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Utility of Genomic Testing after Renal Biopsy

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Keywords
Renal biopsy · Pathology · Chronic kidney disease · Genetics · Genetic polymorphism

Abstract
Background: Renal biopsy is the mainstay of renal pathological diagnosis. Despite sophisticated diagnostic techniques, it is not always possible to make a precise pathological diagnosis. Our aim was to identify a genetic cause of disease in patients who had undergone renal biopsy and determine if genetic testing altered diagnosis or treatment. ***Methods:*** Patients with suspected familial kidney disease underwent a variety of next-generation sequencing (NGS) strategies. The subset of these patients who had also undergone native kidney biopsy was identified. Histological specimens were reviewed by a consultant pathologist, and genetic and pathological diagnoses were compared. ***Results:*** Seventy-five patients in 47 families underwent genetic sequencing and renal biopsy. Patients were grouped into 5 diagnostic categories based on pathological diagnosis: tubulointerstitial kidney disease (TIKD; $n = 18$); glomerulonephritis (GN; $n = 15$); focal segmental glomerulosclerosis and Alport Syndrome ($n = 11$); thrombotic microangiopathy (TMA; $n = 17$); and nonspecific pathological changes ($n = 14$). Thirty-nine patients (52%) in 21 families (45%) received a genetic diagnosis; 13 cases (72%) with TIKD, 4 (27%) with GN, 6 (55%) with focal segmental glomerulosclerosis/Alport syndrome, and 10 (59%) with TMA and 6 cases (43%) with nonspecific features. Genetic testing resulted in changes in understanding of disease mechanism in 21 individuals (54%) in 12 families (57%). Treatment would have been altered in at least 26% of cases (10/39). ***Conclusions:*** An accurate genetic diagnosis can result in changes in clinical diagnosis, understanding of pathological mechanism, and treatment. NGS should be considered as a complementary diagnostic technique to kidney biopsy in the evaluation of patients with kidney disease.

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Introduction

As a procedure, the percutaneous renal biopsy is nearly 70 years old. Since it was first described by Iversen and Braun [1], kidney biopsy has become the gold standard for renal pathological diagnosis [2]. Light microscopy, immunofluorescence, and electron microscopy have been refined over time to provide increasingly precise classification of kidney disease pathology. Standardized classifications guide therapy and define objective endpoints for treatment [3, 4].

Kidney biopsy is a safe procedure with a high diagnostic yield. It gives useful clinical information in 80% of cases [5, 6]. A prospective study of 80 patients by Turner et al. [7] showed that renal biopsy modified diagnosis in 44% and therapeutic approach in 31% of patients. Other studies have shown that treatment is modified in up to 54% of patients [8].

Despite its utility as a therapeutic tool, pathological findings from renal biopsies are not completely accurate or precise. Even with the implementation of international guidelines, a significant degree of interobserver variability continues to exist [9]. Interobserver agreement is as low as 45% in some reports [10]. Alone, renal biopsy may be inadequate to distinguish different phenotypes of kidney disease and provide a precise diagnosis. Approximately 15% of all incident patients in the United Kingdom who reach end-stage renal disease (ESRD) do not have a primary renal diagnosis [11].

Next-generation sequencing (NGS) technology and associated diagnostic techniques have led to a reclassification of the etiology of many forms of kidney disease. There are now >600 genes known to harbor variants that are associated with kidney disease [12]. A recent study showed that whole exome sequencing can yield a genetic diagnosis in nearly 10% of patients with chronic kidney disease (CKD), including 17% of those with nephropathy of unknown origin [12].

The addition of molecular techniques to kidney biopsy as a diagnostic modality may improve precision and lead to more refined diagnosis, more reliable predictions of prognosis and a wider choice of therapeutic options. It may give better diagnostic certainty for patients and families and facilitates screening and genetic counseling. This may offer direct benefits in terms of an earlier diagnosis, and screening of potential living related renal donors who are twice as likely to develop ESRD as unrelated kidney donors [13].

The Irish Kidney Gene Project was established in 2015 to define the prevalence of a positive family history in a cohort of adult patients with CKD in Ireland and to apply NGS techniques to determine genetic causes of kidney disease in this cohort. Our aim was to identify the genetic cause of kidney disease in a cohort of patients who had previously undergone percutaneous kidney biopsy and to review the initial pathological diagnosis in light of this new information. We aimed to determine if genetic diagnosis would lead to a change in understanding of disease mechanism and if this changed understanding of disease mechanism would have implications for the treatment plan.

Methods

Patient Population

Participants were recruited from patients who attended nephrology services in Ireland from January 2014 to December 2017. Informed consent was obtained from all patients. The study was approved by the medical ethics board at the recruitment sites.

