOM	
3	49115274	USP19	Missense	rs150605229	G	A	HOM	

18	57706561	ATP8B1	Missense	rs34719006	C	T	HOM	Path VUS
3	49664477	BSN	Missense	rs149315260	C	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
21	42770791	PDE9A	Missense	rs75225742	T	G	HOM	
19	58128917	ZNF329	Missense	rs34681367	T	C	HOM	
19	56191870	ZSCAN5B	Missense	rs757976812	C	T	HOM	
5	140552044	SRA1	Missense	rs202193903	C	G	HOM	
15	88856792	ACAN	Missense	rs12899191	A	G	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
1	12725527	AADACL3	Missense	rs7513079	T	G	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
10	50129923	FAM21A	Missense		C	A	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
9	65737984	FOXD4L4	Missense	rs10796795	G	C	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
8	2101041	MYOM2	Missense		G	A	HOM	
1	149082016	NBPF9	Missense		A	T	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
9	133360384	SURF2	Missense		G	A	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
7	142912583	TRPV5	Missense		T	C	HOM	
7	100773851	ZAN	Missense	rs78193191	A	G	HOM	
19	44477666	ZNF180	Missense	rs1897820	G	C	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
3	75738859	ZNF717	Missense	rs151311432	A	C	HOM	
6	132538470	TAAR9	Stopgain	rs2842899	A	T	HOM	
10	116624043	PNLIPRP2	Unknown	-9	G	A	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.4.21 Family 21 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
3	64550972	ADAMTS9	Missense	rs80311637	C	T	HOM	
10	47503470	AGAP9	Missense	rs200283865	G	A	HOM	
10	47502781	AGAP9	Missense	-9	T	C	HOM	
10	47502343	AGAP9	Missense	-9	T	C	HOM	
10	47502604	AGAP9	Missense	-9	A	G	HOM	
10	47502991	AGAP9	Missense	-9	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
19	44513190	CEACAM20	Missense	rs1465723	C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense	-9	C	T	HOM	
19	39906242	FCGBP	Missense	-9	T	C	HOM	
19	39902287	FCGBP	Missense	-9	A	G	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
7	74797024	GTF2IRD2	Missense	-9	G	T	HOM	
7	74798013	GTF2IRD2	Missense	-9	G	A	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
16	21647413	IGSF6	Missense	rs189739425	T	C	HOM	
19	54847989	KIR2DS2	Missense	-9	G	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146140007	NBPF10	Missense	-9	C	T	HOM	
1	146135471	NBPF10	Missense	rs3926769	T	C	HOM	
1	146974762	NBPF12	Missense	-9	C	A	HOM	
16	14872311	NOMO1	Missense	-9	A	C	HOM	
1	248638309	OR2T35	Missense	rs150878651	G	A	HOM	
1	248638310	OR2T35	Missense	rs143981271	C	T	HOM	
11	54603136	OR4C46	Missense	rs11246609	T	C	HOM	
2	200920259	ORC2	Missense	rs541263745	G	A	HOM	
12	81375821	PPFIA2	Missense	-9	G	C	HOM	
12	27672474	PPFIBP1	Missense	rs76499984	A	G	HOM	
8	144414297	SLC39A4	Missense	-9	C	G	HOM	
8	144415811	SLC39A4	Missense	-9	A	G	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	
19	54074853	TARM1	Missense	-9	T	C	HOM	
12	11186063	TAS2R42	Missense	-9	T	C	HOM	
10	49945053	TIMM23B	Missense	-9	G	C	HOM	

19	54173068	TMC4	Missense	-9	T	C	HOM	
19	54160472	TMC4	Missense	-9	C	G	HOM	
5	14270866	TRIO	Missense	rs146453151	C	T	HOM	
12	121960614	WDR66	Missense	rs149064276	G	A	HOM	
19	57492212	ZNF419	Splicing	rs2074071	G	A	HOM	

