A targeted gene panel illuminates pathogenesis in young people with unexplained kidney failure

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Abstract

Background

Kidney failure in young people is often unexplained and a significant proportion will have an underlying genetic diagnosis. National Health Service England pioneered a comprehensive genomic testing service for such circumstances accessible to clinicians working outside of genetics. This is the first review of patients using this novel service since October 2021, following its introduction into clinical practice.

Methods

The 'Unexplained Young-Onset End-Stage Renal Disease' (test-code R257) gene panel uses targeted next generation sequencing to analyse 175 genes associated with renal disease in patients under 36 years of age. All tests undertaken between October 2021 and February 2022 were reviewed. Phenotypic data was extracted from request forms and referring clinicians contacted where additional details were required.

Results

71 patients underwent R257 testing over the study period. 23/71 patients (32%) were confirmed to have a genetic diagnosis and 2/71(3%) had a genetically suggestive variant. Nephronophthisis and Alport syndrome were the most common conditions identified, (4/23 (17%) with pathogenic variants in *NPHP1* and 4/23 (17%) with pathogenic variants in *COL4A3/COL4A4*). Positive predictors of a genetic diagnosis included a family history of renal disease (60% of positive cases) and extra-renal disease manifestations (48% of positive cases).

Conclusion

This is the first study to evaluate the R257 gene panel in unexplained young-onset kidney failure, freely accessible to patients meeting testing criteria in England. A genetic diagnosis was identified in 32% of patients. This study highlights the essential and expanding role that genomic testing has for children and families affected by renal disease today.

Keywords: R257, gene panel, unexplained paediatric kidney failure, next generation sequencing.

What is already known?

- The National Genomic Test Directory provides an overview of all genomic tests available to NHSE patients.
- Previous data demonstrates that 30% children with chronic kidney disease are estimated to have an underlying monogenic cause, compared with 5-30% of adult cohorts. Adult rates varied due to their diagnostic category (low rates in congenital abnormalities of the kidney and urinary tract versus high rates in Alport syndrome).
- Recent evidence demonstrates comparable rates of monogenic kidney disease in families with childhood-onset and adult-onset disease.

What this study adds

- The R257 panel is a new, fully-funded, comprehensive genomic test for young-onset unexplained kidney failure. Previous papers have focused on the translational research of genetic testing, whereas this study provides 'real-life' data, looking at the clinical utility and impact on the investigative work-up of unexplained kidney failure. 32% patients were identified as having a pathogenic or likely pathogenic variant in a gene associated with kidney failure. A positive result correlated with a family history of a renal condition, clinical suspicion of a genetic diagnosis and extra-renal features.

How this study might affect research, practice or policy

- We aim to raise awareness of the clinical benefits of genomic testing in young people with unexplained kidney failure. In particular, we advise adapting current practice to incorporate the early use of this test in the clinical pathway (where appropriate), potentially avoiding more invasive procedures (renal biopsy).
- Establishing a diagnosis using the R257 panel has numerous potential benefits. For patients, screening for extra-renal manifestations, understanding the molecular mechanisms of disease and developing targeted treatments. For families, identifying at-risk family members by cascade testing, guiding decisions around future reproductive choices and determining suitability for live kidney donation.

Introduction

Currently over 900 children are living in the United Kingdom (UK) with kidney failure [1]. This includes patients with chronic kidney disease (CKD) stage 5 receiving either medical management, dialysis or transplantation. Congenital abnormalities of the kidney and urinary tract are the leading cause of kidney failure in children in the UK [2]. There is also an increasing awareness of the interplay between genetics and young-onset kidney failure. To date, more than 600 genes have been implicated in monogenic kidney diseases [3]. It is estimated that 30% children with chronic kidney kidney disease, have an underlying genetic diagnosis [4]. Comparable rates are predicted in adults looking at familial studies, estimating that over 1 in 5 adults having an underlying genetic aetiology for their unexplained kidney failure [5, 6]. This lack of diagnosis can have a significant psychological impact on patients and families, causing feelings of vulnerability and overwhelming uncertainty [7].

In the UK, health care is funded through general taxation by the National Health Service (NHS). In 2012, the NHS funded the 100,000 Genomes Project with the objective of sequencing 100,000 genomes from individuals affected by rare diseases [8]. Data from the project supported the use of whole genome sequencing (WGS) technology in routine diagnostic pathways for NHSE patients. Approximately 10% of all NHS genomic testing is now run through WGS technology.

Historically, genetic testing was expensive, limited and time-consuming [9]. Through current next generation sequencing technologies (NGS), turnaround is rapid and increasingly affordable [10]. Diagnostic rates for people affected by rare diseases continue to improve due to genomic testing. A molecular diagnosis provides a number

of advantages, improving access to clinical trials for new treatments and reducing drug-related side effects through more precise prescribing practices based on an understanding of specific disease mechanisms [11].

The Genomic Medicine Service (GMS) set up in England in 2018, provides NHS funded genomic testing through 7 genomic laboratory hubs (GLHs) [12]. The National Genomics Test Directory (NGTD) provides a comprehensive list of all the tests commissioned for use in England [13]. There are currently 12 renal indications for testing, one of these being the 'Unexplained young-onset end-stage renal disease' panel, test-code R257 [13].

