RESEARCH ARTICLE

REVISED The diagnostic yield of whole exome sequencing as a first approach in consanguineous Omani renal ciliopathy syndrome patients [version 2; peer review: 2 approved]

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Abstract

Background: Whole exome sequencing (WES) is becoming part of routine clinical and diagnostic practice. In the investigation of inherited cystic kidney disease and renal ciliopathy syndromes, WES has been extensively applied in research studies as well as for diagnostic utility to detect various novel genes and variants. The yield of WES critically depends on the characteristics of the patient population.
Methods: In this study, we selected 8 unrelated Omani children, presenting with renal ciliopathy syndromes with a positive family history and originating from consanguineous families. We performed WES in affected children to determine the genetic cause of disease and to test the yield of this approach, coupled with homozygosity mapping, in this highly selected population.
DNA library construction and WES was carried out using SureSelect Human All Exon V6 Enrichment Kit and Illumina HiSeq platform. For variants filtering and annotation Qiagen Variant Ingenuity tool was used. Nexus copy number software from BioDiscovery was used for evaluation of copy number variants and whole gene deletions. Patient and parental DNA was used to confirm mutations and the segregation of alleles using Sanger sequencing.
Results: Genetic analysis identified 4 potential causative homozygous variants each confirmed by Sanger sequencing in 4 clinically relevant ciliopathy syndrome genes, (*TMEM231*, *TMEM138*, *WDR19* and *BBS9*), leading to an overall diagnostic yield of 50%.
Conclusions: WES coupled with homozygosity mapping provided a diagnostic yield of 50% in this selected population. This genetic

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approach needs to be embedded into clinical practise to allow confirmation of clinical diagnosis, to inform genetic screening as well as family planning decisions. Half of the patients remain without diagnosis highlighting the technical and interpretational hurdles that need to be overcome in the future.

Keywords
renal ciliopathy, cystic kidney disease, Oman, whole exomes sequencing

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REVISED **Amendments from Version 1**

In this new version we have added a new table (Table 3) which details variants that were observed in the 4 unsolved cases of renal ciliopathies in children from Oman. We present a list of variants in cystogenes that may be contributing to the phenotype but are not sufficient enough on their own to verify as solved. This addition data provides a useful discussion of the use of whole exome sequencing as a first line diagnostic approach. We have expanded the discussion to include the points regarding assess variants at RNA level, validating whole exome sequence findings by Sanger sequencing and determining pathogenicity of genomic variants at the transcriptome level.

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Introduction

There are over 750 million people worldwide affected with chronic kidney disease (CKD), a disease burden that is much higher than those living with diabetes, cancer or even AIDS/HIV[1]. Inherited kidney diseases and renal ciliopathy syndromes are one of the major contributors to CKD burden, where up to 10% of adults and over 70% of children reaching end stage kidney disease (ESKD) are expected to harbour genetic causes[2]. Renal ciliopathy syndromes typically lead to cystic kidney disease and include autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease and nephronophthisis, with a growing number of genetic causes implicated. The most common genetic causes of autosomal recessive renal ciliopathies would include *PKHD1*, *NPHP1*, *INVS*, *NPHP3*, *NPHP4*, *IQCB1*, *CEP290* and *TMEM67*[3]. However, studying such rare diseases has considerable challenges mainly due to the small size of patient cohorts negatively affecting progress of treatments and commercial feasibility. Collaborative research and progress of new technologies and methodologies are strategic to overcoming these challenges.

WES is becoming part of routine clinical and diagnostic practice[2]. Focusing only on protein-coding regions through WES decreases the sequencing costs and produces manageable genetic data for interpretation, which enhances its extensive usage in diagnosis leading to the discovery of previously unrecognized renal disease genes and disorders[2,4]. In the case of heterogeneous renal ciliopathies, WES has been extensively applied in research studies as well as for diagnostic utility to detect various novel genes and variants[5,6]. In this study, WES was used to determine the genetic causes of cystic kidney disease and renal ciliopathy syndromes in a group of 8 unrelated Omani children from consanguineous families, carefully selected with regard to clinical phenotype and in whom no genetic testing had previously been performed. As this study shows, the focus of nephrogenetics in Oman is primarily to establish an accurate genetic diagnosis to explain clinical phenotypes using the significantly improved diagnostic power of genomic technologies.

Methods

Ethical approvals and patients' inclusion and clinical evaluation

This study was approved by the North East-Newcastle & North Tyneside 1 Research Ethics Committee (18/NE/350).

Patients were identified and recruited from paediatric referrals for investigation of inherited kidney disease to the nephrology services within the Ministry of Health Hospital, Muscat, Oman between 2015 and 2018. Whole blood (1.5-2.5 ml in EDTA) samples were collected specifically for this study and used for extraction of genomic DNA. DNA samples from affected and other family members were given an anonymised sample number. All patients had clinical features strongly suggestive of an inherited renal ciliopathy. Written and informed consent was obtained from the parents / guardians of each patient, and any family members (including parents and siblings) involved in this study.