Patients were included if they were aged >18 years, capable of giving consent and had either a self-reported family history of CKD, or extrarenal features consistent with an inherited cause of kidney disease as adjudged by the treating nephrologist. They were excluded if they had not undergone percutaneous native renal biopsy. Demographic and clinical information and family history was obtained from participants. DNA was extracted from blood or saliva samples.

Genetic Diagnosis

A specific genetic diagnosis was obtained by NGS via one of the following 3 methods. Some samples were tested using multiple techniques:

1. In the first cohort of 138 participants, whole exome sequencing was performed in Boston Children's Hospital, Massachusetts, as previously described by Connaughton et al. [14].
2. A second cohort consisted of 54 individuals with autosomal dominant tubulointerstitial kidney disease (ADTKD) who were suspected of having ADTKD-*MUC1* or ADTKD-*UMOD*. Gene testing for *MUC1* C+ insertions was performed at the Broad Institute, Massachusetts, using techniques described elsewhere [15]. *UMOD* mutational analysis was performed in all *UMOD* exons by the Rare Inherited Kidney Disease team of Wake Forest School of Medicine, Winston-Salem, NC [16, 17].
3. A subsequent third cohort of 44 patients was sequenced using targeted NGS. Samples were sequenced in the Royal College of Surgeons in Ireland by targeted NGS using a custom Roche NimbleGen SeqCap or a Roche NimbleGen HeatSeq panel (genes listed in online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000504869) as per the manufacturer's instructions, using 500 ng of input gDNA. Sequencing was performed on an Illumina MiSeq or NextSeq. Sequence data were analyzed using a custom, in-house pipeline. Sequence data were aligned to the NCBI 138/hg38 reference genome and processed using a Burrows-Wheeler Aligner and Picard. Variants were identified using the Genome Analysis ToolKit best practices protocol and annotated using ANNOVAR.

Table 1. Clinical characteristics of 76 individuals who underwent NGS and kidney biopsy

	Total patients (n = 75)	Patients with a genetic diagnosis (n = 39)	Patient with no genetic diagnosis (n = 36)	p value
Age at biopsy, years, median (range)	36 (7–69)	33 (10–61)	38 (7–69)	0.11
Gender, male, n (%)	49 (65)	26 (66)	27 (75)	0.3
Family history, n (%)	69 (92)	37 (95)	32 (89)	0.33
Histological diagnosis, n (%)				
TIKD	18 (24)	13 (33)	5 (14)	
GN	15 (20)	4 (10)	11 (31)	
FSGS/Alport	11 (15)	6 (15.5)	5 (14)	
TMA	17 (23)	10 (26)	7 (19)	
Nonspecific features	14 (18)	6 (15.5)	8 (22)	
Creatinine at biopsy, μmol/L, median (IQR)	153 (101–208)	154 (99–201)	154 (112–258)	0.88
Developed ESRD	52 (69)	28 (72)	24 (66)	0.63
Time in years from initial biopsy and diagnosis to NGS, median (range)	15 (1–46)	17 (1–45)	15 (1–46)	0.24

NGS, next-generation sequencing; TIKD, tubulointerstitial kidney disease; TMA, thrombotic microangiopathy; IQR, interquartile range; ESRD, end-stage renal disease; GN, glomerulonephritis.

Sequences with a minimum coverage of ≥10× were included for analysis. Rare variants (minor allele frequency <0.01 (homozygotes/compound heterozygotes) or minor allele frequency < 0.001 (heterozygotes) in gnomAD control database), functional (exonic/splicing variant), predicted damaging by at least 2 prediction software tools, and in a relevant disease gene (as per Online Mendelian Inheritance in Man) were selected for discussion at a multidisciplinary team meeting.

In all cases, potentially causative variants were classified as pathogenic, likely pathogenic, a variant of unknown significance, likely benign or benign as per the guidelines of the American College of Medical Genetics [18].

Pathological Diagnosis

We identified all sequenced patients who had undergone a renal biopsy. Biopsies were reviewed independently by an experienced renal histopathologist (A.D.) in Beaumont Hospital, Dublin (online suppl. Table 2). Where available, electron micrographs were also reviewed. The histopathologist reassessed the histological slides and compared them to the original results. If there was a discrepancy between the 2, the diagnosis was changed to reflect the diagnosis on re-assessment. The histopathologist was blinded to the gene sequencing results. Where review could not be performed due to inadequate condition or suitability, the original pathological diagnosis was used. Original slides were available and in acceptable condition in 92% of all cases. Electron microscopy was available in 79% of cases.

The medical and histological diagnosis of all patients were reviewed and recorded, including glomerular, interstitial, vascular, and tubular features as well as percentage fibrosis.