There are limitations to the current testing methods in mainstream use, including the inability to detect epigenetic (environmental) factors and certain types of structural variant (large inversions). Nor is testing designed to identify conditions known to be linked to common variants in multiple genes (polygenic inheritance). A negative genetic test may reduce the likelihood that a genetic diagnosis exists, but cannot exclude it altogether.

The NHS is one of the first in the world to pioneer a comprehensive, freely accessible genetic panel for patients with young-onset unexplained kidney failure. It is therefore of great interest to determine the clinical utility of this test for our patients affected by significant renal disease. We will also review the presenting features of these patients to ascertain whether there are any factors pertaining to a genetic diagnosis. Whilst previous studies focus on translational research, there is very little 'real-life' data. This is the first study to evaluate patients using the R257 service since its introduction into clinical care.

Methods

This service evaluation retrospectively reviewed clinical and laboratory records for NHS patients undergoing testing using the R257 panel. Patients and families provided informed consent for genetic testing to their primary clinician. Genomic analysis was undertaken at the South West and North Thames Genomic Laboratory Hubs between October 2021 and February 2022.

Eligible patients meeting clinical criteria pre-specified in the National Genomic Test Directory (NGTD) included those under 36 years of age with unexplained kidney failure, with no identifiable cause detectable by renal biopsy, biochemistry, imaging or clinical assessment [13]. Genetic variants were collated and relevant phenotypic data extracted.

Testing is possible for patients outside of the above categories following discussion with a geneticist, including antenatal patients (part of the post-mortem), patients over 36 years of age or patients with CKD stage 3/4 and patients without a renal biopsy where a strong suspicion of a monogenic (caused by the inheritance of a single gene mutation) diagnosis exists. These cases were included in the evaluation data and data was collected using the pro-forma (Supplement 1).

Referring clinicians were contacted to provide further clinical information where this was required. The gene content of R257 is available to review through PanelApp (Supplement 2), a Genomics England resource providing up-to-date evidence on disease-associated genes [14].

Genes included in panel tests require ‘diagnostic level evidence’ for a disease association before they are signed off as ‘green’ and are included in the clinical test. Known single tandem repeats (STRs) and copy number variants (CNVs) will also be tested where relevant. Those genes with only moderate evidence for a disease association remain on the ‘amber’ list for potential future inclusion in the test [14]. The panels are curated and reviewed by international scientific experts.

Next Generation Sequencing (NGS)

Both laboratories are accredited by the UK Accredited Service (UKAS) for NGS services, utilised to interrogate the genome in order to identify disease causing sequence variants.

The Bristol Genetics Laboratory employs a custom-designed Twist BioSciences probeset with Illumina Nextera DNA flex library preparation in order to target 175 genes included on the R257 panel. NGS data was analysed on the Illumina NextSeq platform, followed by sequence analysis using an open source in-house pipeline with hg19 human genome as a reference. Variant filtering was performed by registered Clinical Scientists using a bespoke in-house database, capable of detecting both sequence variants and larger CNVs. Sequence variants were analysed and interpreted according to the American College of Medical Genetics and Genomics (ACMG) and Association for Clinical Genomic Science (ACGS) best practice guidance [15, 16].

The North Thames Genomic Lab Hub employs the TWIST BioSciences Exome v1.0 with added custom content. Sequencing is conducted on the Illumina NovaSeq with paired-end 150bp reads, with subsequent data analysis is performed using an in-house developed pipeline. Variant analysis is restricted to a virtual panel of the genes from R257, with assessment according to ACMG and ACGS guidelines [15, 16].

Results

71 patients with unexplained kidney failure underwent R257 panel testing. Patients were referred from different centres across England. The age of patients ranged from 0 (antenatal) to 53 years, with a median age of 13 years (Supplement 3 and 4).

Cases with confirmed monogenic diagnosis (Table 1 and 2)

23/71 patients (32%) had a monogenic diagnosis confirmed, a further 2/71 had a genetically suggestive variant detected. 16/23 positive cases (70%) were referred from clinicians reporting a strong clinical suspicion of a genetic aetiology. 8 /16 cases (50%) suspected a ciliopathy from the outset, 1/16 (6%) suspected polycystic kidney disease (PKD) and 7/16 (44%) a specific syndrome (see Table 1). 14/23 positive cases (60%) had a family history of CKD, of which 12/14 (86%) were in a first-degree family member. In 3/14 cases (21%), the underlying condition had previously been defined. 11/23 (48%) confirmed cases had extra-renal features. 5/11 (45%) had more than one extra-renal manifestation. 8/23 (35%) had both extra-renal features and a positive family history.

The R257 gene panel provided a diagnosis for paediatric (18/ 23, 78% patients with a monogenic diagnosis were children) and adult patients (5 /23, 22% patients were over 18 years). For 2/23 (9%) patients with a confirmed monogenic diagnosis, there was no information suggesting a high index of suspicion about a genetic cause, no family history and no extra-renal features.

Of those patients with a confirmed monogenic diagnosis, 8/23 (35%) patients presented acutely with kidney failure, 3/23 (13%) patients presented in the antenatal period and 3/23 (13%) at birth. 5 of these patients sadly passed away, either in the antenatal period, undergoing genetic testing as part of the post mortem or shortly after birth. 2/23 (9%) patients were tested due to a family history and were noted to have kidney failure. One patient was lost to follow up after being noted to have a “renal problem” as a child.

