Title: Diagnostic Utility of Genetic Testing in Patients Undergoing Renal Biopsy

Running Title: Genetic Testing in Renal Biopsy Patients

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ABSTRACT:

Background: High throughput DNA testing is becoming established as a standard diagnostic test in the renal clinic. Previously published studies on cohorts of patients with unexplained chronic kidney disease of a suspected genetic aetiology have suggested a diagnostic yield for genomic sequencing of up to 18%. Here we determine the yield of targeted gene panel in a clinically un-screened cohort of patients referred for percutaneous native renal biopsy.

Methods: Patients who underwent renal biopsy for investigation of chronic kidney disease were sequenced using genomic sequencing panel covering 227 genes in which variation is known to be associated with monogenic CKD. Candidate disease-causing variants were assessed for pathogenicity using guidelines from the American College for Medical Genetics and Genomics.

Results: Fifty CKD patients were recruited and sequenced. A molecular diagnosis was obtained for two patients (4%).

Conclusions: A molecular diagnosis is possible using genomic testing in around 4% of clinically unscreened patients undergoing renal biopsy. Genetic screening may be useful for diagnosis in a subset of CKD patients, but is most valuable when applied to patients with suspected heritable forms of kidney disease.

INTRODUCTION:

Genetic testing is becoming increasingly available as a viable first line diagnostic test in chronic kidney disease (CKD) (Bullich et al. 2018; Harris 2018). Recent studies have shown that genomic sequencing may provide a molecular diagnosis in up to 10% of all patients with CKD and 18% of those who have CKD of unknown origin (Groopman et al. 2019). The diagnostic yield from genetic testing may be even higher in patients with a family history of renal disease (Connaughton et al. 2019). These recent studies sequenced patients referred by treating clinicians who suspected inherited kidney disease. Here, we assess the diagnostic yield of targeted genomic sequencing in a patient cohort early in their diagnostic journey, referred for percutaneous native renal biopsy, without clinical screening for suspected inherited kidney disease.

REUSLTS:

In total, 237 biopsy samples were screened, of which 84 were renal transplant biopsies. Of the remaining 154 native renal biopsies, a further 52 were excluded because DNA was unavailable or because sampling was inadequate for diagnosis at the time of biopsy, or biopsy was not ultimately performed. Following review, another 51 samples were excluded (see Figure 1). Ultimately, 50 samples underwent sequencing.

The median age at biopsy was 48 years, 60% were male. The most common reason for renal biopsy was a deterioration in renal function (22%) measured as rise in serum creatinine concentration or fall in glomerular filtration rate (GFR), and the development of nephritic syndrome (30%), followed by nephrotic syndrome (12%), haematuria and proteinuria (12%) and isolated proteinuria (8%) or haematuria (8%). On biopsy, the most common histological diagnosis was IgA Nephropathy, accounting for 20 patients (40%), 9 (16%) with other forms of glomerulonephritis, 6 (12%) with arteriosclerosis, 8 (16%) with chronic thrombotic microangiopathy (TMA) and five (10%) with thin basement membrane nephropathy (TBMN) and one with Alport syndrome (2%). Two patients (4%) had mixed pathological findings. Diagnostic variants were identified in two patients (2/50, 4%) (see Table 1).

68 Table 1: Variants Considered for Pathogenicity (n=50 total patients)

Manuscript ID	Gene	GT	Gene Inheritance Pattern	ACMG classification	ACMG evidence	MAF (gnomAD)	Type	RefSeq	HGVS	AA position	ClinVar ID	Total coverage	Average coverage	% Above 20X	Phenotype
213	EHHADH	het	AD	VUS	PVS1	7.37E-05	frameshift deletion	NM_001966.4	c.594_595del	p.L198fs	SCV001328294	7768	194.7	100	Iga Nephropathy
8	FN1	comphet (dom)	AD	VUS	PP2, PP3, BS1	0.0047	missense	NM_212482.4	c.4486C>T	p.R1496W	SCV001328286	7517	310.64	100	Non specific
8	FN1	comphet (dom)	AD	VUS	PP2, PP3, BS1	0.0024	missense	NM_212482.4	c.1070G>A	p.G357E	SCV001328267	9471	306.53	100	Non specific
249	COL4A1	het	AD	VUS	PP2, PP3, BP6	0.0029	missense	NM_001845.6	c.161C>T	p.P54L	SCV001328270	3222	216.24	100	Iga Nephropathy
256	COL4A3	het	AR/AD	VUS	PP3	4.47E-05	missense	NM_000091.5	c.4700T>G	p.I1567S	SCV001328289	3570	212.54	100	Arteriosclerosis
261	CFH	het	AD	Likely pathogenic	PVS1, PM2	0	stopgain	NM_000186.4	c.2517C>A	p.C839Ter	SCV001305469	5199	245.25	100	Arteriosclerosis