Following review of biopsy material, renal pathological diagnosis was divided into 5 categories:

- Tubulointerstitial kidney disease (TIKD)
- Chronic glomerulonephritis (GN)
- Focal segmental glomerulosclerosis (FSGS) and Alport syndrome
- Thrombotic microangiopathy (TMA)
- Nonspecific pattern of injury

Statistical Analysis

Descriptive statistics were expressed using frequencies and proportions.

Unpaired t tests and chi-square were used to test for significance between those in whom a genetic diagnosis was obtained and those in whom one was not obtained. A p value of <0.05 was considered statistically significant.

Results

A total of 75 individuals in 47 families had undergone renal biopsy and genetic testing. Of those 75 patients, a pathogenic or likely pathogenic, disease-causing variant that met American College of Medical Genetics criteria (online suppl. Table 3) was detected in 39 cases (52%) in 21 families (45%). In the remaining 36 patients (48%) and 26 families (55%), we were unable to identify a pathogenic variant. A family history was present in 69 patients (92%).

The mean age of patients at the time of renal biopsy was 36 years and 65% were male. There were no statistical differences in age at biopsy, sex, risk of progressing to ESRD, creatinine at biopsy, or presence of a family history between those who obtained a genetic diagnosis and those that did not (Table 1). The median time from

Table 2. Information on genetic diagnosis in 75 individuals who underwent NGS and histological diagnosis by renal pathological diagnostic group

Pathological diagnosis	Genetic diagnosis	Number affected, *n* (%)
TIKD (*n* = 18)	*MUC1*	6 (34)
	UMOD	4 (22)
	HNF1B	1 (5.5)
	NPHP 1	1 (5.5)
	IFT140	1 (5.5)
	No diagnosis	5 (27.5)
Chronic GN (*n* = 15)	*COL4A5*	2 (13)
	UMOD	1 (7)
	MUC1	1 (7)
	No diagnosis	11 (73)
Focal segmental glomerulosclerosis/ Alport Syndrome (*n* = 11)	*COL4A5*	5 (45)
	FANCI	1 (10)
	No diagnosis	5 (45)
TMA (*n* = 17)	*UMOD*	2 (11.5)
	HNF1B	2 (11.5)
	MUC1	1 (6)
	INF2	4 (24)
	IFT140	1 (6)
	No diagnosis	7 (41)
Nonspecific causes (*n* = 14)	*COL4A5*	1 (7)
	C3	1 (7)
	WNK4	1 (7)
	SLC3A1	1 (7)
	HNF1B	1 (7)
	INF2	1 (7)
	No diagnosis	8 (58)

NGS, next-generation sequencing; TIKD, tubulo-interstitial kidney disease; TMA, thrombotic microangiopathy; GN, glomerulonephritis.

biopsy to genetic diagnosis was 15 years (range 1–46 years).

Following review of the pathological diagnosis, TIKD accounted for the histological diagnosis in 18 cases (24%) and 6 families (13%), chronic GN in 15 patients (20%) and 8 families (17%), FSGS and Alport Syndrome in 11 cases (15%) and 10 families (21%), TMA in 17 cases (23%) and 4 families (9%), and nonspecific findings in 14 patients (18%) or 11 families (23%; Table 2). In the additional 8 families (17%), there was a conflicting pathological diagnosis between 2 or more family members. Six of these families had at least 1 family member whose biopsy showed TMA.

Of the 39 patients in whom a genetic diagnosis was made, the genetic diagnosis was provided by testing in cohort 1 in 13 patients (33%) and had been previously reported by Connaughton et al. [14]. The diagnostic rate in this cohort was 39%. Cohort 2 provided diagnosis in 13 (33%) of all patients. Diagnostic rate was 72%. Cohort 3 provided a genetic diagnosis in 13 patients (33%). Diagnostic rate was 52%.

In the 18 patients with a preexisting pathological diagnosis of TIKD, a genetic diagnosis was made in 13 cases (72%; *MUC1, n = 6; UMOD, n = 4; HNF1B, n = 1; IFT140, n = 1; NPHP1, n = 1*) and 6 families (Table 3). In all 13 cases, there was concordance between the *a priori* histological subtype and the genetic diagnosis. In 3 families, the diagnosis confirmed a suspected clinical and pathological diagnosis (ADTKD-MUC1, ADTKD-UMOD). In 1 family, it helped confirm the cause of extrarenal features (*IFT140* causing Mainzer-Saldino syndrome) in a case of suspected nephronophthisis, in 2 further families (*NPHP1* & *HNF1B*) it helped to identify a diagnosis in patients that had previously only been identified as nonspecific TIKD

Table 3. Information on pre-NGS histological diagnosis and post-NGS genetic diagnosis in the 39 patients in whom a pathogenic variant was identified