Heterozygous variants in Autosomal Recessive Disease

2/71 patients had genetically suggestive results, showing heterozygous variants detected in conditions associated with autosomal recessive inheritance (*PKHD1* and *FAT1*). There was a strong phenotypic correlation in both patients for specific diseases (*PKHD1*: antenatally detected polycystic kidneys, *FAT1*-related disorder: ptosis, nephropathy and syndactyly as well as a family history of CKD).

Compound heterozygous variants

Sanger sequencing confirmed bi-parental inheritance in 2/23 patients (*PKHD1* and *CC2D2A*). Parental testing was not performed in a child with *NPHP1* who presented

with kidney failure, polyuria and a family history of CKD. One variant was a whole gene deletion, therefore the second variant is assumed to be on a different allele. As both parents were unaffected it is inferred these variants are inherited in trans.

Cases without a confirmed monogenic diagnosis

46/71 patients remained 'unexplained' without confirmation of a monogenic diagnosis. For 11/46 (24%), the clinician specified a high index of suspicion about a genetic diagnosis (6/11 (54%) referenced *HNF1B*, 3/11 (27%) a ciliopathy and 2/11 a syndromic diagnosis (18%)). 10/46 (21%) 'Unexplained' patients had a family history of CKD (70% in a first degree relative), although the cause of the familial CKD had not been defined. 12/46 patients (26%) had extra-renal features (3/12 with more than 1 extra-renal manifestation (Table 2)). 2 /46 (4%) had both extra-renal features and a family history.

Table 1: Patient characteristics with and without a confirmed monogenic diagnosis

Patient Characteristics	All patients (n = 71)	Confirmed monogenic diagnosis (n = 23)	Suggestive monogenic diagnosis (n=2)	No diagnosis confirmed (n = 46)
Female	36	15	1	20
Male	28	5	0	23
Foetus	7	3	1	3
Antenatal	7	3	1	3
Child < 5 years	11	3	0	8
Child 6 – 18 years	33	12	0	21
Adult	20	5	1	14
Clinical suspicion present	29	16	2	11
Ciliopathy suspected	11	8	0	3
Polycystic kidney suspected	2	1	1	0
HNF1B variant suspected	6	0	0	6
Other syndrome suspected	10	7	1	2
Positive Family History	25	14	1	10
First degree affected	20	12	1	7
Multiple family members	12	6	1	5
Extra-renal features	24	11	1	12
>1 extra-renal feature	9	5	1	3

Table 2: Extra-renal features noted in patients with and without a monogenic diagnosis

Extra-renal Features in patients with a confirmed monogenic diagnosis	(n=11)	**Extra-renal Features in patients with no diagnosis confirmed**	(n = 12)
Encephalocele	2	Developmental Delay	1
Ocular symptoms (combined with developmental delay/ thyroid involvement or ear tags)	2	Hearing loss/ squint and delay	2
		Short stature/ IUGR	3
Bilateral undescended testes	1	Undescended testes	1
Microcolon and bladder symptoms	2	Cerebellar hypoplasia	1
Liver involvement	1	Liver involvement	1
Cardiac failure	1	Infections	1
Anhydramnios	2	Anhydramnios	1
		Trisomy	1

Legend –IUGR refers to intra-uterine growth restriction.

Diagnostic variants (Figure 1):

23 patients had a confirmed monogenic diagnosis. Pathogenic variants in *NPHP1* (4/23) and *COL4A3/COL4A4* (4/23) were the most common. This was followed by *PKHD1* (2/23), *PAX2* (2/23) and *ACTG2* (2/23) confirmed as causative in a total of 7/23 cases.

