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ORIGINAL ARTICLE

Panel sequencing distinguishes monogenic forms of nephritis from nephrosis in children

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ABSTRACT

Background. Alport syndrome (AS) and atypical hemolytic–uremic syndrome (aHUS) are rare forms of chronic kidney disease (CKD) that can lead to a severe decline of renal function. Steroid-resistant nephrotic syndrome (SRNS) is more common than AS and aHUS and causes 10% of childhood-onset CKD. In recent years, multiple monogenic causes of AS, aHUS and SRNS have been identified, but their relative prevalence has yet to be studied together in a typical pediatric cohort of children with proteinuria and hematuria. We hypothesized that identification of causative mutations by whole exome sequencing (WES) in known monogenic nephritis and nephrosis genes would allow distinguishing nephritis from nephrosis in a typical pediatric group of patients with both proteinuria and hematuria at any level.

Methods. We therefore conducted an exon sequencing (WES) analysis for 11 AS, aHUS and thrombotic thrombocytopenic purpura-causing genes in an international cohort of 371 patients from 362 families presenting with both proteinuria and hematuria before age 25 years. In parallel, we conducted either WES or high-throughput exon sequencing for 23 SRNS-causing genes in all patients.

Results. We detected pathogenic mutations in 18 of the 34 genes analyzed, leading to a molecular diagnosis in 14.1% of families (51 of 362). Disease-causing mutations were detected in 3 AS-causing genes (4.7%), 3 aHUS-causing genes (1.4%) and 12 NS-causing genes (8.0%). We observed a much higher mutation detection rate for monogenic forms of CKD in consanguineous families (35.7% versus 10.1%).

Conclusions. We present the first estimate of relative frequency of inherited AS, aHUS and NS in a typical pediatric cohort with proteinuria and hematuria. Important therapeutic and preventative measures may result from mutational analysis in individuals with proteinuria and hematuria.

Keywords: genetics, monogenic renal disease, nephritis, nephrotic syndrome, pediatrics

INTRODUCTION

Alport syndrome (AS) is a rare, progressive hereditary nephropathy that accounts for 1.6% of chronic kidney disease (CKD) manifesting before age 25 years [1, 2]. It is characterized by hematuria, proteinuria and extrarenal manifestations such as ocular and cochlear abnormalities. Patients with AS usually display severe decline of renal function, with 50% of males reaching end-stage renal disease (ESRD) by age 25 years and 15% of females reaching ESRD by age 40 years [3]. In contrast, thin basement membrane nephropathy (TBMN) is characterized by largely asymptomatic hematuria that is rarely associated with proteinuria and ESRD. Recessive mutations have been identified in the *COL4A3* and *COL4A4* genes as causative for AS and dominant mutations in the *COL4A3* and *COL4A4* genes have been identified as causative for TBMN [4–6]. Mutations in the *COL4A5* gene cause X-linked AS [4–6]. To date, >1000 different mutations in *COL4A3*, *COL4A4* and *COL4A5* have been described. In patients with *COL4A4* mutations, the ability to distinguish early which patients will exhibit severe renal disease and which patients will display benign hematuria can help reduce or delay the decline of renal function [7–10]. Previously, other groups have been able to identify disease-causing mutations in genes encoding for type IV collagen in 80% of patients with AS [11].

Atypical hemolytic–uremic syndrome (aHUS) is another rare nephropathy, characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury. It accounts for ∼2% of CKD cases that manifest before age 25 years [1, 2]. Mutations in nine genes have been identified as causative for aHUS [1, 2, 12–15]. The approval of eculizumab in 2011 has

opened the door for new therapeutic approaches to the treatment of chronic aHUS. Thus, distinguishing between hereditary and nonhereditary forms of HUS has major implications for treatment approaches. In contrast, steroid-resistant nephrotic syndrome (SRNS) is a more common form of nephropathy that is characterized by proteinuria, hypoalbuminemia and edema. SRNS accounts for ∼10% of all CKD manifesting before age 25 years [1, 2, 16]. A monogenic cause of SRNS can be identified in ∼30% of cases that manifest before age 25 years [10, 16, 17].