Fam ID	ID	Gender	Fam Hx	Age at Bx	Histological diagnosis	Cr. at biopsy, µmol/L	Fibrosis on Bx, %	Genetic Dx	Chr position	c. change p. change	Zygosity	MAF	ACMG	Type
TIKD														
2	2A	M	Yes	38	TIKD/gouty nephropathy	232	50	*UMOD*	16	c.G767G>A p.Cys256Tyr	Het	0	Likely path.	Non-synonymous SNV
2	2B	F	Yes	22	TI fibrosis	–	50	*UMOD*	16	c.G767G>A p.Cys256Tyr	Het	0	Likely path.	Non-synonymous SNV
2	2C	M	Yes	18	TI fibrosis	201	65	*UMOD*	16	c.G767G>A p.Cys256Tyr	Het	0	Likely path.	Non-synonymous SNV
3	3A	F	Yes	47	Familial TIKD	–	80	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
3	3B	F	Yes	38	Familial TIKD	–	70	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
3	3C	F	Yes	43	Active TI nephritis	150	75	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
3	3D	M	Yes	42	Familial TIKD	140	70	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
3	3E	M	Yes	46	Familial TIKD	177	75	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
3	3F	F	Yes	53	TI fibrosis	–	10	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
4	4A*	F	Yes	38	Early TI fibrosis	146	–	*HNF1B*	17	c.544+3_544+6del/	Het	0	Path.	Deletion
5	5A*	F	Yes	19	TI inflammation	1,355	50	*NPHP1*	17	c.555_556insA p.Pro186Hisfs*2	Hom	0	Path.	Non-synonymous SNV
6	6A*	M	Yes	26	Early nephronophthisis	46	<10	*IFT140*	16	c.634G>A p.Gly212Arg	Hom	5.4×10^{-5}	Path.	Non-synonymous SNV
15	15A	F	Yes	54	TIKD	638	50	*UMOD*	16	c.317G>A p.Cys106Tyr	Het	0	Path.	Non-synonymous SNV
GN														
2	2D	M	Yes	52	MPGN	101	20	*UMOD*	16	c.G767G>A p.Cys256Tyr	Het	0	Likely path.	Non-synonymous SNV
7	7A	F	Yes	55	Proliferative GN	67	10	*MUC1*	1	c.ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
8	8A	M	Yes	65	IgA GN	90	30	*COL4A5*	×	c.2959_2976del p.987_992del	Het	0	Likely path.	Non-FS deletion
9	9A	M	Yes	41	Focal proliferative GN	80	<10	*COL4A5*	×	c.3427G>A p.Gly1143Ser	Hemi	0	Likely path.	Non-synonymous SNV
FSGS/Alport syndrome														
11	11A*	M	Yes	20	FSGS	1,350	80	*COL4A5*	×	c.2605G>A p.Gly869Arg	Hemi	0	Path.	Non-synonymous SNV
12	12A*	F	Yes	33	Alport syndrome	100	10	*COL4A5*	×	c.2396G>A p.Gly799Asp	Het	0	Likely path.	Non-synonymous SNV
13	13A*	M	Yes	24	Alport syndrome	170	10	*COL4A5*	×	c.1423+1G>T	Hemi	0	Path.	ESS
14	14A*	M	No	13	FSGS	165	>50	*FANCI*	15	c.217A>T p.Ile73Phe	Hom	1.4×10^{-5}	Likely path.	Non-synonymous SNV

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Table 3. (continued)