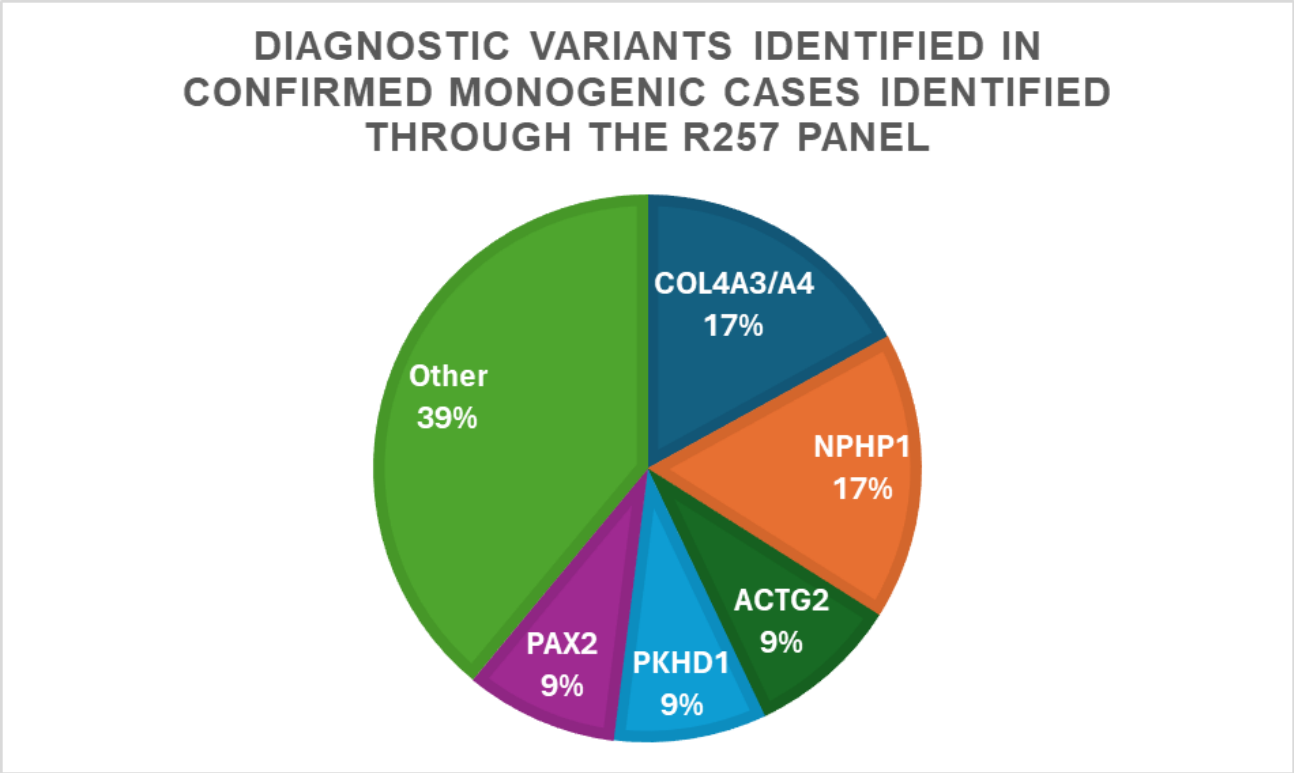

Figure 1 – Diagnostic variants

39% patients presented with 'other' mutations (outlined in Table 3.)These include diagnostic variants in *ACE, COQ8B, CC2D2A, FAT1, ICQB1, NUP93, SDCCAG8, TMEM138, UMOD and WT1.*

Table 3 – Patients with a confirmed monogenic diagnosis

Genes	Condition	Transcript	cDNA	Zygosity	AR/ AD	Phenotype	Urine	Renal USS	Extra- renal features	FHX	Consanguinity	Biopsy done
ACE	Renal tubular dysgenesis	NM_000789.3	c.2305+208_2702delinsTT	Hom	AR	Renal dysgenesis at birth.	NA	Renal dysgenesis	Yes – pulmonary anhydramnios	Yes	Yes	
ACTG2	Megacystis microcolon intestinal hypoplasia	NM_001615.3	c.118C>T	Het	AD	Antenatal- Megacystitis microcolon &intestinal hypoperistalsis.	NA	Megacystitis	Yes -Microcolon and megacystitis			
ACTG2	Megacystitis microcolon intestinal hypoplasia	NM_001615.4	c.532C>T	Het	AD	Birth - Gastrointestinal obstruction and raised creatinine		Megacystitis	Yes – Microcolon and megacystitis			
CC2D2A ***CC2D2A***	Meckel Gruber syndrome	NM_001080522.2	Gene 1 c.3774dup Gene 2- c.439-2A>G Sanger sequencing confirmed biparental inheritance	C. Het	AR	Post mortem, antenatal	NA	Polycystic	Yes – encephalocele and polydactyly			
COL4A4	Alport syndrome spectrum	NM_000092.4	c.2628_2654dup	Het	AR/ AD	Progressive renal decline		Singe kidney	No	Yes		
COL4A4	Alport syndrome spectrum	NM_000092.4	c.1598G>A	Hom	AR/ AD	Kidney failure ? cause	Haematuria + proteinuria	Small kidneys	No hearing loss			
COL4A3 and NPHS2	Alport syndrome spectrum	NM_000091.4 NM_014625.3	Gene 1- c.2189G>A Gene 2- c.686G>A	Het Het	AR/ AD	Acute presentation kidney failure and fatigue.	Proteinuria	Small, scarred kidneys	No hearing loss	Yes - likely Alports		
COL4A3	Alport syndrome spectrum	NM_000091.4	c.3499G>A	Het	AR/ AD	CKD 5. Diagnosed as child but lost follow up		Small kidneys	No	Yes		
COQ8B	Primary coenzyme Q10 deficiency	NM_024876.3:	c.1447G>T	Hom	AR	Acute presentation of kidney failure	Proteinuria	Large and bright	No			Interstitial fibrosis, glomerulo-sclerosis
ICQB1	Senior Løken Syndrome	NM_001023570.3	c.488-1G>A	Hom	AR	Acute presentation of kidney failure and fatigue		Small, bright kidneys	Yes - developmental delay, ocular		Yes	
NPHP1 ***NPHP1***	Nephronophthisis	NM_000272.4	Gene 1- Whole gene deletion Gene 2- c.1885-1G>C	C. Het	AR	Acute history of lethargy and presented in kidney failure. Polyuria,	Trace blood 1+ protein	Bright, cysts lobulated	No	Yes		Non-specific findings