In the setting of a pediatric nephrology clinic, presentation with a combination of proteinuria and hematuria poses a common diagnostic challenge. Molecular screening approaches for hereditary forms of CKD have been applied to well-defined disease cohorts, but few studies have tested for hereditary forms of CKD in such a typical pediatric patient population with both proteinuria and hematuria. We therefore hypothesized that identification of causative mutations by whole exome sequencing (WES) in known nephritis and nephrosis (NS) genes would allow distinguishing nephritis from nephrosis in a typical pediatric group of patients with proteinuria and hematuria. To examine the prevalence of hereditary forms of AS, aHUS and NS in a pediatric cohort of 371 CKD patients with proteinuria and hematuria manifesting before 25 years of age, we sequenced the coding regions of 11 AS-, aHUS- and thrombotic thrombocytopenic purpura (TTP)-causing genes and in parallel sequenced the coding regions of 23 common SRNS-causing genes. Causative mutations in AS-, aHUS- or NS-causing genes could be identified in 14.1% of individuals with childhood-onset proteinuria and hematuria and mutation analysis provides a safe approach for arriving at an etiologic diagnosis that can help distinguish nephritis from nephrosis in a pediatric population.

MATERIALS AND METHODS

Human subjects

This study was approved by the institutional review boards of Boston Children's Hospital and the University of Michigan. DNA samples were collected from 2854 individuals between 2003 and 2014 after obtaining informed consent, clinical data and pedigree information (www.renalgenes.org). Inclusion criteria were defined by the clinical presentation of both any level of proteinuria and any level of hematuria. The majority of patients had nephrotic-range proteinuria as defined by >2.5 g of proteinuria per day or a urine protein:creatinine ratio >2 g/g of creatinine [18]. The subjects had an onset of proteinuria and hematuria before an age of 25 years. It has previously been reported that the overall prevalence of monogenic CKD is >20% in patients manifesting before age 20 years [1], with 29.5% of nephrotic syndrome cases caused by single gene mutations [16]. A separate, previous study molecularly solved 83% of patients with AS with an average age of molecular diagnosis of 26 years [11]. Based on these previous studies, there is good evidence to support the use of an age cutoff of 25 years, with a high likelihood of monogenic CKD etiology in patients presenting before age 25 years. Thus a total of 362 families (371 patients) who met the inclusion criteria were included in this study, which consisted of 193 male and 178 female subjects. There was a bias against inclusion of patients positive for *WT1* mutations due to initial prescreening of patients with phenotypically described Denys–Drash syndrome, Frasier syndrome or Wilms tumor. Our patient cohort had partial overlap with a previously published cohort, as discussed below [16].

Mutation analysis

In order to screen patients for monogenic forms of AS, aHUS and SRNS, we took a two-pronged approach (Supplementary data, Figure S1). For monogenic forms of AS, aHUS and TTP, we screened all 362 families using barcoded multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS), even if they had previously undergone WES screening for mutations in SRNS-causing genes before the start of this study. This was done to ensure that we had thoroughly and uniformly screened every single patient for any mutations in any of the AS-, aHUS- and TTP-causing genes by the time of completion of this study. For monogenic forms of SRNS, some of our patients had been screened previously for pathogenic mutations in SRNS-causing genes in a previously published barcoded multiplex PCR and NGS study [16]. Of the 362 families, 315 had been previously screened for monogenic forms of SRNS using either WES or barcoded multiplex PCR and NGS, as alluded to above [16]. Thus there were 47 remaining families who had never been screened for monogenic forms of SRNS who were subsequently screened for monogenic forms of SRNS in this study using barcoded multiplex PCR and NGS. In summary, by the end of this study all 362 families were newly screened for mutations in AS-, aHUS- and TTP-causing genes and 47 families that had never been screened for mutations in NS-causing genes were newly screened for NS-causing mutations (Supplementary data, Figure S1).