261	COL4A4	het	AR/AD	Pathogenic	PVS1, PM2, PP3	0	frameshift deletion	NM_000092.5	c.4603_4604del	p.Q1535fs	SCV001305364	79477	228.38	100	Arteriosclerosis
264	SLC9A3R1	het	AD	VUS	PP3, PP5	0.0019	missense	NM_004252.5	c.458G>A	p.R153Q	SCV001328287	53389	232.13	100	TMA
286	COL4A4	het	AR/AD	Pathogenic	PVS1, PS1	6.90E-05	stopgain	NM_000092.5	c.2906C>G	p.S969Ter	SCV001305529	35958	210.28	100	TBMN
14	APOA1	het	AD	VUS	PM4, BS1	0.0002	nonframeshift deletion	NM_001318018.2	c.391_393del	p.131_131del	SCV001328284	146178	220.15	100	Non specific
203	CFHR5	het	AD	VUS	PVS1, BS1	0.0021	frameshift insertion	NM_030787.4	c.480dupA	p.P160fs	SCV001328290	46564	232.82	100	Iga Nephropathy
203	CFHR5	het	AD	VUS	PP3, PP5, BS1	0.0014	missense	NM_030787.4	c.622T>C	p.C208R	SCV001328296	74760	318.13	100	Iga Nephropathy
16	DSTYK	het	AD	VUS	PP3, BS1	0.0009	missense	NM_015375.3	c.2776G>T	p.D926Y	SCV001328277	63745	252.96	100	TMA
16	WT1	het	AD	VUS	PM2, PP3	0	missense	NM_024426.6	c.576G>T	p.Q192H	SCV001328293	148187	212.61	100	TMA
43	ZNF423	het	AR/AD	VUS	PP2, PP3	6.92E-05	missense	NM_015069.4	c.2251C>T	p.R751C	SCV001328275	944847	290.19	100	MPGN
20	APOA1	het	AD	VUS	PM1, PP3	0	missense	NM_001318018.2	c.625G>A	p.G209S	SCV001328297	944847	290.19	100	TBMN
139	ACTN4	het	AD	VUS	PP2, PP3	4.51E-05	missense	NM_004924.6	c.928C>T	p.R310W	SCV001328306	176954	266.5	100	Minimal change disease

142	DSTYK	het	AD	VUS	PP3, BS1	0.0009	missense	NM_015375.3	c.2776G>T	p.D926Y	SCV001328277	75958	257.48	100	AIN
152	EHHADH	het	AD	VUS	PP3, BS1	0.0097	missense	NM_001966.4	c.2108C>T	p.S703F	SCV001328274	27071	172.43	100	Membranous
158	UMOD	het	AD	VUS	PP2, PP3	2.03E-05	missense	NM_003361.3	c.1243C>T	p.R415C	SCV001328268	31225	151.58	100	Iga Nephropathy
159	FREM1	het	AD	VUS	PP3, BS1	0.0001	missense	NM_144966.7	c.1640C>G	p.A547G	SCV001328271	74568	191.2	100	Iga Nephropathy
159	SOX17	het	AD	VUS	PM4, BS1	0.0072	nonframeshift insertion	NM_022454.4	c.948_949insCACCAG	p.Q316delinsQHQ	SCV001328307	105825	108.32	100	Iga Nephropathy
1	CFHR1	hom	AR/AD	VUS	PVS1,PP3	0.0018	splicing	NM_002113.3	c.790+1G>A		SCV001328302	42400	170.97	100	Iga Nephropathy
1	COL4A3	het	AR/AD	VUS	PP3	0.0003	missense	NM_000091.5	c.1886C>T	p.T629M	SCV001328273	64232	302.98	100	Iga Nephropathy
1	FN1	het	AD	VUS	PP2, PP3, BS1	0.0002	missense	NM_212482.4	c.5954C>A	p.P1985H	SCV001328295	86861	277.51	100	Iga Nephropathy
1	FN1	het	AD	VUS	PP2, PP3	5.28E-05	missense	NM_212482.4	c.3130G>A	p.V1044M	SCV001328281	36521	262.74	100	Iga Nephropathy
175	PKD1	het	AD	VUS	PP3, PP5	0.0009	missense	NM_001009944.3	c.12460C>T	p.R4154C	SCV001328269	141278	263.58	100	TBMN
186	SLC7A9	het	AR/AD	VUS	PVS1, PM2	0	stopgain	NM_001985.3	c.292C>T	p.R98C	SCV001328279	83790	301.4	100	Iga Nephropathy