Fam ID	ID	Gender	Fam Hx	Age at Bx	Histological diagnosis	Cr. at biopsy, μmol/L	Fibrosis on Bx, %	Genetic Dx	Chr position	c. change p. change	Zygosity	MAF	ACMG	Type
16	16A	M	Yes	34	Alport syndrome	169	10	*COL4A5*	×	c. 1762G>A p.Gly588Ser	Hem	0	Likely path.	Non-synonymous SNV
17	17A	M	Yes	20	Alport syndrome	72	30–55	*COL4A5*	×	c.3310G>T p.Gly1104Cys	Hem	0	Likely path.	Non-synonymous SNV
TMA														
4	4B*	M	Yes	43	Chronic TMA	135	20	*HNF1B*	17	c.544+3_544+6del /	Het	0	Likely Path.	Deletion
6	6B*	F	Yes	11	TMA and TBMN	301	60–70	*IFT140*	16	c.634G>A p.Gly212Arg	Hom	5.4×10^{-5}	Path.	Non-synonymous SNV
15	15B	M	Yes	44	Chronic TMA/FSGS	400	75	*UMOD*	16	c.317G>A p.Cys106Tyr	Het	0	Path	Non-synonymous SNV
15	15C	M	Yes	42	Chronic TMA	133	30	*UMOD*	16	c.317G>A p.Cys106Tyr	Het	0	Path.	Non-synonymous SNV
18	18A	M	Yes	24	TMA and TBMN	99	40	*INF2*	14	c.640C>T p.Arg214Cys	Het	4.08×10^{-6}	Likely path	Non-synonymous SNV
18	18B	F	Yes	23	TMA and TBMN	75	50	*INF2*	14	c.640C>T p.Arg214Cys	Het	4.08×10^{-6}	Likely path.	Non-synonymous SNV
18	18C	M	Yes	28	TMA and TBMN	94	20	*INF2*	14	c. 640 C>T p.Arg214Cys	Het	4.08×10^{-6}	Likely path.	Non-synonymous SNV
18	18D	M	Yes	34	TMA and TBMN	154	60	*INF2*	14	c.640C>T p.Arg214Cys	Het	4.08×10^{-6}	Likely path.	Non-synonymous SNV
19	19A	M	Yes	30	TMA and TBMN	106	20	*MUC1*	1	c. ins(3n+1) in VNTR p. MUC1fs	Het	–	Path.	FS insertion
20	20A	F	Yes	42	Acute TMA	–	15	*HNF1B*	17:36064929	c.1255_1256del p.Ala419fs	Het	0	Likely path.	FS deletion
Non-specific changes														
20	20B	M	Yes	42	Oligomeganephronia	167	75	*HNF1B*	17	c.1255_1256del p.Ala419fs	Het	0	Likely path.	FS deletion
8	8B	M	Yes	56	Arteriosclerosis with fibrosis	225	70	*COL4A5*	×	c.2959_2976del p.987_992del	Hem	0	Likely path.	Non-FS deletion
21	21A*	M	Yes	18	Within normal limits	60	0	*C3*	19	c.4534C>T p.Arg1512Cys	Het	8.12×10^{-6}	Likely path.	Non-synonymous SNV
21	21B*	F	Yes	20	Mesangial proliferation	170	60–70	*INF2*	14	c.353T>A p.Ile118Asn	Het	0	Path.	Non-synonymous SNV
22	22A*	F	Yes	32	Arteriosclerosis	62	5	*WNK4*	17	c.506C>T p.Pro169Leu	Het	0	Path.	Non-synonymous SNV
23	23A*	M	No	25	Severe fibrosis	191	>70	*SLC3A1*	2	c.1799G>A p.Gly600Glu	Het	7×10^{-5}	Likely Path.	Non-synonymous SNV

* Genetic diagnosis as reported by [14].

NGS, next-generation sequencing; Fam ID, family identity number; ID, personal identity number; Fam Hx, family history; Bx, biopsy; Cr, creatinine; Dx, diagnosis; Chr, chromosome; c. change, nucleotide change; p. change, amino acid change; MAF; minor allele frequency; ACMG, American College of Medical Genetics; A, adenine; M, male; F, female; TIKD, tubulointerstitial kidney disease; TI, tubulointerstitial; GN, glomerulonephritis; FSGS, focal segmental glomerulosclerosis; TBMN, thin basement membrane nephropathy; del, deleterious; ESS, essential splice site; fs, frameshift mutation; FS, frameshift; C, cytosine; G, guanine; Hem, hemizygous; Het, heterozygous; Hom, homozygous; Ig, immunoglobulin; Path., pathogenic; SNV, single nucleotide variation; T, thymine; TMA, thrombotic microangiopathy.

Table 4. Information on phenotype and histological diagnosis among families and family members, alteration to final diagnosis, and potential alterations to treatment following next generation sequencing