High-throughput mutation analysis by array-based multiplex PCR and NGS

We designed 358 target-specific primer pairs for 300 coding exons and the adjacent splice sites of 11 genes that are known to cause AS, aHUS or TTP when mutated. The genes sequenced were *ADAMTS13, C3, CD46, CFH, CFHR5, CFI, COL4A3, COL4A4, COL4A5, DGKE* and *THBD* (Supplementary data, Table S1). For the 47 families who had not been screened previously for monogenic forms of SRNS, we used 524 target-specific primer pairs for 460 coding exons and the adjacent splice sites of 23 genes that are known to cause NS when mutated [16]. These 524 primer pairs were the same as those used for multiplex PCR and NGS in the patients previously screened for monogenic forms of NS [16]. The genes screened by multiplex PCR and NGS were *ACTN4, ADCK4, ARHGAP24, ARHGDIA, CD2AP, COQ2, COQ6, CRB2, CUBN, INF2, ITGA3, ITGB4, LAMB2, LMX1B, MYO1E, NPHS1, NPHS2, PDSS2, PLCE1, PTPRO, SMARCAL1, TRPC6* and *WT1* (Supplementary data, Table S2). In all multiplex PCRs, amplicon sizes ranged from 200 to 300 base pairs (primer sequences are available from the authors upon request). The use of barcoded multiplex PCR (48.48 Access Arrays system, Fluidigm, South San Francisco, CA, USA) allowed parallel amplification of all 358 amplicons in 362 families while screening AS, aHUS, and TTP genes and all

524 amplicons in the 47 families not previously screened for monogenic causes of SRNS. Subsequently the pooled barcoded PCR product libraries were sequenced on a MiSeq system (Illumina, San Diego, CA, USA) using the v2 chemistry. Sequence reads were aligned to the human reference sequence using CLC Genomics Workbench (CLC bio, Aarhus, Denmark) [19]. Prior to further evaluation, we excluded synonymous variants and variants that occur with a minor allele frequency >1% in the Short Genetic Variations database (dbSNP, version 138).

Homozygosity mapping

For genome-wide homozygosity mapping the GeneChip Human Mapping 250k *Sty*I Array from Affymetrix (Santa Clara, CA, USA) was used. Nonparametric logarithm of odds scores were calculated using a modified version of the program GENEHUNTER 2.1 [20, 21] through stepwise use of a sliding window with sets of 110 single-nucleotide polymorphisms and the program ALLEGRO [22] in order to identify regions of homozygosity as described [23, 24] using a disease allele frequency of 0.0001 and Caucasian marker allele frequencies.

WES

WES and variant burden analysis were performed as described previously [25]. In brief, genomic DNA was isolated from blood lymphocytes and subjected to exome capture using SureSelect human exome capture arrays (Agilent Technologies, Santa Clara, CA, USA) followed by NGS on the HiSeq sequencing platform (Illumina) as previously described.

Mutation calling

Sequence reads were mapped against the human reference genome (National Center for Biotechnology Information build 37/hg19) using the CLC Genomics Workbench (version 6.5.1; CLC bio). Variants with minor allele frequencies <1% in the dbSNP (version 138) were selected and annotated for impact on the encoded protein and for conservation of the reference base and amino acid among orthologs across phylogeny. All patients were evaluated for mutations in genes known to cause AS or aHUS when mutated (Supplementary data, Table S1) and for genes known to cause SRNS when mutated (Supplementary data, Table S2). In all patients with any potentially pathogenic heterozygous variant in *NPHS2*, we further verified for the presence or absence of a second heterozygous c.686 G > A (p.R229Q) mutation [26]. This was done because the allele frequency of the *NPHS2* p.R229Q mutation exceeds the 1% cutoff used in this study and thus would have been missed during our initial analysis unless we explicitly checked for it [26].

Validation of variants

Variants were validated as previously described [16]. Briefly, all variants previously reported as pathogenic in individuals with AS, aHUS, TTP or SRNS were considered as likely disease causing. Novel variants were ranked based on their likelihood to be deleterious for the function of the encoded protein. We considered protein truncation and obligatory splice site mutations as likely disease causing. For missense alleles, evolutionary conservation among orthologues and across phylogeny and bioinformatics prediction programs PolyPhen-2 [27], SIFT [28] and MutationTaster [29] were taken into consideration. All variants that were frequently present in the homozygous state for recessive genes (>1%) heterozygous state for dominant genes (>0.1%), or hemizygous state for X-linked dominant genes in healthy control cohorts [1000 Genomes Browser, Exome Aggregation Consortium (ExAC), Exome Variant Server (EVS) and Genome Aggregation Database (gnomAD)] were excluded unless previous studies demonstrated concrete loss of function or incomplete penetrance for the specific variant. Variants were confirmed in patient DNA using Sanger sequencing. Whenever parental DNA was available, segregation analysis was performed. Final calling of variant pathogenicity was performed by geneticists together with physician scientists who had knowledge of the clinical phenotypes and pedigree structure.