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Patient 261: This 40 year-old female patient had an American College of Medical Genetics and Genomics (ACMG) classified pathogenic (PVS1, PM2, PP3) heterozygous frameshift deletion in Collagen Type IV Alpha 4 Chain (*COL4A4*) (NM_000092.4:exon47:c.4603_4604del:p.Q1535fs), suggestive of autosomal dominant Alport-type disease (Phenotype MIM number 203780) or autosomal dominant TBMN. This variant was absent from the gnomAD database (Karczewski et al. 2019). The patient presented with microscopic haematuria and proteinuria during pregnancy, along with pregnancy-induced hypertension. Her hypertension resolved post-pregnancy, but she had persistent proteinuria and haematuria. Her renal function was preserved. The patient also carried an ACMG- Pathogenic (PVS1 (null variant), PS1 (previously established pathogenic variant), PM2 (absent from controls)) stop-gain variant in Complement Factor H (*CFH*) (NM_000186.3:exon16:c.C2517A:p.C839Ter). Patient 261 presented with haematuria and proteinuria and notably had a low C3 level (consistent with the presence of the pathogenic *CFH* variant). Her proteinuria improved with renin-angiotensin-aldosterone-system (RAAS) blockade, but did not entirely resolve. Biopsy showed TBMN as well as mild arteriosclerosis and atherosclerosis, prominent double contour formation and TMA, with 2/10 sclerosed glomeruli. The patient had no history of hearing loss and no family history of kidney disease or hearing loss. Sixty months of follow-up did not reveal significant loss of renal function over time. Parents were unavailable for further testing. We conclude that this patient has a thin basement membrane causing haematuria as a result of a pathogenic variant in *COL4A4* as well as low C3 as a result of a pathogenic variant in *CFH*.

Patient 286: A heterozygous stopgain variant in *COL4A4* (NM_000092.4:exon32:c.2906C>G:p.S969Ter) was identified in a 39 year-old female patient which was classified using ACMG guidelines as pathogenic (PVS1 (null variant), PS1 (previously established pathogenic variant)). The patient had normal renal function, with a history of loin pain and microscopic haematuria. She had been treated multiple times for urinary tract infection on the basis of dipstick haematuria, but did not self-report any other symptoms of urinary tract infections. The patient reported an extensive family history of loin pain and at least one relative with advanced CKD. She did not have any self-reported hearing or visual disturbance. Biopsy results indicated TBMN. We conclude that this patient has thin basement membrane nephropathy due to the presence of a heterozygous *COL4A4* variant.

Variants of Unknown Significance: Additionally, 25 ACMG-classified variants of unknown significance (VUS) were identified in 18 patients, including four loss-of-function variants and two truncating variants (see Table 1). Five patients without a molecular diagnosis carried more than one VUS. An *SLC7A9* (solute carrier family 7, member 9) stop-gain variant (NM_001985:exon3:c.C292T:p.R98C) in patient 186, was classified as Likely Pathogenic using ACMG guidelines, but following clinical review, was determined to be a poor phenotypic match and re-classified as a VUS.