Table 11.4.22 Family 22 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
2	55180508	CLHC1	Splicing	rs114931154	A	T	HOM	
18	46253736	C18orf25	Deletion		CTG	-	HOM	
8	24489230	ADAM7	Missense	rs200420185	C	T	HOM	
17	36013244	CCL23	Missense		T	C	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
14	102964517	CDC42BPB	Missense	rs200090522	T	C	HOM	
19	44513190	CEACAM20	Missense	rs1465723	C	T	HOM	
10	68432283	DNA2	Missense	rs201999986	C	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
10	48180858	FRMPD2	Missense	rs61840030	C	T	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
3	160268535	IFT80	Missense	rs137853116	C	G	HOM	Path
1	14960756	KAZN	Missense	rs763136249	C	T	HOM	
10	17849701	MRC1	Missense	rs606231248	G	A	HOM	Other
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	19256051	MRTO4	Missense	rs138723852	G	A	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
1	149063750	NBPF9	Missense		C	A	HOM	
19	3453859	NFIC	Missense	rs201510249	C	T	HOM	
4	2941692	NOP14	Missense	rs61740573	G	A	HOM	
2	43697264	PLEKHH2	Missense	rs200313721	G	A	HOM	
8	103998243	RIMS2	Missense	rs182266368	G	A	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
9	39360952	SPATA31A1	Missense	rs62550833	G	A	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	

19	54074853	TARM1	Missense		T	C	HOM	
12	11186063	TAS2R42	Missense		T	C	HOM	
19	54173068	TMC4	Missense		T	C	HOM	
19	54160472	TMC4	Missense		C	G	HOM	
1	25343040	TMEM50A	Missense	rs3093647	C	T	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.4.23 Family 23 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
11	66560624	ACTN3	Stopgain	rs1815739	C	T	HOM	Conflict
2	62864775	EHBP1	Missense	rs140051312	T	C	HOM	
11	62607764	EML3	Missense	rs751526266	C	T	HOM	
13	37008146	EXOSC8	Missense		C	A	HOM	
13	32247435	FRY	Missense	rs533637252	G	A	HOM	
4	48550633	FRYL	Missense	rs764716269	C	T	HOM	
3	112125243	GCSAM	Frameshift	rs747627067	A	-	HOM	
4	44691670	GUF1	Missense		G	A	HOM	
10	30626121	LYZL2	Stopgain	rs568515405	G	T	HOM	
1	146135471	NBPF10	Missense	rs3926769	T	C	HOM	
12	6514287	NCAPD2	Missense	rs201733141	C	T	HOM	
16	4463755	NMRAL1	Missense	rs375960349	C	T	HOM	
10	79711875	NUTM2B	Missense	rs61863495	T	C	HOM	
10	79712351	NUTM2B	Missense	rs451438	T	C	HOM	
11	124250452	OR8G1	Stopgain	rs4268525	C	G	HOM	
1	52384560	ORC1	Missense	rs547441862	T	C	HOM	
11	45913855	PEX16	Missense		T	G	HOM	
19	15472096	PGLYRP2	Missense		G	C	HOM	
12	11267400	PRB3	Frameshift		-	G	HOM	
1	156384556	RHBG	Frameshift	rs71591938	-	C	HOM	
19	16862452	SIN3B	Missense	rs564988933	C	T	HOM	
8	133040076	SLA	Missense	rs200019421	G	A	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
2	61204353	USP34	Missense	rs188105570	C	T	HOM	

Table 11.4.24 Family 24: Homozygous rare variants present in both affected siblings

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
16	81141373	PKD1L2	Missense	rs117006360	G	A	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
9	39360952	SPATA31A1	Missense	rs62550833	G	A	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	

Table 11.4.25 Family 25: Homozyogus rare variants present in both affected siblings

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
19	39906242	FCGBP	Missense		T	C	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	