Coverage statistics for multiplex PCRs

While sequencing AS-, aHUS- and TTP-causing genes, we achieved a median sequencing coverage of 200× per individual and 400× per amplicon. Only 11 individuals (3%) and 27 amplicons (7.5%) had a median coverage <20×. While sequencing SRNS-causing genes in the 47 previously unscreened families in our cohort, we achieved a median sequencing coverage of 200× per individual and 200× per amplicon. No individuals (0%) and 43 amplicons (8.2%) had a median coverage <20×. Coverage statistics for our patients previously sequenced for mutations in SRNS-causing genes were previously reported [16].

Web Resources

- 1000 Genomes Browser, http://browser.1000genomes.org
- Biobase, https://portal.biobase-international.com/hgmd/pro/search_gene.php?
- Ensembl Genome Browser, http://www.ensembl.org
- Exome Aggregation Consortium, exac.broadinstitute.org
- Exome Variant Server, http://evs.gs.washington.edu/EVS/
- Genome Aggregation Database, http://gnomad.broadinstitute.org
- Human Gene Nomenclature Committee, http://www.genenames.org/
- MutationTaster, http://www.mutationtaster.org/ [29]
- Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
- PolyPhen2, http://genetics.bwh.harvard.edu/pph2/ [27]
- Primer3, http://primer3.ut.ee/
- Sorting Intolerant from Tolerant (SIFT), http://sift.jcvi.org/ [28]
- UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway

Software

- CLC Genomics Workbench, version 6.5.1 (CLC bio, Aarhus, Denmark)
- Alamut Visual, version 2.7, revision 1) (Interactive Biosoftware, Rouen, France)

RESULTS

Mutation detection

In a pediatric cohort of 371 patients (362 families) who had proteinuria and hematuria with an onset before 25 years of age, we examined for mutations in 11 genes that are known monogenic causes of AS (3 genes), aHUS (7 genes) or TTP (1 gene) if mutated (Supplementary data, Table S1) and for 23 genes that are known as monogenic causes of SRNS (Supplementary data, Table S2). Consanguinity was present in 56 of the 362 families screened (15.5%). We detected mutations in three of the three AS-causing genes and in three of the seven aHUS-causing genes (Table 1). We did not detect any mutations in the TTP-causing gene *ADAMTS13*. We detected causative mutations in 12 of the 23 SRNS-causing genes (Table 1). Mutations that likely explained the molecular etiology of disease were detected 51 of 362 unrelated families (14.1%) (Table 1).

Genes with pathogenic variants

Variants were validated as previously described in the methods and in Sadowski *et al.* [16]. Mutations were detected in three AS-causing genes in 17 families: *COL4A5* (10 families), *COL4A3* (6 families) and *COL4A4* (1 family) (Tables 2 and 3). Mutations were detected in three aHUS-causing genes in five families: *CFHR5* (three families), *CFH* (one family) and *CFI* (one family) (Tables 2 and 3).

In addition, mutations were detected in 12 SRNS-causing genes in 29 families: *NPHS1* (5 families), *NPHS2* (5 families), *LMX1B* (4 families), *PLCE1* (4 families), *LAMB2* (3 families), *SMARCAL1* (2 families), *ACTN4* (1 family), *ARHGDIA* (1 family), *COQ2* (1 family), *CUBN* (1 family), *INF2* (1 family) and *TRPC6* (1 family) (Tables 4 and 5). No pathogenic variants were found in the following 16 genes: *ADAMTS13*, *ADCK4*, *ARHGAP24*, *C3*, *CD2AP*, *CD46*, *COQ6*, *CRB2*, *DGKE*, *ITGA3*, *ITGB4*, *MYO1E*, *PDSS2*, *PTPRO*, *THBD* and *WT1*. Of the 55 different disease-causing mutations detected in this study, 19 (34.5%) were novel variants that had never previously been reported in databases containing human disease-causing mutations.