DISCUSSION:

Genetic testing using DNA from peripheral blood in an undifferentiated population of patients undergoing renal biopsy led to a diagnostic rate of 4% in a cohort of 50 patients. Testing in a larger group of patients is merited. Whole exome and genome sequencing may increase the rate of diagnosis, however panel-based sequencing is already in widespread

use as a first line test for screening those with suspected inherited kidney disease and reflects current clinical practice (Mallett et al. 2017; Heyne et al. 2019; Lata et al. 2018; Bullich et al. 2018). We expect the diagnostic yield to rise as databases mature and further evidence emerges in the literature.

Both patients carried heterozygous, diagnostic variants in *COL4A4*. Both had histology consistent with TBMN. Though this gene has typically been associated with recessive Alport syndrome, multiple reports (Marcocci et al. 2009; Hines et al. 2018; Longo et al. 2002) have demonstrated that patients with heterozygous *COL4A4* or *COL4A3* variants can develop significant renal disease and can benefit from early initiation of renin-angiotensin-aldosterone system blockade (Stock et al. 2017).

The diagnostic yield obtained (4%) is lower than that reported in previous studies of CKD patients (10-18%)(Groopman et al. 2019), but this yield is notable given that these patients are not clinically screened for suspected heritable forms of kidney disease. These results suggest that genetic screening may be useful for diagnosis in a subset of CKD patients. However, without careful selection, clinical acumen and examination of the pedigree by an experienced clinician or clinical geneticist, the yield of testing in an un-screened cohort may be low. An effort to define the health economic as well as clinical utility of genomic testing in unscreened cohorts of CKD patients may be an area for future research.

METHODS:

Samples were obtained from the North Dublin Renal Biobank (NDRBB) which was established in 2010 to obtain tissue samples from patients with renal disease. Blood, urine and renal tissue samples were collected prospectively from those undergoing percutaneous renal biopsy. We recruited sequential individuals who underwent renal biopsy for investigation of chronic kidney disease in Beaumont Hospital between 2010 and 2018, if they were over the age of 18 and capable of giving informed consent. Patients were excluded from analysis if:

- They underwent transplant renal biopsy
- They underwent re-biopsy to reassess a known condition
- They were thought to have an acute kidney injury (AKI) secondary to a defined insult. This was considered to be the case if they had an acute rise in creatinine **and** a diagnosis on biopsy of acute tubular necrosis (ATN) or acute interstitial nephritis (AIN)
- They had a positive ANCA and a biopsy showing pauci-immune vasculitis
- They were a known diabetic and had a diagnosis consistent with diabetic nephropathy

DNA was extracted from blood lymphocytes. Genomic sequencing was performed in-house, with library preparation using a previously described targeted renal disease gene panel (Cormican et al. 2019). This sequencing method is unable to detect small insertions and deletions in the variable number tandem repeat region of *MUC1*. Exonic and splicing variants were prioritised for multi-disciplinary team discussion if they had a minor allele frequency (MAF) <1%. Synonymous variants were excluded from analysis. Variant

pathogenicity was classified according to the American College of Medical Genetics and Genomics guidelines (Richards et al. 2015). A variant was classified as diagnostic if it was categorised by the ACMG guidelines as 'Likely Pathogenic' or 'Pathogenic' and was a good phenotypic match. Sequencing and variant interpretation was conducted in a research (non-accredited) capacity, diagnostic variants were confirmed using an accredited test from a service provider and reported back to patients.

Supplementary Table 1: Sequencing Statistics

Sequencing Type	Custom Targeted Panel
Library Preparation Method	Roche HyperPrep
Number of Samples per Run	96
Illumina Sequencing Machine	Illumina NextSeq
Sequencing Kit	Illumina NextSeq 500/550 Mid Output Kit v2
Read Type	Paired end sequencing
Read Length	2x150bp
Average Total Reads per Sample (Range)	192745257 (133513493 - 247284970)
Average Coverage per Sample (Range)	206X (143-265X)
Average % Bases above 20X (Range)	99.3% (98.4-99.7%)

ADDITIONAL INFORMATION

Data Deposition and Access

All variants discussed in this manuscript have been submitted to ClinVar (see Table 1) and requests for access to raw sequence data can be made via direct contact with the corresponding author.