			inherited in trans (unaffected parents and whole gene deletion)			polydipsia longstanding but not investigated.						
NPHP1	Nephronophthisis	NM_000272.4	Whole gene deletion	Hom	AR	Investigated due to FHX and in kidney failure, polyuria				Yes		
NPHP1	Nephronophthisis	NM_000272.4	Whole gene deletion	Hom	AR	Kidney failure			No	Yes		
NPHP1	Nephronophthisis	NM_000272.4	Whole gene deletion	Hom	AR	Acute CKD 4 Months of malaise. No polyuria/ failure to thrive.	1+ Blood 3+ protein	Small kidneys	Nil ocular	Yes		
NUP93	Nephrotic syndrome	NM_014669.4	c.1772G>T	Hom	AR	Acute kidney failure. Nephrotic and hypertensive	Proteinuria	Echogenic Normal size	Yes - Hypertensive cardiac failure	Yes		Non specific
PAX2	Renal coloboma syndrome	NM_003990.4	c.74G>A	Het	AD	Post-natal bilateral renal dysplasia, oligohydramnios	Heavy proteinuria	Renal dysplasia	No	Yes		
PAX2	Renal coloboma syndrome	NM_003987.4	c.76dup	Het	AD	Kidney failure	proteinuria	Small bright kidneys	Yes – ocular and ear tags	Yes		
PKHD1 ***PKHD1***	Autosomal recessive polycystic kidney disease (ARPKD)	NM_138694.3	Gene 1- c.107C>T Gene 2- c.2452C>T (Sanger sequencing confirmed inheritance from both parents)	C. Het	AR	Polycystic kidneys, kidney failure.	NA	Polycystic kidneys	Yes - likely liver	Yes ARPKD		
PKHD1	Autosomal recessive polycystic kidney disease	NM_138694.3	c.11215C>T	Hom	AR	Polycystic kidneys on antenatal scan, followed up at birth and confirmed.	NA	Polycystic kidneys	Yes - anyhydramnios		Yes	
SDCCAG8	Nephronophthisis	NM_006642.4	c.513_514dup	Hom	AR	Acute kidney failure. Polyuria, normotensive			No			
TMEM138	Joubert syndrome	NM_016464.4	c.380C>T	Hom	AR	Antenatal-cystic kidneys	NA	Large, cystic	Yes- encephalocele	Yes		

UMOD	Uromodulin kidney disease	NM_003361.4	c.278_289delinsCCGCCTCCT	Het	AD	Investigated due to FHX and hypertension, detected CKD 4	Haematuria, 1+ protein		No	Yes		Interstitial fibrosis
WT1	Denys Drash	NM_024426_449AAs.3	c.1168C>T	Het	AD	Male, acute kidney failure, anuric, pulmonary oedema.	Proteinuria	Small, Increased echogenicity	Yes - Bilateral undescended testes	Yes		

Legend: ***FHX refers to family history of an underlying renal disease (including chronic kidney disease, syndromes with associated renal problem and structural kidney problems) . CKD to chronic kidney disease. Inheritance - AR refers to autosomal recessive, AD refers to autosomal dominant. Hom refers to homozygous, Het to heterozygous and C. Het to compound heterozygous. Patients highlighted in red passed away in the antenatal period or shortly after birth. All reporting was returned to the clinician followed ACMG / ACGS recommendations. These refer to the phenotypic specificity, the size of the gene panel and the clinical sensitivity of the test to determine the significance of a single variant in a heterozygous state [15, 16]. Supplement 5 describes the genetic conditions named above [17].***

Discussion

We present data for 71 patients with unexplained young-onset kidney failure who underwent R257 testing between October 2021 and February 2022. Results demonstrate the high diagnostic yield of the R257 gene panel, with a pathogenic variant detected in 32% of patients and an average turnaround time of 84 days. These results are similar to those from comparable NGS based renal tests such as in nephrolithiasis [18].

At the time of requesting, an underlying genetic disorder was suspected in almost three quarters (70%) of patients with a confirmed monogenic diagnosis. This was in contrast to 24% patients with a negative result. Over half of patients (60%) with a monogenic diagnosis had a family history of CKD, 86% of which affected a first degree relative. This is consistent with studies showing a three to nine-fold greater risk of CKD in those with a positive family history [19]. In contrast, 21% had a positive family history of CKD in the group with negative genetic results. Extra-renal features were present in almost half (48%) of patients who received a genetic diagnosis. This was true of only the minority (26%) of patients with a negative result. Our study supports data showing a similar diagnostic rates in paediatrics (15/44, 34%; excluding antenatal patients) and adults (5/20, 25%) [5].

There are well established benefits of confirming a molecular diagnosis for patients with renal disease, including the potential to avoid invasive tests (renal biopsy), screening for extra-renal manifestations enabling early intervention and providing cascade testing for at-risk family members.

We are seeing numerous benefits due to the improved understanding of molecular mechanisms of renal disease through evolving targeted therapies (Lumasiran and Nedisoran, small interfering RNA therapies for the primary hyperoxalurias, gene therapy for steroid-resistant nephrotic syndrome and Sparsetan, a Dual Endothelin Angiotensin Receptor Antagonist in Alport syndrome [20,21]). Furthermore genetic testing provides an opportunity to screen living kidney donors for genetic variants associated with an increased risk of progressive CKD, ascertaining suitability [22].

The versatility of the R257 gene panel in real-life practice is clearly demonstrated by the variety of diagnostic variants detected and the wide age range of patients (antenatal to adulthood). Our results show *NPHP1* to be the most commonly implicated gene in those with a monogenic diagnosis. This is consistent with the literature on nephronophthisis, the leading monogenic cause of kidney failure in the first three decades of life [23].