Table 1. Distribution of causative mutations detected in 18 of 34 sequenced genes for AS, aHUS, TTP and SRNS in 56 families presenting with proteinuria and hematuria before age 25 years

Gene symbol ($n = 34$)	Number of families with molecular genetic diagnosis ($n = 51$)	Percentage of total families (=100%)
Alport syndrome		
COL4A5	10	2.76
COL4A3	6	1.66
COL4A4	1	0.28
aHUS		
CFHR5	3	0.84
CFH	1	0.28
CFI	1	0.28
C3	0	0
CD46	0	0
DGKE	0	0
THBD	0	0
TTP		
ADAMTS13	0	0
Nephrotic syndrome		
NPHS1	5	1.38
NPHS2	5	1.38
LMX1B	4	1.10
PLCE1	4	1.10
LAMB2	3	0.82
SMARCAL1	2	0.56
ACTN4	1	0.28
ARHGDIA	1	0.28
COQ2	1	0.28
CUBN	1	0.28
INF2	1	0.28
TRPC6	1	0.28
ADCK4	0	0
ARHGAP24	0	0
CD2AP	0	0
COQ6	0	0
CRB2	0	0
ITGA3	0	0
ITGB4	0	0
MYO1E	0	0
PDSS2	0	0
PTPRO	0	0
WT1	0	0
Total	51	14.1

DISCUSSION

Rate of mutation detection in AS and aHUS genes versus previous studies

Here we screened 362 families of 371 patients presenting with proteinuria and hematuria before age 25 years. We sequenced the coding regions of 34 genes known to cause monogenic AS, aHUS or SRNS and identified a causative mutation in 51 of 362 families (14.1%). AS, aHUS and SRNS often progress to ESRD, placing a significant health burden on patients and their families and adversely impacting quality of life [2]. Multiple monogenic causes of AS, aHUS and SRNS have been identified [4–6, 12, 13, 15, 43, 65, 44–46, 57, 58, 63, 66–89]. The frequency of single-gene mutations in typical, pediatric patients with proteinuria and hematuria has not yet been studied systematically in these genes. Our detection rate of AS-causing genes in 17 of 362 families (4.7%) was much lower than the 80% previously reported in a cohort of 101 patients with suspected or diagnosed AS [11]. The difference in mutation detection rates between our study and the previous study is likely due to the preselection of a cohort highly enriched for AS phenotypes in previous studies. Our mutation detection rate for AS-causing genes of 4.7% was reflective of broad inclusion criteria of proteinuria and hematuria instead of a distinct AS phenotype.

Mutation detection rate in SRNS genes versus previous studies

In 29 (8.0%) of 362 families, we identified pathogenic mutations in SRNS-causing genes. This percentage is much lower than previously reported mutation detection rates of 29.5% [16], 28.3% [17] and 31.7% [90] in three pediatric NS cohorts. This discrepancy is partially due to our exclusion of patients

Table 2. Pathogenic variants detected by multiplex PCR and NGS in 362 families (371 patients) with proteinuria and hematuria with an age of onset <25 in 11 genes that if mutated, cause AS, aHUS or TTP