Table 11.4.26 Family 26 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
18	46253736	C18orf25	Deletion		CTG	-	HOM	
1	12719616	AADACL3	Missense	rs3010877	C	T	HOM	
10	47502343	AGAP9	Missense		T	C	HOM	
14	34773641	BAZ1A	Missense		A	C	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
12	96740035	CFAP54	Missense	rs115819507	G	A	HOM	
12	96743779	CFAP54	Missense	rs111287403	C	T	HOM	
1	16049817	CLCNKB	Missense	rs148870670	G	T	HOM	
6	43047017	CUL7	Missense	rs564703357	C	T	HOM	
15	22882981	CYFIP1	Missense	rs139635799	C	T	HOM	
15	22903836	CYFIP1	Missense		C	T	HOM	
17	37624364	DDX52	Missense		C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
5	95428662	FAM81B	Missense	rs1541797	G	A	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	39899634	FCGBP	Missense		C	T	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
19	39899636	FCGBP	Missense		A	C	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
13	38850041	FREM2	Missense		C	A	HOM	
5	177435064	GRK6	Missense	rs143935970	G	A	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
8	2101041	MYOM2	Missense		G	A	HOM	
1	146135471	NBPF10	Missense	rs3926769	T	C	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
2	24758086	NCOA1	Missense	rs150066931	A	G	HOM	
9	137214866	NDOR1	Missense	rs144580113	A	T	HOM	
12	112967487	OAS3	Missense	rs539747098	C	T	HOM	
1	248638310	OR2T35	Missense	rs143981271	C	T	HOM	
1	248638309	OR2T35	Missense	rs150878651	G	A	HOM	
3	16286445	OXNAD1	Missense		A	G	HOM	
6	43520353	POLR1C	Missense	rs776965617	A	C	HOM	
10	97381435	RRP12	Missense	rs139484717	C	T	HOM	
10	97400324	RRP12	Missense	rs201596788	T	G	HOM	

6	35955504	SLC26A8	Missense	rs566366898	C	T	HOM	
15	45486716	SLC30A4	Missense	rs201823030	T	C	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
9	133402955	STKLD1	Missense		G	A	HOM	
1	24383997	STPG1	Missense	rs200647962	T	C	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
1	27334251	TMEM222	Missense	rs150291870	T	G	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
9	132400231	TTF1	Missense	rs148256256	A	C	HOM	
1	12368532	VPS13D	Missense		A	G	HOM	
3	75741362	ZNF717	Missense	rs149568659	T	C	HOM	
18	63712604	SERPINB11	Stopgain	rs4940595	G	T	HOM	
6	44282544	TCTE1	Stopgain	rs138414421	G	A	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.4.27 Family 27 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
8	141494938	MROH5	Splicing	rs6578193	C	T	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
6	148514458	SASH1	Splicing		-	A	HOM	
11	72139111	FOLR3	Frameshift		TA	-	HOM	
16	88533296	ZFPM1	Frameshift		-	CC	HOM	
1	16759590	MST1L	Frameshift	rs200532237	-	C	HOM	
9	136508276	NOTCH1	Missense		C	T	HOM	
19	7032419	MBD3L5	Missense		G	C	HOM	
8	144138810	HGH1	Missense		G	C	HOM	
10	45825953	AGAP4	Missense		C	T	HOM	
3	75737230	ZNF717	Missense	rs141124538	T	C	HOM	
4	9226202	USP17L13	Missense		G	A	HOM	
1	149584102	PPIAL4C	Missense		C	T	HOM	
8	144379425	ADCK5	Missense	rs533374578	C	A	HOM	
1	145872994	ANKRD35	Missense	rs41315701	T	C	HOM	
1	145873487	ANKRD35	Missense	rs6670984	G	A	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
9	41996327	CNTNAP3B	Missense	rs62554986	A	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	

17	74866471	FDXR	Missense	rs690514	T	C	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46550427	GPRIN2	Missense	rs3127679	T	C	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
1	146135471	NBPF10	Missense	rs3926769	T	C	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
1	149071644	NBPF9	Missense		T	C	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141180333	PCDHB8	Missense	rs2740583	C	T	HOM	
10	120355038	RPL21	Missense	rs12781587	T	A	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
4	131416	ZNF718	Missense		G	C	HOM	
19	21808727	ZNF43	Stopgain		C	T	HOM	
1	171208951	FMO2	Stopgain	rs6661174	C	T	HOM	