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Ethics Statement:

Written, informed consent was obtained from all participants of this study. Ethical approval was provided from the Ethics committee of Beaumont Hospital, Dublin, Ireland (REC 12/75).

Conflict of Interest Statement:

None declared

Author Contributions:

KAB, SLM, GLC, CG and PJC conceived the idea and planned the experiments. SLM, PJC and DS facilitated recruitment of patients to this study. AD, BD and SLM assessed the renal biopsy samples. SLM and KAB drafted the manuscript with support from PJC and GLC. KAB conducted the sequencing and bioinformatic analysis of sequencing data. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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238 Figure 1

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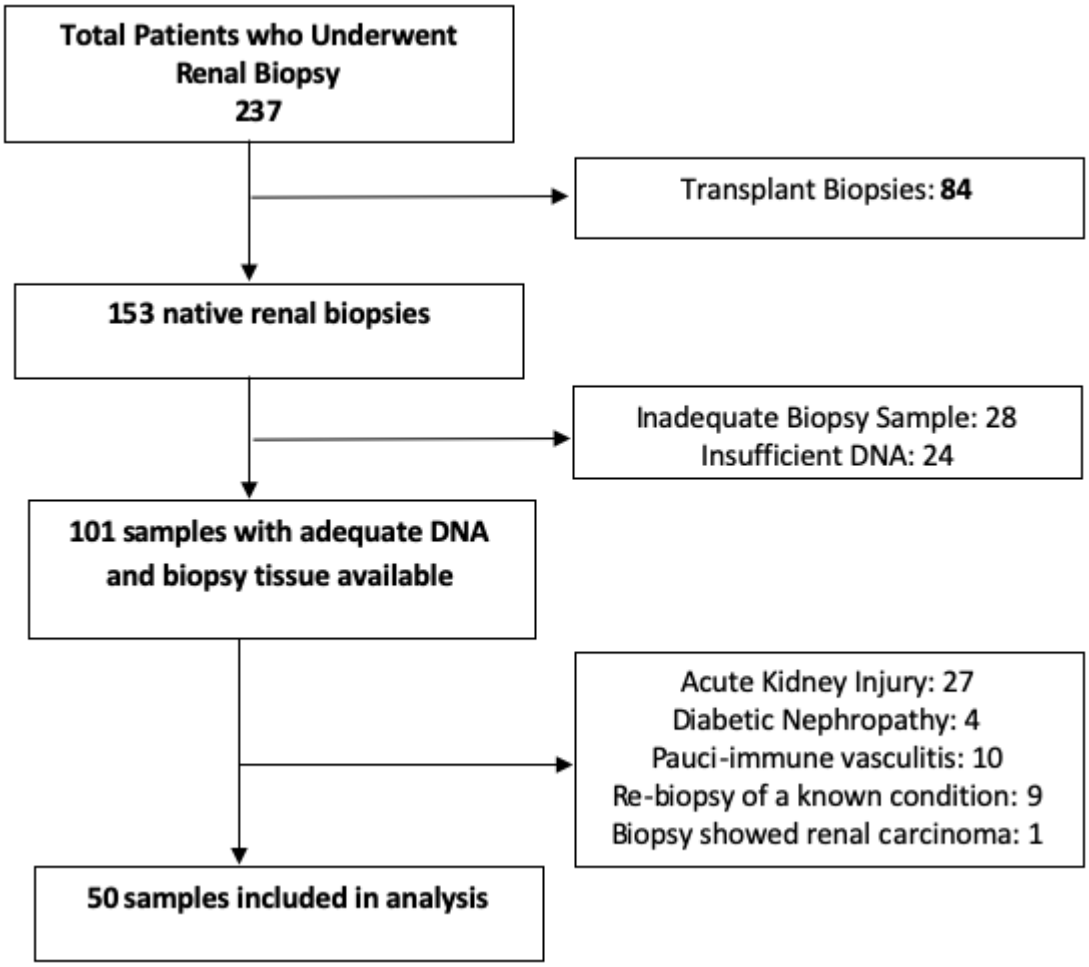

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