We identified no patients with pathogenic variants in *HNF1B* (known to contribute to a significant proportion of kidney failure in children) [24]. This may partly relate to our small sample size, but also due to the availability of targeted *HNF1B* gene testing, readily available before the R257 panel. Patients with an *HNF1B* related kidney disease may have been identified previously (and not within our cohort of ‘unexplained’ kidney failure patients). Furthermore *HNF1B*-related kidney disease may not have

been detected by NGS in cases where there is a large deletion or insertion. As the R257 panel is now employing WGS techniques, the ability to detect such structural variants is expected to be more precise.

Similarly, 2 patients with heterozygous variants in autosomal recessive diseases (*PKHD1 and FAT1)* were noted in our cohort. It can be challenging to determine the true significance of a variant, whether a second pathogenic variant has been missed during analysis or whether it is incidental and has no bearing on the CKD at all. As both patients had strong clinical phenotypes associated with specific diseases it is highly likely that a second variant has been unidentified. Cautious clinical interpretation is essential when reviewing inconclusive results, however the use of precise WGS technology is likely to improve our understanding.

We also wish to highlight an interesting patient, with variants in 2 genes (*COL4A3* and *NPHS2*). A literature review highlighted such cases can be associated with progression to kidney failure, warranting close monitoring. It may well be that the decline in renal function can be delayed by initiating preventative strategies (SGLT2 inhibitors) at an early stage [25].

Three other Alport syndrome spectrum patients presented with kidney failure, one patient had a homozygous variant in *COL4A4* and two adults with heterozygous variants in *COL4A3* and *COLA4* respectively, with a strong family history of CKD. Traditionally heterozygous variants were deemed to have a more favourable course. The variant alone may not always explain the phenotype and therefore a second renal diagnosis would be considered (for example IgA nephropathy). In other cases the variant may provide a clue towards the aetiology, guiding the need for further

investigations. Recent studies acknowledge the wider range of phenotypes in patients with heterozygous *COL4A3* and *COL4A4* and their association with kidney failure [26]. A clinical suspicion of Alport syndrome was not reported in these cases and the classical history of hearing loss was not present. There is not a national screening programme in the UK to detect hearing loss after the neonatal period or haematuria/proteinuria. In many instances patients present in kidney failure and these clinical signs may evade detection. This demonstrates the role of genetic testing in establishing a diagnosis in patients with indistinct phenotypes.

In other cases, the genetic diagnosis will have simply added to what was already clinically suspected. 2/4 patients with *NPHP1* presented with symptoms of polyuria, polydipsia and kidney failure and nephronophthisis was suspected. The clinician still needs to rule out alternative possibilities for renal failure and a genetic diagnosis makes this possible.

Unfortunately, many children presenting with CKD have a delayed diagnosis and present with significant kidney failure [27]. Over one third of patients (36%) in this study presented acutely with kidney failure (including all but one of the *NPHP1* patents and 3 /4 with Alport syndrome. 2/23 (9%) patients with a confirmed monogenic diagnosis were tested due to a family history and were found to be in kidney failure.

The late diagnosis of kidney failure in a significant proportion of our patients with a monogenic cause is of great concern. There are a wider range of pharmaceutical options for reno-protection available (SGLT2 inhibitors) and it is therefore of paramount importance that we focus efforts on identifying these cases early, before kidney failure ensues [28]. Genetic testing will provide a solution in some cases and

we must therefore see its increasing availability in mainstream practice as a significant advantage.

Reflecting on prevention and early identification of genetic renal disorders, the UK National Screening Committee are piloting WGS in 2023 to sequence 100,000 babies as part of a UK new-born screening programme (The Generation Study). They predict that screening will identify 9 children born every day with a rare, treatable, genetic disease where early intervention is crucial [29]. Renal conditions will be amongst those chosen for screening (precise details yet to be decided).

In our study we make note of five patients (*PKHD1* (2), *TMEM138* (1), *CC2D2A* (1) and *ACE* (1)) who presented with kidney failure from birth and sadly passed away. For these families, a genetic diagnosis influenced decisions about withdrawal of care, seeking early specialist input and further reproductive choices (for example pre-implantation genetic diagnosis). There is evidence to suggest that a diagnosis provided by a post-mortem can help families to grieve, providing an explanation for the death [30].

Limitations are intrinsic to NGS based platforms used by the R257 panel, including the inability to detect relevant intronic variants, some copy number variants and certain types of structural variants. WGS technology will improve the ability of the test to detect diagnostic variants across all of these domains. Good phenotyping information (where possible using Human Phenotype Ontology terms) will continue to be essential for interpretation of the genomic data.

Conclusion

This study is the first to evaluate a bespoke genomic testing panel in patients with young-onset unexplained kidney failure, commissioned by NHSE. Results demonstrate a high diagnostic yield with a relatively short turnaround time (3 months if non-urgent). Providing detailed clinical information at the point of requesting is essential, including the patients' family history, suspicion of a particular genetic diagnosis and the presence or absence of extra-renal features, which correlate with a higher likelihood of a monogenic diagnosis being confirmed. The R257 service transitioned to a WGS platform in April 2022 and future studies will aim to compare results from our evaluation of the R257 gene panel with those achieved through WGS.

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Conflict of interest: The authors declare that they have no conflict of interest.