Table 11.4.28 Family 28 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	Clin Var
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
16	21404635	NPIPB3	Unknown		T	A	HOM	
16	21404639	NPIPB3	Unknown		G	A	HOM	
16	21404219	NPIPB3	Unknown		C	A	HOM	
16	21404629	NPIPB3	Unknown		G	T	HOM	
16	21404630	NPIPB3	Unknown		T	G	HOM	
16	21405126	NPIPB3	Unknown		C	T	HOM	
5	177307847	MXD3	Stopgain	rs750671130	C	A	HOM	
9	65738362	FOXD4L4	Stopgain		G	A	HOM	
8	104491058	LRP12	Missense	rs374702794	C	T	HOM	
12	21642075	LDHB	Missense	rs532712842	G	A	HOM	
9	68357302	PGM5	Missense		G	T	HOM	
12	50731453	DIP2B	Missense		G	C	HOM	
1	200603909	KIF14	Missense	rs373895990	C	T	HOM	

16	3767765	CREBBP	Missense	rs555109138	C	A	HOM	
16	2090912	PKD1	Missense	-9	G	A	HOM	
1	200663972	DDX59	Missense	rs558924701	C	T	HOM	
10	113577182	HABP2	Missense	rs78201625	C	T	HOM	
10	128116523	MKI67	Missense	rs41306644	C	T	HOM	
5	179113943	ADAMTS2	Missense	rs756227371	T	C	HOM	
1	205918866	SLC26A9	Missense	rs3811428	C	T	HOM	
19	21808757	ZNF43	Missense		T	G	HOM	
4	139719526	MAML3	Missense	rs762492535	T	G	HOM	
1	207584841	CR1	Missense	rs574578676	G	A	HOM	
15	74821119	LMAN1L	Missense	rs138585415	C	T	HOM	
12	42097591	GXYLT1	Missense	rs770582666	G	C	HOM	
4	9243880	USP17L17	Missense	rs758355781	T	G	HOM	
2	240042136	PRR21	Missense		G	A	HOM	
10	79706060	NUTM2B	Missense	rs199845914	C	T	HOM	
3	75741293	ZNF717	Missense	rs141084845	G	A	HOM	
10	120355038	RPL21	Missense	rs12781587	T	A	HOM	
4	131416	ZNF718	Missense		G	C	HOM	
19	44386639	ZNF285	Missense	rs12610859	C	T	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
7	100773851	ZAN	Missense	rs78193191	A	G	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
10	46584599	SYT15	Missense	rs3127785	G	C	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
8	144415811	SLC39A4	Missense		A	G	HOM	
1	120890035	PPIAL4B	Missense		G	A	HOM	
5	141188034	PCDHB9	Missense	rs11167743	T	C	HOM	
5	141180333	PCDHB8	Missense	rs2740583	C	T	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141184133	PCDHB16	Missense	rs17844651	A	G	HOM	
5	141184136	PCDHB16	Missense		C	A	HOM	
5	141184153	PCDHB16	Missense	rs2697532	G	A	HOM	
10	46027444	NCOA4	Missense	rs10761581	A	C	HOM	
1	149063750	NBPF9	Missense		C	A	HOM	
1	149071644	NBPF9	Missense		T	C	HOM	
1	146074378	NBPF10	Missense		T	G	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
7	130733862	KLF14	Missense	rs111400400	G	A	HOM	
7	130733894	KLF14	Missense		G	T	HOM	
8	142664633	JRK	Missense	rs2976399	T	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	

7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
20	35434589	GDF5	Missense	rs224331	C	A	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
17	79731150	ENPP7	Missense	rs8074547	C	T	HOM	
9	41996327	CNTNAP3B	Missense	rs62554986	A	T	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
17	36013244	CCL23	Missense		T	C	HOM	
1	206116320	AVPR1B	Missense	rs33990840	C	G	HOM	
1	145872994	ANKRD35	Missense	rs41315701	T	C	HOM	
22	23622099	DRICH1	Deletion		CAT	-	HOM	
6	89867988	CASP8AP2	Deletion	rs781114556	GAC ATC TTT GCC CAG	-	HOM	
1	196994128	CFHR5	Frameshift	rs565457964	-	A	HOM	
7	2513251	LFNG	Frameshift		-	GATG	HOM	
19	4511713	PLIN4	Frameshift	rs747124466	T	-	HOM	
7	100773854	ZAN	Frameshift		G	-	HOM	
1	202567832	PPP1R12B	Splicing		-	T	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
12	121626866	ORAI1	Splicing		GCC CC	-	HOM	
9	65682859	CBWD5	Splicing		T	C	HOM	
13	30713842	ALOX5AP	Splicing		-	TG	HOM	