Clinical information relating to patient presentation, phenotype and family pedigree structure, with an emphasis on familial kidney disease was obtained, following informed consent for access to the medical records. Family pedigrees were drawn using Invitae© online tool (https://familyhistory.invitae.com).

DNA isolation, library preparation and exome sequencing

gDNA was isolated from whole blood of patients and the available family members using Hamilton's Microlab® STAR™, according to the manufacturer protocol. DNA extraction was performed in the National Genetic Centre in Oman. DNA library construction and WES were outsourced to EuroFins GATC Biotech (Germany) or Novogene Co., Ltd (China). SureSelect Human All Exon V6 Enrichment Kit (Agilent Technologies, CA, USA) and Illumina HiSeq platform (Illumina, San Diego, CA, USA) were used. Analyses of raw data (FASTQ format) were performed including sequence reads mapping to the human reference genome hg19 using BWA (Li and Durbin, 2009), removal of PCR duplicates using Picard (http://broadinstitute.github.io/picard/), alignment refinement using GATK, coverage analysis and SNP and indel calling using GATK's Haplotype Caller (McKenna *et al.*, 2010).

Variant and CNV detection and annotation

SNP and indel VCF files were investigated using Qiagen Variant Ingenuity tool for variants filtration and annotation. Nexus copy number software from BioDiscovery (9.0) was used for CNVs analysis and visualization. To detect regions of homozygosity, WES genotype data were used to create homozygosity mapping using the online homozygosity mapper tool (http://www.homozygositymapper.org/).

Variant validation by Sanger sequencing

Sanger sequencing was utilized to confirm suspected disease-causing variants and their segregation if DNA samples from parents and other family members were available. Primer3

was utilized to design primer sequences (http://primer3.ut.ee/) (Extended Data Table 1[7]). PCR amplification was performed using *Taq* PCR master mix (Qiagen) kit, as per the manufacturer instructions. Sanger sequencing was outsourced to EuroFins GATC Biotech (Germany). The obtained sequences were assembled and aligned compared to a reference sequence using the SequencePilot 4.2.2 software (JSI Medical Systems GmbH).

Results

Patient characteristics

WES was carried out for 8 unrelated paediatric patients with an age range of 3 months to 6 years of age (5 female, 3 male) with a clinical suspicion of a renal ciliopathy syndrome and known consanguinity as demonstrated by pedigree diagrams (Extended data Figure 1[7]). This was a diagnostic-naïve population without prior genetic analysis. Patients had a variety of clinical features, renal and extra-renal, with 5 probands reaching ESKD within 5 years of life (Table 1). Seven out of 8 had a positive family history of kidney disease and 6 had extra-renal manifestations typical of ciliopathy syndromes which included Senior-Løken syndrome, Joubert syndrome, Meckel syndrome and Bardet-Biedl syndrome (Table 1).

Exome sequencing data

Quality control of WES revealed that >99% of the reads were properly mapped to the reference genome. The details of the depth, coverage and target sequences covered are summarized in Extended data Table 2[7]. The average coverage depth was 145.9. Comparable coverage of target coding regions was achieved among the 8 cases with an average of 96.4% of the exome being covered at least 20-fold (Extended data Table 2[7]). Homozygosity mapping of all patients confirmed large regions of homozygosity, typical of known parental consanguinity (Extended data Figure 2[7]).

Molecular genetic findings

A molecular genetic diagnosis was obtained in 4 out of the 8 patients (Figure 1), leading to an overall diagnostic yield of 50% (Table 2). Four different homozygous single nucleotide variants (SNVs) were detected in 4 known ciliopathy genes (*TMEM231*, *TMEM138*, *WDR19* and *BBS9*) and were confirmed by Sanger sequencing (Figure 1). Three of the mutations were missense mutations affecting highly conserved amino acids (Extended Data Figure 3[7]) whilst the fourth was a splice-site mutation (Figure 2). All tested samples were examined for mutations in ACMG actionable genes but none were identified.

The identified causative variant in M46 was novel (c.710A>G; p.Y237C in *TMEM231*) and has not been previously reported in any databases. This homozygous missense change is found in a large region of homozygosity on Chromosome 16 (Extended data Figure 2[7]) and is predicted by Sorting Intolerant from

Table 1. Clinical characteristics of Omani patients.