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Informed consent: Written consent for participating in this service evaluation was not required apart from the index case who has signed a consent form. However informed consent was obtained from all participants/ parents for R257 gene panel analysis.

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Supplementary Information

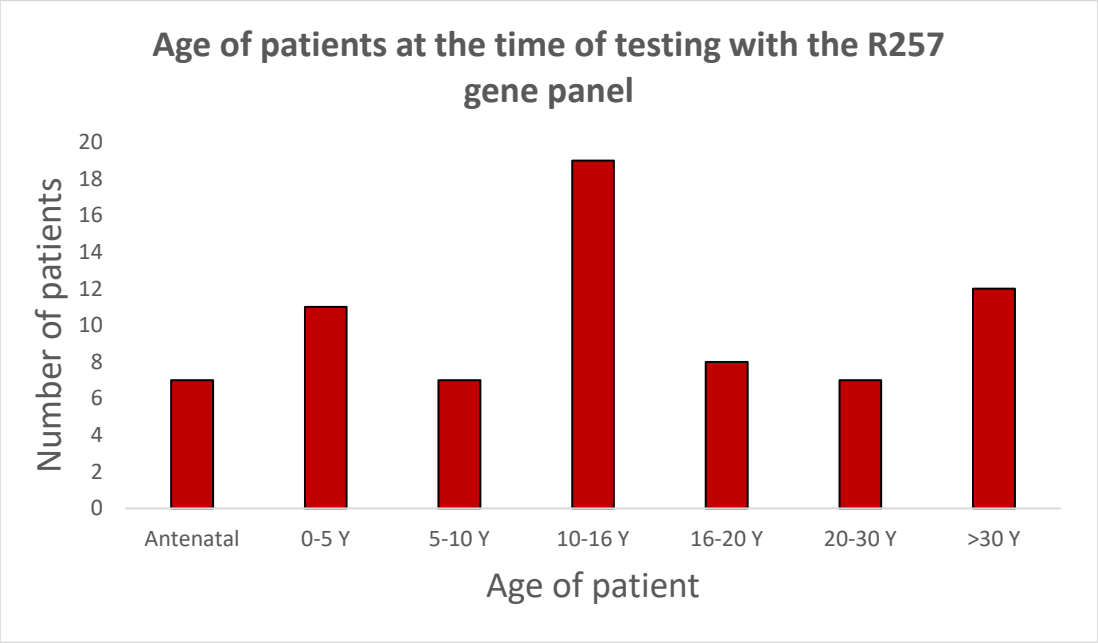
1) Supplement 1 – Pro-forma used for data collection

1. Clinical presentation of the patient
2. Age of presentation
3. Was there a strong clinical suspicion of a genetic disorder at the time of testing?
4. Was there any family history of an underlying renal disease?
5. Were there any extra-renal manifestations identified?
6. Was there a pathogenic or likely pathogenic variant identified? ***If yes, which?***