Table 11.4.29 Family 29 Filtered Variants

Chr	Start	Gene	Effect	avsnp144	Ref	Alt	GT	ClinVar
1	175098515	TNN	Missense	rs756343208	C	T	HOM	
1	27107657	SLC9A1	Missense		G	A	HOM	
11	16341047	SOX6	Missense	rs142511858	C	T	HOM	
14	91974664	TRIP11	Missense	rs141259390	G	A	HOM	
1	51322136	TTC39A	Missense	rs765707131	G	T	HOM	

1	119948561	NOTCH2	Missense		G	A	HOM	
1	53461589	DMRTB1	Missense	rs138758029	C	T	HOM	
17	36013244	CCL23	Missense		T	C	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
1	27382530	CD164L2	Missense	rs2504779	C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
7	74798013	GTF2IRD2	Missense		G	A	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
7	74797024	GTF2IRD2	Missense		G	T	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
1	150308112	MRPS21	Missense	rs10480	T	C	HOM	
8	2101041	MYOM2	Missense		G	A	HOM	
19	11416089	RGL3	Missense	rs167479	T	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	
19	54173068	TMC4	Missense		T	C	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
19	44497294	ZNF180	Missense	rs2571108	A	G	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.3.30 Family 30: Heterozygous rare variants present in both parents

Chr	Start	Gene	Effect	avsnp144	Ref	Alt	GT	ClinVar
13	42313061	AKAP11	Missense	rs778705489	G	A	HET	
11	77093730	CAPN5	Missense		G	A	HET	
16	89649443	CHMP1A	Missense	rs185258649	G	A	HET	
19	1037719	CNN2	Missense	rs199741851	G	T	HET	
19	1037716	CNN2	Missense	rs200177867	T	C	HET	
19	1037671	CNN2	Missense	rs199840457	A	G	HET	
19	1037680	CNN2	Missense	rs200505828	C	T	HET	
11	122851780	CRTAM	Missense		G	A	HET	
4	168471824	DDX60L	Missense	rs755913066	G	A	HET	
6	37021533	FGD2	Stopgain		C	T	HET	
5	154898477	GEMIN5	Missense	rs35899504	C	T	HET	
12	42106012	GXYLT1	Stopgain	rs77688235	G	A	HET	
12	42106000	GXYLT1	Missense	rs78540738	C	A	HET	

12	42106035	GXYLT1	Missense	rs79888973	A	G	HET	
12	42106023	GXYLT1	Missense	rs76034661	A	C	HET	
12	42106015	GXYLT1	Missense		A	T	HET	
11	47625713	MTCH2	Missense	rs76666113	A	G	HET	
11	47622719	MTCH2	Stopgain		C	T	HET	
11	47638743	MTCH2	Missense		A	G	HET	
11	47625686	MTCH2	Missense	rs78071782	A	G	HET	
11	47638749	MTCH2	Stopgain		T	A	HET	
8	100712378	PABPC1	Missense	rs202060459	G	A	HET	
8	100712713	PABPC1	Missense		C	T	HET	
8	100709214	PABPC1	Missense	rs766099049	G	A	HET	
8	100712696	PABPC1	Missense		G	A	HET	
8	100712669	PABPC1	Missense		T	C	HET	
8	100709464	PABPC1	Missense		G	A	HET	
6	138930042	REPS1	Missense	rs373049732	G	A	HET	
4	102905528	SLC9B1	Stopgain	rs77618489	T	A	HET	
4	102905612	SLC9B1	Stopgain	rs200075071	G	A	HET	
10	86960006	SNCG	Missense	rs781623510	G	A	HET	
7	67083465	TYW1	Stopgain		G	A	HET	
7	67067402	TYW1	Missense		C	T	HET	
7	93358405	VPS50	Missense		C	T	HET	
7	73834973	WBSCR27	Missense		T	G	HET	