Patient ID	Gender	Age at referral	Clinical features	Additional clinical features	CKD stage	Family history of kidney disease	Parental consanguinity
M43	F	3 y	Nephronophthisis	DD, right hip dysplasia, failure to thrive.	5 (ESKD at 3 y)	Yes	Yes
M44	F	2 y	Cystic kidney disease	Hypertension, liver fibrosis	5 (ESKD at 2 y)	Yes	Yes
M46	F	3 m	Meckel syndrome with cystic kidneys	Dysmorphic features, occipital encephalocele, polydactyly, diaphragmatic hernia	5 (ESRD at 1 y)	Yes	Yes
M47	F	5 y	Cystic kidney disease	Retinitis pigmentosa, conductive hearing loss	5 (ESKD at 5 y)	Yes	Yes
M48	M	3 y	Joubert syndrome with cystic kidneys	DD, hypotonia, poor visual acuity, brain MRI showed molar tooth malformation	1	Yes	Yes
P3	M	3 y	Cystic kidney disease		1	No	Yes
P18	M	6 y	Nephronophthisis	Hypertension, DD and retinal dystrophy.	5 (ESKD at 5 y)	Yes	Yes
N36	F	1 y	Cystic kidney disease	Post-axial polydactyly	1	Yes	Yes

CKD, chronic kidney disease; DD, developmental delay; ESKD, end stage kidney disease; F, female; M, male; m, month; y, year.

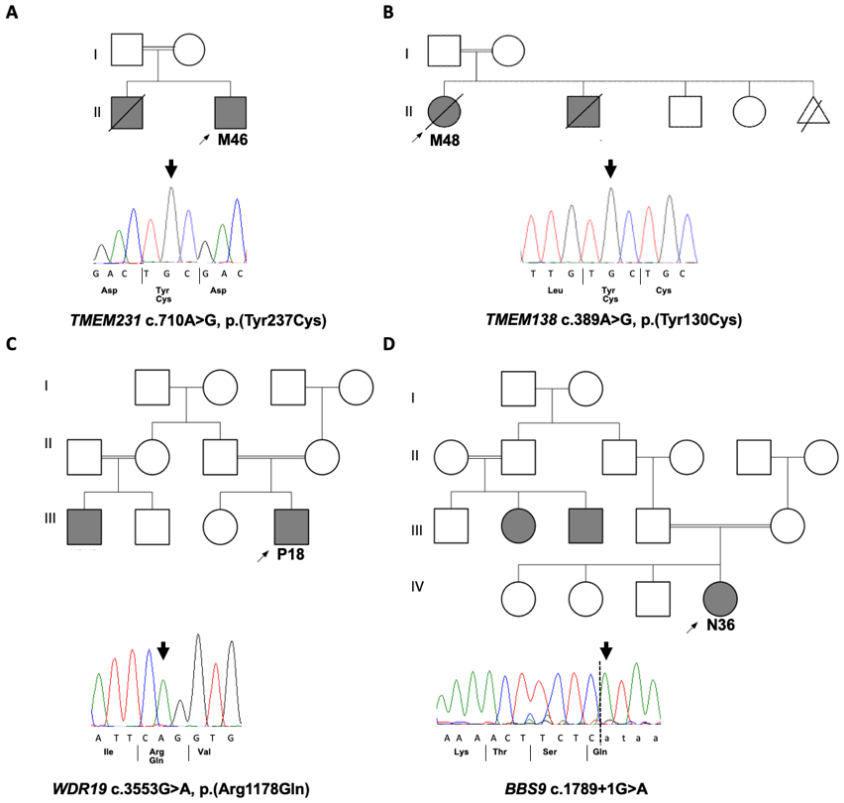

Figure 1. Family structures and Sanger sequencing in solved renal ciliopathy cases. Pedigrees of solved families with Sanger sequecing chromatograms confirming the disease causative variants that were identified by WES in four families. **A**. M46 with homozygous missense variant in *TMEM231*. **B**. M48 with homozygous missense variant in *TMEM138*. **C**. P18 with homozygous missense variant in *WDR19* **D**. N36 with homozygous splice site variant in *BBS9*.

Tolerant (SIFT) to be damaging, PolyPhen-2 to be possibly damaging and MutationTaster to be disease causing. The Y237 amino acid in TMEM231 is conserved to *Caenorhabditis elegans* (Extended data Figure 3[7]). Mutations in *TMEM231* are known to cause both Joubert syndrome and Meckel syndrome (Extended Data Table 3[7]), and the clinical phenotype of patient M48, which included encephalocele, polydactyly and polycystic kidney disease and early onset ESKD, is consistent with a Meckel-like ciliopathy syndrome.

The identified causative variant in M48 was a known allele (c.389A>G; p.Y130C in *TMEM138*) and has been previously reported in a child with Joubert syndrome and a cerebello-retinal-renal phenotype[8]. This homozygous missense change is found in a narrow region of homozygosity on Chromosome 11 (Extended data Figure 2[7]) and is predicted by SIFT to be deleterious, PolyPhen-2 to be probably damaging and MutationTaster to be disease causing. The Y130 amino acid in TMEM138 is conserved to *Danio rerio* (Extended data Figure 3[7]). Mutations in *TMEM138* are known to cause Joubert syndrome (Extended data Table 4[7]), and the clinical phenotype of patient M48, which included molar tooth sign, visual loss and cystic kidney disease, is consistent with a Joubert syndrome ciliopathy.