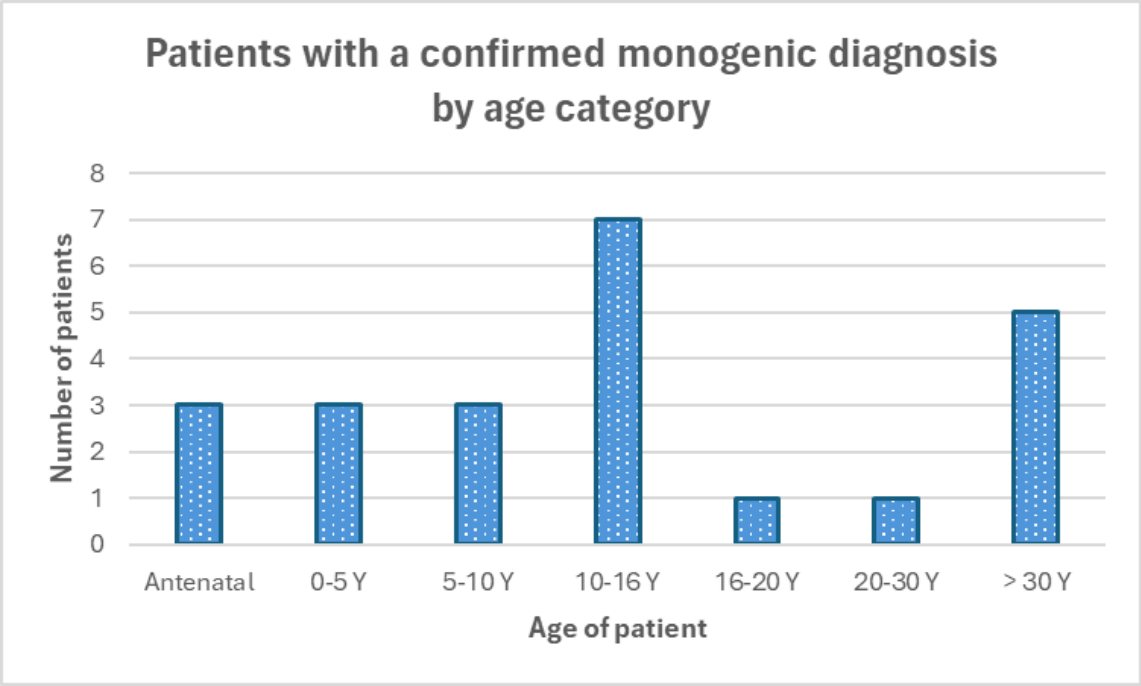
ACE	BB55	COL4A1	FRAS1	ITGA3	NPHP3	RRM2B	TMEM67
ACTG2	BB59	COL4A3	FREM1	ITGA8	NPHP4	SALL1	TP53RK
ACTN4	BNC2	COL4A5	FREM2	ITSN1	NPHS1	SCARB2	TRAF3IP1
AGT	B5ND	C002	GANAB	KIAA0586	NPHS2	SDCCAG8	TRAP1
AGTR1	C3	COQ6	GATA3	KIAA0753	NUP107	SEC61A1	TRIM8
AGXT	C5orf42	C008B	GATM	KIF7	NUP133	SGPL1	TRPC6
AHI1	CC2D2A	CRB2	GLA	KYNU	NUP85	SIX5	TSC1
ALMS1	CD46	CSPP1	GLI3	LAGE3	NUP93	SLC22A12	TSC2
AMN	CENPF	CTNS	GRHPR	LAMB2	OCRL	SLC2A9	TTC21B
ANK56	CEP104	CUBN	GRIP1	LMX1B	OFD1	SLC3A1	TTC8
ANOS1	CEP164	DDX59	HAAO	LRIG2	OSGEP	SLC7A9	TXNDC15
ARHGDIA	CEP290	DGKE	HNF1B	LZTFL1	PAX2	SMARCAL1	UMOD
ARL13B	CEP41	DHCR7	HOGA1	MAGI2	PBX1	TBC1D8B	VHL
ARL6	CEP83	DLC1	HPSE2	MAPKBP1	PKD1	TBX18	VIPAS39
ARMC9	CFB	DNAJB11	HYLS1	MK51	PKD2	TCTN1	VPS33B
B9D2	CFH	DSTYK	ICK	MMACHC	PKHD1	TCTNZ	WDPCP
B9D2	CFHR1	DYNC2H1	IFT122	MT-TE	PLCE1	TCTN3	WDR19
BB51	CFHR3	DZIP1L	IFT43	MUC1	PMM2	TMEM107	WDR35
BB510	CFHR5	EMP2	INF2	MYH9	PODXL	TMEM138	WDR60
BB512	CFI	EYA1	INPP5E	MY01E	REN	TMEM216	WDR73
BB52	CHD7	FAN1	INVS	NEK8	RET	TMEM231	WT1
BB54	CLCN5	FAT1	IQCB1	NPHP1	RPGRIP1L	TMEM237	XDH

Supplementary Figure 2 – Genes tested on the R257 gene panel

Accessible through PanelApp (https://nhsgms-panelapp.genomicsengland.co.uk/) [18]



Supplementary Figure 3 - graph showing the age distribution of all patients at the time of testing with the R257 gene panel



Supplementary Figure 4 – Graph demonstrating the age distribution of patients with a positive genetic result for a pathogenic or likely pathogenic variant

Supplement 5 - Genetic Conditions [30]

Alport syndrome – characterised by progressive kidney failure associated with haematuria, sensorineural hearing loss, and ocular involvement (anterior lenticonus). In this paper we have used the term Alport syndrome spectrum as some patients did not have sensorineural hearing loss.

Autosomal recessive polycystic kidney disease – Often presents at birth or in the neonatal period with polycystic kidneys, hypertension, renal impairment and can be lethal. Other features include pulmonary hypoplasia due to oligohydramnios and may develop hepatic fibrosis and portal hypertension.

Denys-Drash – characterised by kidney disease - glomerulosclerosis, high risk of developing a Wilms' tumour and males present with gonadal dysgenesis

FAT1 related disorder – Glomerular tubulopathy of Steroid-resistant nephrotic syndrome associated with haematuria, ptosis, nephropathy and syndactyly.

Joubert's syndrome – Cerebellar malformation, hypotonia, developmental delay and kidney failure. Associated encephalocele, ocular, liver and endocrine abnormalities

Juvenile Nephronophthisis - a renal ciliopathy characterised by impaired renal concentration, cystic renal disease and progression to kidney failure.

Meckel Gruber syndrome - is a multisystem disorder, with a poor life expectancy due to pulmonary hypoplasia. It is associated with three classic features, occipital encephalocele, polycystic kidneys and polydactyly

Megacystis microcolon intestinal hypoplasia syndrome – typically presents with neonatal intestinal obstruction due to decreased/ absent intestinal peristalsis, a very small colon and largely dilated non-obstructed urinary bladder

Primary coenzyme Q10 deficiency – can cause steroid resistant nephrotic syndrome, cerebellar ataxia, ocular abnormalities and hypertrophic cardiomyopathy

Renal coloboma syndrome – causing small underdeveloped kidneys and malformation of the optic nerve sometimes with retinal involvement. This can progress to CKD stage 5 and visual loss

Renal tubular dysgenesis - severe kidney disorder characterized by abnormal development of the kidneys before birth. Associated with oligohydramnios and pulmonary anhydramnios.

Senior Løken Syndrome – causes nephronophthisis presenting with polyuria, polydipsia and fatigue as well as ocular involvement due to retinal dystrophy

Uromodulin kidney disease - causes slowly progressive kidney disease to CKD stage 5 and can be associated with high uric acid levels and gout