Table 11.4.31 Family 31: Pathogenic and Likely Pathogenic Variants on ClinVar

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
6	26092913	HFE	Missense	rs1800562	G	A	HET	Conflicting
1	15445717	CTRC	Missense	rs121909293	C	T	HET	Conflicting
6	18130687	TPMT	Missense	rs1142345	T	C	HET	Pathogenic
1	114693436	AMPD1	Stopgain	rs17602729	G	A	HET	Pathogenic
6	18138997	TPMT	Missense	rs1800460	C	T	HET	Pathogenic
11	66560624	ACTN3	Stopgain	rs1815739	C	T	HET	Conflicting
7	101128436	SERPINE1	Missense	rs6092	G	A	HET	Pathogenic
11	68794860	CPT1A	Missense	rs2229738	C	T	HET	Conflicting
5	1294051	TERT	Missense	rs61748181	C	T	HET	Conflicting
1	155236376	GBA	Missense	rs2230288	C	T	HET	Conflicting
14	94380925	SERPINA1	Missense	rs17580	T	A	HET	Other, Pathogenic
8	142912850	CYP11B2	Missense	rs61757294	A	G	HET	Pathogenic
15	82538982	RPS17	Synonymous	rs6991	A	G	HET	Conflicting

Table 11.4.32 Family 32: Pathogenic and Likely Pathogenic Variants on ClinVar

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
1	152312601	FLG	Frameshift	rs558269137	ACTG	-	HET	Pathogenic |VUS
5	178211964	PHYKPL	Missense	rs142181517	T	A	HET	Pathogenic
1	15445717	CTRC	Missense	rs121909293	C	T	HET	Other |Pathogenic
13	50945445	RNASEH2B	Missense	rs75184679	G	A	HET	Pathogenic
3	129528906	RHO	Missense	rs28933394	C	T	HET	Pathogenic
1	46189457	POMGNT1	Splicing	rs386834024	C	A	HET	Likely pathogenic
18	59480228	CCBE1	Missense	rs121908250	A	T	HET	Pathogenic
13	48411859	LPAR6	Missense	rs121434309	C	T	HET	Pathogenic
11	64751627	PYGM	Frameshift	rs769960481	A	-	HET	Likely pathogenic
1	97883329	DPYD	Missense	rs1801265	A	G	HET	Pathogenic

Table 11.4.33 Family 33: Pathogenic and Likely Pathogenic Variants on ClinVar

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
12	52571381	KRT74	Missense	rs147962513	A	G	HET	Pathogenic
10	97584837	HOGA1	Missense	rs764396564	C	T	HET	Pathogenic
19	7125507	INSR	Missense	rs1799816	C	T	HET	Pathogenic /other
3	15645186	BTD	Missense	rs13078881	G	C	HET	Pathogenic
7	87452957	ABCB4	Missense	rs58238559	T	C	HET	Pathogenic
17	12996585	ELAC2	Missense	rs5030739	C	T	HET	Pathogenic
17	7224973	ACADVL	Missense	rs148584617	G	A	HET	Other/ Likely pathogenic
12	6333477	TNFRSF1A	Missense	rs4149584	C	T	HET	Conflicting
1	197090994	ASPM	Synonymous	rs143931757	A	G	HET	Pathogenic /VUS
9	6589230	GLDC	Synonymous	rs121964976	C	T	HET	Pathogenic
2	166277030	SCN9A	Missense	rs12478318	T	G	HET	Pathogenic / other