Family ID	Number of affected individuals	ID	Phenotype	Histological diagnosis	Potential change in diagnosis	Genetic diagnosis	Final diagnosis (OMIM phenotype MIM no.)	Material change in diagnosis	Potential treatment change	Nature of change
2	4	2A	Progressive CKD, onset in 20s and early onset gout	TIKD or gouty nephropathy	Yes	*UMOD*	ADTKD–*UMOD* (603860)	No	No	
		2B	Progressive CKD, onset in 20s and early onset gout	TI fibrosis	Yes	*UMOD*	ADTKD–*UMOD* (603860)	No	No	
		2C	Progressive CKD, onset in 20s and early onset gout	TI fibrosis	Yes	*UMOD*	ADTKD–*UMOD* (603860)	No	No	
		2D	Progressive CKD, onset in 20s and early onset gout	MPGN/DDD	Yes	*UMOD*	ADTKD–*UMOD* (603860)	No	No	
3	6	3A	Progressive non-proteinuric CKD, detected age 35	Familial TIKD	Yes	*MUC1*	ADTKD–*MUC1* (174000)	No	No	
		3B	Progressive non-proteinuric CKD detected age 38	Familial TIKD	Yes	*MUC1*	ADTKD–*MUC1* (174000)	No	No	
		3C	Progressive non-proteinuric CKD detected mid-30s	Acute TI fibrosis	Yes	*MUC1*	ADTKD–*MUC1* (174000)	No	No	
		3D	Progressive non-proteinuric CKD detected mid-30s	Familial TIKD	Yes	*MUC1*	ADTKD–*MUC1* (174000)	No	No	
		3E	Progressive non-proteinuric CKD age 40	Familial TIKD	Yes	*MUC1*	ADTKD–*MUC1* (174000)	No	No	
		3F	Progressive non-proteinuric CKD detected mid-30s	TI fibrosis	Yes	*MUC1*	ADTKD–*MUC1* (174000)	No	No	
4	2	4A*	CKD mid-30s, diabetes mellitus and annulara pancreas	TIKD	Yes	*HNF1B*	ADTKD–*HNF1B* (137920)	No	Yes	Liver and parathyroid screening
		4B*	CKD age 42, diabetes mellitus	TMA	Yes	*HNF1B*	ADTKD–*HNF1B* (137920)	No	Yes	Liver and parathyroid screening
5	1	5A*	CKD, age 21, small cystic kidneys on renal US	TI nephritis	Yes	*NPHP1*	Nephronophthisis 1, juvenile (256100)	No	No	
6	2	6A*	Small cystic kidneys, retinitis pigmentosa, mild learning disability	Early nephronophthisis	Yes	*IFT140*	Mainzer-Saldino syndrome (266920)	No	No	
		6B*	Small cystic kidneys, retinitis pigmentosa, mild learning disability	TMA and TIKD	Yes	*IFT140*	Mainzer-Saldino syndrome (266920)	No	No	
7	1	7A	Low complement (C3), gout, arthropathy, family history	Proliferative glomerulonephritis	Yes	*MUC1*	ADTKD–*MUC1* (174000)	Yes	Yes	Steroid avoidance

Table 4. (continued)

Family ID	Number of affected individuals	ID	Phenotype	Histological diagnosis	Potential change in diagnosis	Genetic diagnosis	Final diagnosis (OMIM phenotype MIM no.)	Material change in diagnosis	Potential treatment change	Nature of change
8	2	8A	Microscopic hematuria and CKD III	IgA nephropathy	Yes	*COL4A5*	Alport syndrome I, X linked (301050)	Yes	Yes	ENT and ophthalmology review
		8B	Progressive CKD detected in 40s, hematuria detected in 20s	Arteriosclerosis with fibrosis	Yes	*COL4A5*	Alport syndrome I, X linked (301050)	Yes	Yes	ENT and ophthalmology review
9	1	9A	Hypertension, proteinuria and haematuria	Focal proliferative GN	Yes	*COL4A5*	Alport syndrome I, X linked (301050)	Yes	Yes	ENT and ophthalmology review
11	1	11A	Progressive CKD, glaucoma and hearing impairment	FSGS	Yes	*COL4A5*	Alport syndrome I, X linked (301050)	Yes	No	
12	1	12A*	Hematuria and proteinuria, nephew with hearing loss	Alport syndrome	No	*COL4A5*	Alport syndrome I, X linked (301050)	No	No	
13	1	13A*	Progressive hematuria, CKD and hearing loss	Alport syndrome	No	*COL4A5*	Alport syndrome I, X linked (301050)	No	No	
14	1	14A*	Bilateral small kidneys, gout, retinitis pigmentosa, anemia and pseudotumor cerebri	FSGS	Yes	*FANCI*	Fanconi Anaemia, complementation group I (609053)	Yes	Yes	Cancer screening
15	3	15A	Progressive CKD	TIKD	Yes	*UMOD*	ADTKD–*UMOD* (603860)	Yes	No	
		15B	Progressive CKD in mid-50s, Bechet's disease	Chronic TMA/FSGS	Yes	*UMOD*	ADTKD–*UMOD* (603860)	Yes	No	
		15C	Sarcoidosis, CKD	Chronic TMA	Yes	*UMOD*	ADTKD–*UMOD* (603860)	Yes	No	
16	1	16A	Hematuria, progressive CKD and hearing loss	Alport syndrome	No	*COL4A5*	Alport syndrome I, X linked (301050)	No	No	
17	1	17A	Hematuria, progressive CKD and hearing loss	Alport syndrome	No	*COL4A5*	Alport syndrome I, X linked (301050)	No	No	
18	4	18A	Progressive CKD, 1.8 g proteinuria, no evidence of systemic TMA	TMA and TBMN	Yes	*INF2*	Glomerulosclerosis, focal segmental, 5 (613237)	Yes	No	
		18B	Proteinuria but normal renal function, age 42, no evidence of systemic TMA	TMA and TBMN	Yes	*INF2*	Glomerulosclerosis, focal segmental, 5 (613237)	Yes	No	
		18C	Proteinuria, progressive CKD, no evidence of systemic TMA	TMA and TBMN	Yes	*INF2*	Glomerulosclerosis, focal segmental, 5 (613237)	Yes	No	
		18D	Progressive CKD, ESRD age 36, no evidence of systemic TMA	TMA and TBMN	Yes	*INF2*	Glomerulosclerosis, focal segmental, 5 (613237)	Yes	No	