The identified causative variant in P18 was a known allele (c.3553G>A; p.R1178Q) in *WDR19* and has been previously reported in cases of nephronophthisis (NPHP)-related ciliopathies with retinal and liver involvement[9–11], Senior-Løken syndrome[12] and more complex ciliopathies[13]. This homozygous missense change is found in a large region of homozygosity on Chromosome 4 (Extended data Figure 2[7]) and segregation of the pathogenic causative allele in *WDR19* with P18's family members was confirmed. The missense allele is predicted by SIFT to be tolerated, PolyPhen-2 to be probably damaging and MutationTaster to be disease causing. The R1178 amino acid in WDR19 is conserved to *C.elegans* (Extended data Figure 3[7]). Mutations in *WDR19* are associated with a wide spectrum of ciliopathies (Extended data Table 5[7]), and the clinical phenotype of patient P18, which included NPHP and early onset ESKD and retinal dystrophy is consistent with a Senior-Løken syndrome.

The identified causative variant in N36 was a known splice-site allele (c.1789+1G>A in *BBS9*) and has been previously reported in patients with Bardet-Biedl syndrome (BBS)[14,15]. This homozygous missense change is found in a region of homozygosity on Chromosome 7 (Extended data Figure 2[7]) and is predicted to cause loss of splice donor site. Mutations in

Table 2. Molecular Genetic Findings in four Omani children with renal ciliopathy syndromes.

Family - individual	Gene name	Nucleotide change	Amino acid change	Zygosity	Amino acid conser.	ACMG Classification	dbSNP ID	MAF	CADD score	SIFT Pred	PolyPhen-2 Pred	MutationTaster	Reference
M46	*TMEM231*	c.710A>G	p.Y237C	Hom	*C.elegans*	Uncertain significance	NA	Not found	22.7	Damaging	Possibly Damaging	Disease causing	N/A
M48	*TMEM138*	c.389A>G	p.Y130C	Hom	*D.rerio*	Likely pathogenic	rs387907135	3.98×10^{-6} (gnomAD)	25.7	Deleterious	Probably damaging	Disease causing	Lee *et al.* (2012)[8]
P18	*WDR19*	c.3553G>A	p.R1178Q	Hom	*C.elegans*	Likely pathogenic	rs79436363	6.35×10^{-5} (gnomAD)	24.6	Tolerated	Probably Damaging	Disease causing	Halbritter *et al.* (2013)[9]
N36	*BBS9*	c.1789+1G>A	Splice donor site loss	Hom	N/A	Pathogenic	rs201938124	7.96×10^{-6} (gnomAD)	25	N/A	N/A	Disease causing	Nishimura *et al.* (2005)[14]

Reference sequence IDs: *TMEM231*: NM_001077416; *TMEM138:* NM_016464; *WDR19*: NM_025132; *BBS9*: NM_198428

Abbreviations: CADD score, combined annotation dependant depletion; conser, conservation; gnomAD, Genome Aggregation Database; Hom, homozygous; N/A, not available

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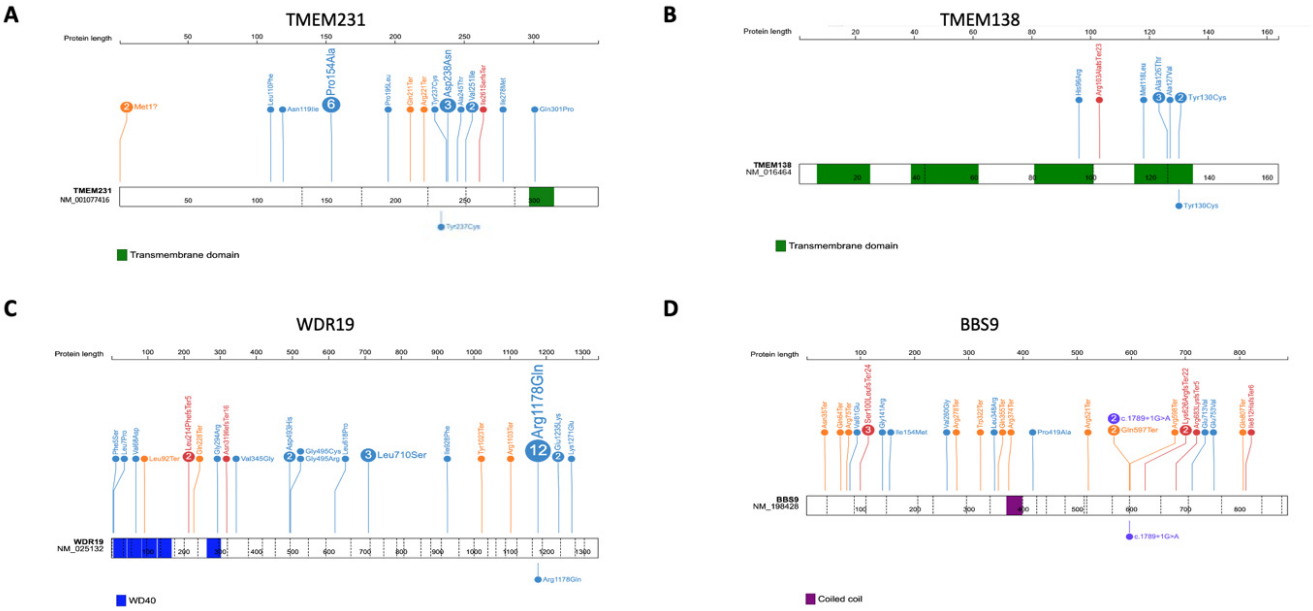