Table 11.4.34 Family 34 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
17	7513775	POLR2A	Splicing		CA	-	HOM	
1	158766425	OR6N1	Frameshift		G	-	HOM	
22	36191798	APOL4	Frameshift		CT	-	HOM	
3	58205500	DNASE1L3	Frameshift	rs751206379	TG	-	HOM	
11	72139111	FOLR3	Frameshift		TA	-	HOM	
7	100773854	ZAN	Frameshift		G	-	HOM	
4	78911414	BMP2K	Missense		T	C	HOM	
16	30583200	ZNF785	Missense	rs35215913	A	C	HOM	
8	144379425	ADCK5	Missense	rs533374578	C	A	HOM	
1	145873487	ANKRD35	Missense	rs6670984	G	A	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
17	74866908	FDXR	Missense	rs1688149	C	T	HOM	
17	74866471	FDXR	Missense	rs690514	T	C	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
7	74797697	GTF2IRD2	Missense	rs707394	G	C	HOM	
2	171976274	HAT1	Missense	rs73976541	T	C	HOM	
8	142664633	JRK	Missense	rs2976399	T	C	HOM	
17	38297242	MRPL45	Missense	rs11559007	C	T	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
5	141137592	PCDHB5	Missense	rs400562	C	T	HOM	
14	21523492	SALL2	Missense	rs1263810	G	C	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
19	54074853	TARM1	Missense		T	C	HOM	
12	11186144	TAS2R42	Missense		C	T	HOM	
12	11186063	TAS2R42	Missense		T	C	HOM	
10	49945053	TIMM23B	Missense		G	C	HOM	
7	100773851	ZAN	Missense	rs78193191	A	G	HOM	
10	47368615	ZNF488	Missense		G	A	HOM	
10	47368085	ZNF488	Missense	rs3814160	G	A	HOM	
1	171208951	FMO2	Stopgain	rs6661174	C	T	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	

Table 11.4.35 Family 35 Filtered Variants

Chr	Start	Gene	Effect	DbSNP	Ref	Alt	GT	ClinVar
17	7426367	C17orf74	Deletion	rs751636242	CGC CGC CGC	-	HOM	
1	27382554	CD164L2	Missense	rs2474297	C	T	HOM	
4	2449967	CFAP99	Missense	rs3108494	C	T	HOM	
7	142865498	EPHB6	Missense	rs8177146	G	T	HOM	
8	144355665	FBXL6	Missense		C	T	HOM	
19	7689276	FCER2	Missense	rs145322667	C	T	HOM	
19	39906242	FCGBP	Missense		T	C	HOM	
19	39883386	FCGBP	Missense		C	T	HOM	
8	143213308	GPIHBP1	Missense	rs11538389	T	G	HOM	
10	46550427	GPRIN2	Missense	rs3127679	T	C	HOM	
10	46549613	GPRIN2	Missense	rs3127822	A	G	HOM	
9	5164001	INSL6	Missense	rs773353870	T	C	HOM	
19	5694425	LONP1	Missense	rs373182816	G	A	HOM	
19	4538112	LRG1	Missense	rs144112190	C	T	HOM	
1	146974762	NBPF12	Missense		C	A	HOM	
10	46027444	NCOA4	Missense	rs10761581	A	C	HOM	
19	54906696	NCR1	Missense		C	A	HOM	
5	141101254	PCDHB3	Missense		C	G	HOM	
16	81141373	PKD1L2	Unknown	rs117006360	G	A	HOM	
17	7513775	POLR2A	Splicing		CA	-	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
8	144414297	SLC39A4	Missense		C	G	HOM	
7	75501512	SPDYE5	Missense	rs62477724	G	C	HOM	
5	140552044	SRA1	Missense	rs202193903	C	G	HOM	
12	11186063	TAS2R42	Missense		T	C	HOM	
12	11186144	TAS2R42	Missense		C	T	HOM	
7	142929454	TRPV5	Missense		T	C	HOM	
7	142912583	TRPV5	Missense		T	C	HOM	
7	100773851	ZAN	Missense	rs78193191	A	G	HOM	
19	44477666	ZNF180	Missense	rs1897820	G	C	HOM	
19	44479350	ZNF180	Missense	rs2253563	G	C	HOM	
19	44386639	ZNF285	Missense	rs12610859	C	T	HOM	

Key:

Alt alternate, CHET compound heterozygous, Conflicting reported as benign and pathogenic on ClinVar, Chr chromosome, Deletion nonframeshift deletion, Frameshift frameshift deletion and/or insertion, GT genotype, HET heterozygous, HOM homozygous, Insertion nonframeshift insertion, Ref reference

11.5. Publications arising from this thesis

Elucidating the genetic architecture of Adams-Oliver syndrome in a large European cohort.