Table 4. (continued)

Family ID	Number of affected individuals	ID	Phenotype	Histological diagnosis	Potential change in diagnosis	Genetic diagnosis	Final diagnosis (OMIM phenotype MIM no.)	Material change in diagnosis	Potential treatment change	Nature of change
19	1	19A	Progressive CKD, no systemic evidence of TMA	TMA	Yes	*MUC1*	ADTKD–*MUC1* (174000)	Yes	No	
20	2	20A	Cystic kidney with slowly progressive CKD, raised liver enzymes, no evidence of systemic TMA	Acute TMA	Yes	*HNF1B*	ADTKD–*HNF1B* (137920)	Yes	Yes	Diabetic screening
		20B	Congenital abnormality of the kidney	Oligomegonephronia	Yes	*HNF1B*	ADTKD–*HNF1B* (137920)	Yes	Yes	Diabetic screening
21	2	21A*	Low complement (C3) levels and normal renal function	Within normal limits	Yes	*C3*	C3 deficiency (612925)	Yes	No	
		21B*	ESKD age 23, bland urinalysis	Mesangial proliferation	Yes	*INF2*	Glomerulosclerosis, focal segmental, 5 (613237)	Yes	No	
22	1	22A*	CKD diagnosed aged 26, hypertension, father and sister with history of CKD	Arteriosclerosis	Yes	*WNK4*	Pseudo-hypoaldosteronism – hypertensive CKD (614491)	Yes	Yes	Salt avoidance and use of thiazides
23	1	23A*	Gout and progressive kidney disease and nephrotic range proteinuria in mid-20s	Severe fibrosis	Yes	*SLC3A1*	Cystinuria (220100)	Yes	Yes	Stone prevention, increased fluid intake

* Genetic diagnosis as reported by [14].

NGS, next-generation sequencing; CKD, chronic kidney disease; TI, tubulointerstitial; IgA, immunoglobulin A; TMA, thrombotic microangiopathy; TBMN, thin basement membrane nephropathy; FSGS, focal segmental glomerulosclerosis; TIKD, tubulointerstitial kidney disease; GN, glomerulonephritis; DDD, dense deposit disease.

(Table 4). In the 5 cases in which a diagnosis could not be made, a family history was present in all cases.

In the chronic GN group, a genetic diagnosis was made in 4 cases (27%; *COL4A5, n = 2; MUC1, n = 1; UMOD, n = 1*) in 4 families (Table 3). In each case, a genetic diagnosis was advanced which indicated an alternative diagnosis of kidney disease. In those in whom a *COL4A5* variant was identified, one had a biopsy diagnosis of IgA nephropathy and the other a diagnosis of focal proliferative GN. In those in whom a TIKD-associated gene was identified, 1 patient (*UMOD*) had membranoproliferative GN on biopsy. The other patient (*MUC1*) had a history of gout and multiple family members with kidney disease but had initially presented with a clinical as well as histological phenotype consistent with systemic lupus erythematosus (Table 4).

In the FSGS and Alport Group, genetic diagnosis was made in 6 cases (55%) (*COL4A5*, *n* = 5; *FANCI*, *n* = 1; Table 3) in 6 families. Four patients with an a priori diagnosis of Alport syndrome had their diagnosis confirmed (*COL4A5*). A further patient who had previously been simply labeled FSGS was also found to have a diagnosis of *COL4A5*.

In the TMA group, 10 cases (59%) in 6 families received a genetic diagnosis (*UMOD, n = 2; HNF1B, n = 2; MUC1, n = 1; INF2, n = 4; IFT140, n = 1*; Table 3). No patient had a phenotype consistent with a primary TMA or hemolytic uremic syndrome. In the nonspecific findings group, a genetic diagnosis was made in 6 cases (43%; *COL4A5, n = 1; C3, n = 1; WNK4, n = 1; SLC3A1, n = 1; HNF1B, n = 1; INF2, n = 1*; Table 3). This reclassified patients with TMA or nonspecific findings into the TIKD group in 7 cases (*MUC1, UMOD, IFT140, HNF1B*) and into the FSGS and Alport Group in 6 cases (*COL4A5, INF2* related FSGS). Three cases had nonspecific genetic

diagnoses including pseudohypoaldosteronism (*WNK4*), low complement C3 (*C3*), and cystinuria (*SLC3A1*; Table 3).