Figure 2. Distribution of mutations in *TMEM231*, *TMEM138*, *WDR19* and *BBS9*. Positions and the predicted protein alterations are shown for **A**. *TMEM231* **B**. *TMEM138* **C**. *WDR19* **D**. *BBS9*. Exon structure is marked by a dashed line. Protein domains are shown in colored bars. Known mutations are shown above the gene/protein structure with the number showing frequency (if >1) of probands reported for each mutation. Mutations identified in this study are shown below the gene/protein structure.

BBS9 are known to cause BBS (Extended data Table 6[7]), and the clinical phenotype of patient N36, which included features of BBS including post-axial polydactyly affecting all limbs and cystic kidney disease is consistent with a BBS ciliopathy.

Four of the families (M43, M44, M47 and P3) remained genetically 'unsolved' following WES and careful analysis and filtering of potential pathogenic alleles within regions of homozygosity, given their known consanguinity. The disease inheritance in all these four families was consistent with an autosomal recessive pattern given parents were unaffected. Indeed, more than one sibling was affected for families M43, M44 and M47 (Extended data Figure 1[7]). For the single affected sibling in P3 de novo heterozygous alleles need to be considered in addition to biallelic variants. We re-examined WES data for homozygous, compound heterozygous and de novo heterozygous alleles. Table 3 shows some alleles of interest in these families relating to ciliopathy phenotypes. In family M43 we identified biallelic (compound heterozygous) changes in *PKHD1* which were predicted to be benign. In family M44 we observed a single heterozygous allele in *COL4A1* of uncertain significance as well as a very rare synonymous allele in *NPHP3* that might be implicated in a splicing defect and a heterozygous *PKD1* missense allele of uncertain significance. Family M47 had a homozygous missense allele in *C2CD3* which was predicted to be benign whilst family P3 had a homozygous loss of function allele in *IFT140.* This allele however was not confirmed following Sanger sequencing and is likely to be a WES artefact. Finally, P3 has a single heterozygous allele in *ALG9*, which was predicted to be benign.

Discussion

In paediatric populations, CKD is a major contributor to healthcare burden leading to severe morbidity and mortality. At least 17% of those with ESKD are considered as CKD with unknown aetiology, where the primary kidney disease is not clear[16]. In addition, the primary clinical diagnosis of CKD patients is often inaccurate[16]. Thus, in the developing era of precision medicine, WES is used as an essential tool that provides novel diagnostic perspectives for the detection of the causes of CKD. Knowledge of genetic causes has valuable clinical implications in therapeutic intervention, improving prognosis, guide family counselling or managing settings of kidney transplantation[17]. Despite being rare, inherited kidney diseases represent one of the most common causes of CKD and ESKD, accounting for up to 10% of adults and almost all children commencing renal replacement therapy[18]. The possibility of monogenic causes in those with unknown aetiology of CKD or with atypical clinical presentation is assumed to be high[16]. At least 500 different genetic causes have been associated with childhood CKD[19].

In this pilot study, we examined the utility of WES in the diagnosis of 8 different Omani children with childhood onset CKD related to cystic kidney disease and a suspected inherited renal ciliopathy. A conclusive genetic diagnosis was achieved in half of the cases. Positive WES findings allow a precise molecular diagnosis and targeted clinical management as well

Table 3. Alleles of interest in 'unsolved' Omani children with renal ciliopathy syndromes.

Family - individual	Gene name	Nucleotide change	Amino acid change	Zygosity	Amino acid conser.	ACMG Classification	dbSNP ID	MAF	CADD score	SIFT Pred	PolyPhen-2 Pred	MutationTaster
M43	*PKHD1*	c.786A>G	p.L262L	Het	*M.musculus*	Benign	rs570064466	0.000118 (gnomAD)	15.02	N/A	N/A	Disease causing
M43	*PKHD1*	c.2279+13T>G	p.?	Het	*M.musculus*	Benign	rs180914598	0.005672 (gnomAD)	N/A	N/A	N/A	Polymorphism
M44	*COL4A1*	c.1588C>T	p.P530S	Het	*C.elegans*	Uncertain significance	rs145172612	0.00039 (gnomAD)	26.9.	Deleterious	Probably Damaging	Disease causing
M44	*NPHP3*	c.2805C>T	p.G935G	Hom	*M.musculus*	Uncertain significance	rs1281725083	0.000007956	12.92	N/A	N/A	Disease causing
M44	*PKD1*	c.11870G>A	p.G3957D	Het	*D.melanogaster*	Uncertain significance	rs536586062	0.000264 (gnomAD)	16.7	Tolerated	Possibly Damaging	Polymorphism
M47	*C2CD3*	c.6487G>T	p.V2163F	Hom	*M.musculus*	Benign	rs550167325	0.000799 (gnomAD)	<10	N/A	N/A	Polymorphism
P3	*IFT140*	c.2098dupT	p.S700fs*10	Hom	*M.musculus*	Uncertain significance	N/A	N/A	<10	N/A	N/A	Disease causing
P3	*ALG9*	c.1452-14T>C	p.?	Het	*M.musculus*	Benign	Rs187507214	0.007077 (gnomAD)	N/A	N/A	N/A	Disease causing