Meester JAN, Sukalo M, Schröder KC, Schanze D, Baynam G, Borck G, Bramswig NC, Duman D, Gilbert-Dussardier B, Holder-Espinasse M, Itin P, Johnson DS, Joss S, Koillinen H, McKenzie F, Morton J, Nelle H, Reardon W, Roll C, Salih MA, Savarirayan R, Scurr I, Splitt M, Thompson E, **Titheradge H**, Travers CP, Van Maldergem L, Whiteford M, Wieczorek D, Vandeweyer G, Trembath R, Van Laer L, Loeys BL, Zenker M, Southgate L, Wuyts W.

Hum Mutat. 2018 Sep;39(9):1246-1261. doi: 10.1002/humu.23567. Epub 2018 Jul 4.

Human biallelic MFN2 mutations induce mitochondrial dysfunction, upper body adipose hyperplasia, and suppression of leptin expression.

Rocha N, Bulger DA, Frontini A, **Titheradge H**, Gribsholt SB, Knox R, Page M, Harris J, Payne F, Adams C, Sleigh A, Crawford J, Gjesing AP, Bork-Jensen J, Pedersen O, Barroso I, Hansen T, Cox H, Reilly M, Rossor A, Brown RJ, Taylor SI, McHale D, Armstrong M, Oral EA, Saudek V, O'Rahilly S, Maher ER, Richelsen B, Savage DB, Semple RK.

Elife. 2017 Apr 19;6. pii: e23813. doi: 10.7554/eLife.23813

Phenotypic Spectrum in Osteogenesis Imperfecta Due to Mutations in TMEM38B: Unraveling a Complex Cellular Defect.

Webb EA, Balasubramanian M, Fratzl-Zelman N, Cabral WA, **Titheradge H**, Alsaedi A, Saraff V, Vogt J, Cole T, Stewart S, Crabtree NJ, Sargent BM, Gamsjaeger S, Paschalis EP, Roschger P, Klaushofer K, Shaw NJ, Marini JC, Högler W.

J Clin Endocrinol Metab. 2017 Jun 1;102(6):2019-2028. doi: 10.1210/jc.2016-3766.

The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity.

Damgaard RB, Walker JA, Marco-Casanova P, Morgan NV, **Titheradge HL**, Elliott PR, McHale D, Maher ER, McKenzie ANJ, Komander D.

Cell. 2016 Aug 25;166(5):1215-1230.e20. doi: 10.1016/j.cell.2016.07.019. Epub 2016 Aug 11.

11.6. Poster presentations arising from this thesis

Homozygous MFN2 mutation causes an interesting lipodystrophy

H Titheradge, H Cox, C Morgan, R Semple, D McHale, J Harris, N Rocha, D Bulger, ER Maher

Manchester Dysmorphology Conference 2016.

Autosomal recessively inherited autoinflammatory disorder (Weber Christian disease) responsive to tumour necrosis factor alpha inhibition.

H Titheradge, NV Morgan, J Ainsworth, ER Maher.

European Society of Human Genetics Conference 2015

A Familial form of Weber Christian disease

H Titheradge, NV Morgan, J Ainsworth, ER Maher.

Manchester Dysmorphology Conference 2014

11.7. Presentations arising from this thesis

The Role of Detailed Clinical Phenotyping in Genomic Medicine.

Plenary Session, Joint Meeting of the UK/Dutch Clinical Genetics Society and Cancer Genetics Group, March 2018

OTULIN-Related Autoinflammatory syndrome (ORAS).

Sheffield Rare Disease Study Day, September 2017

Genomics for Rheumatologists.

West Midlands Regional Rheumatology meeting, June 2017

Multiple Symmetrical Lipomatosis.

Regional Neurology Meeting, October 2015

High Throughput Next Generation Sequencing Analysis and Clinical Genetics.

Regional Paediatric Endocrinology Meeting, October 2015

Genetic approaches to Novel Pathway and Target Discovery in Human Genetic Disease.

Regional Clinical Genetics Meeting, February 2015