A genetic diagnosis helped to alter or clarify the diagnosis in 31 patients (79%) and 17 families (81%) and materially altered the diagnosis in 21 patients (54%) in 12 families (57%) in whom a genetic diagnosis was made or 28% of patients and 26% of families who underwent biopsy (Table 4). A genetic diagnosis had the potential to alter treatment in 10 cases (26%) of those with a genetic diagnosis and 13% of the total group who underwent biopsy. These potential interventions included screening, with the referral to ophthalmology and hearing assessment in 4 cases of undiagnosed Alport syndrome, diabetic screening in cases of renal cysts and diabetes syndrome, and novel treatments, such as the addition of thiazide diuretics in a patient diagnosed with pseudohypoaldosteronism (Table 4).

Discussion

Renal biopsy remains the gold standard for diagnosis of renal disease and a useful tool in predicting diagnosis and prognosis in patients with CKD. However, it remains imprecise when differentiating certain renal disorders. This is partially due to interobserver variability and partially due to heterogeneity of many kidney diseases. We have demonstrated that NGS sequencing provides a deeper understanding of the mechanism of kidney disease, and this potentially allows for more rational selection of treatment.

In our cohort, genetic diagnosis was most sensitive in TIKD. We made a diagnosis in 72% of those who had been biopsied. However, even in those groups where inherited disease is not suspected, genetic testing may be valuable. One patient diagnosed with TMA, one with MPGN and one with proliferative vasculitis were suggested to have an alternate diagnosis of familial TIKD following review. This is consistent with the findings of Groopman et al. [12] who showed that even in what are traditionally thought to be multifactorial disorders such as hypertensive or diabetic kidney disease, a monogenic diagnosis may still be identified in 1–2.5% of cases. Our findings suggest that *COL4A5* disorders in adults may still be underdiagnosed on biopsy alone. This would be consistent with recent evidence that *COL4A* pathogenic variants are an underrecognized cause of FSGS in patients without the classic hearing loss of Alport syndrome [19, 20]. A recent paper identified monogenic disorders in 9% of adults with FSGS, the majority of which were *COL4A* pathogenic variants [21].

In those in which a genetic cause of kidney disease was identified, we have shown an increased precision or change in diagnosis in 81% of families and 79% of patients. This does not account for any affected family members that did not undergo biopsy, whom are also likely to be affected by genetic diagnosis. There was a potential to alter management in 26% of patients. In particular, it would allow for screening for extrarenal features, such as diabetes in patients diagnosed with diabetes and renal syndrome (*HNF1B*) and hearing loss in Alport syndrome (*COL4A5*). Genetic diagnosis can facilitate avoidance of toxic inappropriate therapies [22, 23]. It may help avoid corticosteroid therapy in patients with the appearance of tubulointerstitial nephritis on biopsy but a genetic diagnosis of ADTKD such as *MUC1*. Though none of our biopsied patients received steroids due to known family histories, many had biopsies consistent with an acute interstitial nephritis, which would traditionally receive corticosteroids.

The limitations of this study are its size. Only 39 patients had both a histological and genetic diagnosis. While care was taken to ensure a correct histological diagnosis, in a handful of cases, not all modalities were available for review, and in 2 cases, only original biopsy reports were available. In addition, it was not possible to rule out the presence of dual diagnoses. For instance, patient 7A presented with arthropathy, low C3 levels, and a biopsy showing acute GN, and they were treated acutely for systemic lupus erythematosus. While presentation of subsequent family members with CKD led to subsequent screening and detection of a pathogenic *MUC1* variant, the retrospective nature of the analysis means it is difficult to assess what role, if any, this played in the patient's initial presentation.

Currently, genetic testing remains time consuming and is unlikely to replace renal biopsy as the gold standard for diagnosis due to rapidity of turnaround. However, with increased availability, development of new technologies, and falling cost, we believe NGS will have a major role to play in combination with kidney biopsy in the diagnosis of CKD and may provide additional information beyond what kidney biopsy may supply.

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Author Contributions

S.L.M.: conception analysis and preparation of paper; A.D. and N.K.F.: review of pathology; K.A.B., D.M.C., C.P.S., K.K., G.L.C., A.J.B., and F.H.: genetic analysis; C.K.: patient recruitment and analysis; L.A.R., K.K., and M.A.L.: data collection; B.D.: paper preparation; P.J.C.: paper conception and writing.

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