Reference sequence IDs: *PKHD1*: NM_138694; *COL4A1*: NM_001845; *NPHP3*: NM_153240; *PKD1*: NM_001009944 Abbreviations: CADD score, combined annotation dependant depletion; conser, conservation; gnomAD, Genome Aggregation Database; Hom, homozygous; N/A, not available

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as informing family planning and facilitating proper genetic counselling. In four of the children (M46, M48, P18 and N36) the molecular genetic findings confirmed the suspected clinical diagnosis. The identification of a molecular genetic diagnosis in all these families can provide accurate genetic advice about the parent's reproductive choices and the possibility of preimplantation genetic diagnosis (PGD) or early genetic testing of a foetus in future pregnancies.

A wide range of genetic studies have been performed in childhood CKD populations and different diagnostic yields were achieved due to differences in the inclusion criteria or patients and the study design. In a study of families with inherited kidney disease, Mallett*, et al.*[20] reported a diagnostic yield of 46%, reflecting the significant ability of WES in underlying the potential genetic causes of most renal phenotypes. In another recent study[2], Groopman *et al.* reported higher diagnostic yield in patients with congenital and cystic kidney disease (23.9%). Furthermore, regardless of the primary kidney diagnosis, higher diagnostic yield was associated with a positive family history of CKD, history of parental consanguinity and presentations of extra-renal features[2,6]. Thus using a combination of homozygosity mapping along with WES genotype data is always recommended as a powerful approach for consanguineous families to identify rare genetic causes[21].

Although WES provides massive amounts of genetic data, 4 patients remained unsolved in this study. Despite an analysis of both homozygous alleles, compound heterozygous alleles and heterozygous de novo alleles, families M43, M44, M47 and P3 remained unsolved. A very rare homozygous allele in *NPHP3* was noted in family M44. Mutations in *NPHP3* have been associated with very early and severe ciliopathy syndromes including Meckel syndrome[22], which matches the phenotype of this family. Proving the pathogenicity of this synonymous change now requires RNA analysis as well as searching for this allele in patients with a similar phenotype. Transcript aware annotation of genomic variants will be the next major step in utilising the data from WES effectively[23,24]. The identification of a novel loss of function allele in *IFT140* in family P3 that failed to be confirmed following Sanger sequencing acts as a cautionary reminder to validate all WES findings before genetic reporting. The WES read depth of this allele was <10 and therefore sequencing coverage remains an important consideration when choosing diagnostic sequencing modalities.

Interpretation of many novel and extremely rare variants is still limited by the incomplete knowledge of the total human protein-coding genes as well as the incorrect annotation of variants pathogenicity and incorrect association of genes with the disease in the literature. At present, up to 70% of protein-coding genes have no recognized human disease phenotype[25]. False gene-disease associations are present in the literature[26,27] and clinically valuable databases of variants pathogenicity, such as Human Gene Mutation Database (HGMD®), comprise various errors causing benign variants being falsely selected out of the data and allocated as plausible diagnosis[28]. This situation is predicted to improve as further genomes are sequenced, including large data collections containing populations of both healthy individuals and patients with rare diseases. In addition, studying more families with similar clinical phenotypes from the same population may facilitate linking novel undiscovered genes to the disease phenotype in those unsolved patients.

In this study, WES confirmed the clinical diagnosis in 4 children. In a similar study of large consanguineous or familial cohort (n = 79) of children clinically diagnosed with NPHP, genetic diagnostic yield of 63% was reported, of which the clinical diagnosis was confirmed in 64% and changed to different molecular diagnosis in the remaining 36%[11].

This study has some limitations, including small sample size that does not give a generalized image of broader childhood renal ciliopathy in the population from Oman. However, an enhanced assessment of the utility of WES in the clinical diagnostic practice of these disorders may be given through systematic WES analysis of a larger, unselected cohort. Moreover, the diagnostic gap in this study may be caused by the common technical limitation of WES, including the missed detection of structural variant breakpoints, sequencing difficulties for regions with repetitive elements or guanine-cytosine (GC)-rich regions, and limited discrimination between highly homologous genomic regions with pseudogenes. These limitations are attributed to the short-read lengths that are utilized to generate high genomic coverage and depth[29]. These limitations are assumed to be resolved through using long-read sequencing platforms that compromise these technical challenges and improve the detection of genetic variants[29]. Thus, the emerging future of long-read sequencing based whole genome sequencing (WGS) could enhance the diagnostic yield of patients with inherited renal ciliopathies and provide more conclusive primary kidney disease diagnosis. This can be supported by recent reports of WGS obtaining higher molecular diagnostic yield compared with WES, where 20–40% of those unsolved by WES were genetically conclusive by WGS[30]. In particular, WGS has recently been used to successfully identify a deep intronic allele in *NPHP3* leading to nephronophthisis[31] and with such approaches, defining deleterious intronic alleles will allow an increase in the diagnostic yield of WGS.

Recent advancements in medical genetics through the use of massively parallel sequencing have not only advanced the discovery of novel causative variants, genes and phenotypes, but also contributed to the re-classification of diseases and phenotypes into novel gene-based ontologies[32]. However, all types of next generation sequencing (NGS)-based testing (Target panel, WES and WGS) have some shared limitations, including the inability to obtain enough coverage of genomic regions with highly repetitive GC-content sequence, such as that in *MUC1* gene. In his study of six unrelated families with medullary cystic kidney disease type 1 (MCKD1)[33], Kirby *et al.* highlighted the challenges of these technologies in detecting

the causative monogenic causes of some Mendelian disorders, such as MCKD1, where only long-range polymerase chain reaction and molecular cloning successfully performed the task. Moreover, in many patients with acquired diseases, NGS testing is of limited importance and transformation of genetic results into clinical setup may be challenging[32]. In the field of kidney disease, the majority of genetic testing studies are narrowed to a research setting, thus until now the knowledge of its diagnostic efficacy in clinical practice is still limited[16]. In addition, managing the medical ethics raised by these technologies, including uncertain variants and incidental findings, and balancing the social concerns is still challenging[34].

Conclusion

WES of patients with different inherited cystic kidney diseases and renal ciliopathies shows promise as a diagnostic tool, especially in well selected patients with a high coefficient of inbreeding and/or with a syndromic presentation. It has the potential to resolve those cases with clear suspicion of renal ciliopathies, as well as those with uncertain aetiology causing CKD. The fact that ~50% of patients remain without genetic diagnosis after WES highlights the need for improved sequencing techniques and interpretation tools, driven by constantly evolving knowledge regarding the genetic architecture of diseases. The clinical impacts of positive WES results on therapeutic choice, genetic counselling and guidance of kidney transplant are critical. Indeed, professional genetic counselling on the prospective effects of a positive test result is crucial, bearing also in mind the possibility of incidental findings. Although further studies from the Omani population are required, we predict an expanding impact of NGS-based diagnosis, both gene panels and WES in clinical practice in the very near future.

Data availability

Underlying data

Figshare: The diagnostic yield of whole exome sequencing as a first approach in consanguineous Omani renal ciliopathy syndrome patients, https://doi.org/10.6084/m9.figshare.13696750.v1[35].

This project contains the following underlying data:

- M46.snps.vcf
- M46.indels.vcf
- M48.snps.vcf
- M48.indels.vcf
- JAS_P18.GATK.snp.vcf
- JAS_P18.GATK.indel.vcf
- JAS_N36.GATK.snp.vcf
- JAS_N36.GATK.indel.vcf

Extended data

Figshare: The diagnostic yield of whole exome sequencing as a first approach in consanguineous Omani renal ciliopathy syndrome patients, https://doi.org/10.6084/m9.figshare.c.5287753.v1[7].

This project contains the following extended data:

- Extended data Table 1. Forward and reverse primer sequences used for WES variants verification (https://doi.org/10.6084/m9.figshare.13675201)
- Extended data Table 2. Whole exome sequence alignment and coverage profile by sample (https://doi.org/10.6084/m9.figshare.13675222)
- Extended Data Table 3. TMEM231 alleles (https://doi.org/10.6084/m9.figshare.13675471)
- Extended data Table 4. TMEM138 alleles (https://doi.org/10.6084/m9.figshare.13675504.v1)
- Extended data Table 5. WDR19 alleles (https://doi.org/10.6084/m9.figshare.13675540)
- Extended data Table 6. BBS9 alleles (https://doi.org/10.6084/m9.figshare.13675546)
- Extended data Figure 1. Pedigree diagrams (https://doi.org/10.6084/m9.figshare.13675552.v1)
- Extended data Figure 2. Homozygosity mapping (https://doi.org/10.6084/m9.figshare.13675558.v1)
- Extended data Figure 3. Clustal alignments of amino acids associated with identified missense mutations (https://doi.org/10.6084/m9.figshare.13675561.v1)

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 19 July 2021

https://doi.org/10.5256/f1000research.58306.r89233

✔ **Paraskevi Goggolidou**
Department of Biomedical Science and Physiology, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK

Having looked at the revised manuscript, I am happy to accept it.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 12 July 2021

https://doi.org/10.5256/f1000research.58306.r89232

✔ **Enza Maria Valente**
[1] Neurogenetics Research Center, IRCCS Mondino Foundation, Pavia, Italy
[2] Department of Molecular Medicine, University of Pavia, Pavia, Italy

I am satisfied with the changes that the authors made to the article. A significant section on negative results has been added which now makes the manuscript more complete and informative.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: neurogenetics, ciliopathies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 06 April 2021

https://doi.org/10.5256/f1000research.43403.r81403

?

Paraskevi Goggolidou
Department of Biomedical Science and Physiology, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK

This manuscript by Al Alawi *et al.* provides an interesting application of Whole Exome Sequencing (WES) in Ciliopathy patients in Jordan. The authors have looked at a small number of Ciliopathy patients and identified mutations of interest in 50% of them. As they are working with rare disease patients, the limitations in sequencing bigger patient populations are understandable. Furthermore, this work provides a very useful proof of concept in the application of WES for genetic diagnosis of rare diseases, such as Ciliopathies.

However, as discussed in the discussion section, WES has got limitations and other approaches such as WGS might have been more insightful for this study. The manuscript would thus benefit from a more thorough discussion of the possibility of identified by WGS cases of non-exonic mutations causing Ciliopathies and a comment on the mechanisms behind this. Furthermore, it is important to comment on whether there were any single heterozygous pathogenic variants identified in known genes and if they checked for genes within stretches of homozygosity in consangeneous families. Finally, for the non-specialist audience a brief description of the key characteristics and genes associated with renal ciliopathies in the introduction section would have been useful.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Renal Ciliopathies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response ( ) 30 Jun 2021

John Sayer, Newcastle University, Newcastle upon Tyne, UK

We agree that WGS may be more powerful than WES in order for us to solve the unsolved cases. We have added some discussion regarding this point as suggested. In response to the comments regarding the "negative cases' we have now expanded the paper to discuss these further as there may, as the reviewer suggests be some useful learning points. As all the families were consanguineous, we focussed on homozygous variants but we have commented now on any significant heterozygous variants in cystogenes. Similarly, we have looked at missense alleles and synonymous changes that have been excluded by pathogenicity filters and comment on these also. Finally, as suggested we report homozygous variants in regions of homozygosity by descent in candidate genes that may shed new light on renal ciliopathies. A new table detailing variants in unsolved cases has been added and the discussion expanded to account for these new data.
We have now added a brief introduction to renal ciliopathies and the common genetic causes has now been added as suggested.

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 March 2021

https://doi.org/10.5256/f1000research.43403.r81405

?

Enza Maria Valente
[1] Neurogenetics Research Center, IRCCS Mondino Foundation, Pavia, Italy

[2] Department of Molecular Medicine, University of Pavia, Pavia, Italy

In this short article, the authors show the importance of Whole Exome Sequencing (WES) approach as a first genetic screening for patients with renal ciliopathy syndromes. They focus on a small cohort of 8 consanguineous Omani probands, who underwent WES. The diagnostic yield was 50%: four out of 8 probands were found to carry homozygous pathogenic variants in known genes. The study was carried out in a proper way, with sound methodology. The identified variants were adequately named and classified following ACMG guidelines. The discussion correctly addresses limitations of WES.

There are two main limitations in this article. The first is the very small cohort size, which does not really allow making a correct estimate of the diagnostic yield of WES in renal ciliopathies. Expansion of the study to a larger group of patients would clearly provide more useful information, also regarding the genetic background of genetic renal ciliopathies in Oman. Second, even maintaining this cohort, it would be very interesting to know more about “negative” cases: where there any single heterozygous pathogenic variants in known genes? Any missense variants classified as VUS? Moreover, since families were consanguineous, the authors should report whether they checked for genes included within stretches of homozygosity by descent, and list the potentially interesting homozygous variants in candidate genes lying within these regions. This would definitely improve the study which, in its present form, does not add substantial new data to current knowledge on the topic, and therefore it remains largely confirmatory.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: neurogenetics, ciliopathies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response ( ) 30 Jun 2021

John Sayer, Newcastle University, Newcastle upon Tyne, UK

Yes we agree that this is a small study size, but we were careful only to include subjects that had not had any previous genetic investigations and fulfilled the criteria of a suspected renal ciliopathy syndrome. In this way this study was seeing the added value in terms of genetic diagnosis to perform whole exome sequencing as a first line approach in contrast to a targeted renal genetics panel. We have added some comments regarding the size of the cohort.

In response to the comments regarding the "negative cases' we have now expanded the paper to discuss these further as there may, as the reviewer suggests be some useful learning points. As all the families were consanguineous, we focussed on homozygous variants but we have commented now on any significant heterozygous variants in cystogenes. Similarly, we have looked at missense alleles and synonymous changes that have been exclude by pathogenicity filters and comment on these also. Finally, as suggested we report homozygous variants in regions of homozygosity by descent in candidate genes that may shed new light on renal ciliopathies. A new table detailing variants in unsolved cases has been added and the discussion expanded to account for these new data.

Competing Interests: No competing interests were disclosed.