Family	Exon (Zygosity)	Nucleotide change	Amino acid change	dbSNP (rs #)	Conserved to	Poly-phen2	SIFT	Mutation taster	Sex	Ethnicity (consan-guinity)	gnO-MAD general population	gnO-MAD closest ethnicity	Bio-base
COL4A3													
A2041	4 (Het)	c.272G>A	p.Gly91Asp	–	*Dr*	0.994	Del	DC	m	Euro (N)	1/30960 (0 hom)	0/14998 (0 hom)	[30]
	48 (Het)	c.4421T>C	p.Leu1474Pro	200302125	*Dr*	1	Del	DC			735/276998 (0 hom)	620/126548 (0 hom)	[31, 32]
A1916	4 (Het)	c.279 + 6T>C	Splice	770953670	NA	−71%	−15.4%	−2.4%	f	Slavic (N)	1/245742 (0 hom)	0/30758 (0 hom)	Novel
A2490	23 (Het)	c.1504 + 6A>C	Splice	760718271	NA	+2.8%	+0.9%	+0.2%	f	Kazakh (N)	1/246078 (0 hom)	1/111616 (0 hom)	Novel
A1479	27 (Het)	c.1978C>A	p.Pro660Thr	773674552	*Gg*	0.661	Del	DC	f	Turk (Y)	5/277150 (0 hom)	5/126688 (0 hom)	Novel
A2358	29 (Hom)	c.2162del	p.Gly721Val*26	–	NA	NA	NA	NA	m	Asian (Y)	3/240354 (0 hom)	2/29504 (0 hom)	Novel
A2609	49 (Het)	c.4487G>A	p.Arg1496Gln	776086781	*Gg*	0.989	Del	DC	m	Euro (N)	11/276940 (0 hom)	1/126550 (0 hom)	Novel
COL4A4													
B789	2 (Hom)	c.71 + 1G>A	Splice	–	NA	−100%	−100%	−100%	f	Cauc (N)	–	–	[33]
COL4A5													
A5192	3 (Hem)	c.231 + 3A>G	Splice	376366035	NA	−32.2%	−0.4%	−11.1%	m	Turk (Y)	2/178120 (2 hem)	2/79762 (2 hem)	Novel
A965	4 (Hem)	c.274_279del	p.Arg92_Gly93del	–	*Dm*	NA	NA	NA	m	Euro (Y)	–	–	Novel
A3676	6 (Hem)	c.322-1G>A	Splice	–	NA	−100%	−100%	−100%	m	Indian (Y)	–	–	[34]
A2917	20 (Hem)	c.1217del	p.Gly406Val*68	–	NA	NA	NA	NA	m	Slavic (N)	–	–	Novel
A3933	24 (Hem)	c.1634G>A	p.Gly545Asp	–	*Dm*	1	Del	DC	m	Indian (N)	–	–	Novel
	24 (Hem)	c.1634G>A	p.Gly545Asp	–	*Dm*	1	Del	DC	m	Indian (N)	–	–	Novel
B711	25 (Hem)	c.1781G>A	p.Gly594Asp	–	*Dm*	0.355	Del	DC	m	Euro (N)	–	–	[35]
A1963	25 (Hem)	c.1931G>A	p.Gly644Asp	–	*Dm*	1	Del	DC	m	Hisp (Y)	–	–	[11, 36]
B28	46 (Hem)	c.4063del	p.Glu1355Asn*22	–	NA	NA	NA	NA	m	Arabic (Y)	–	–	Novel
A4926	48 (Hem)	c.4309C>G	p.Gln1437Glu	143778018	*Dm*	0.407	Del	DC	m	Filipino (N)	19/197664 (3 hom)	2/4713 (0 hom)	Novel
A169	49 (Hem)	c.4439del	p.Pro1480His*74	–	NA	NA	NA	NA	m	Turk (Y)	–	–	Novel
	49 (Hem)	c.4439del	p.Pro1480His*74	–	NA	NA	NA	NA	m	Turk (Y)	–	–	Novel
CFH													
A4035	10 (Het)	c.1507C>G	p.Pro503Ala	570523689	*Ci*	0.746	Del	PMP	f	Euro (N)	4/245456 (0 hom)	4/111226 (0 hom)	[37][a]
CFHR5													
A4967	2 (Het)	c.232T>C	p.Ser78Pro	146025130	NA	0.986	Tol	PMP	m	African (N)	32/277182 (0 hom)	27/24038 (0 hom)	Novel
A2351	4 (Het)	c.486dup	p.Glu163Arg*35	565457964	NA	NA	NA	NA	f	Cauc (N)	564/276160 (0 hom)	395/126014 (0 hom)	[38, 39]
A3422	10 (Het)	c.1615T>G	p.Phe539Val	111989094	NA	0.998	Del	DC	m	Arabic (?)	5/277086 (0 hom)	0/126608 (0 hom)	Novel
CFI													
A2336	13 (Het)	c.1558 + 5G>T	Splice	1114013791	NA	−21.2%	−11.9%	−12.3%	f	Asian (N)	2400/276954 (19 hom)	0/18862 (0 hom)	[40, 41]

The genes sequenced were *ADAMTS13*, *C3*, *CD46*, *CFH*, *CFHR5*, *CFI*, *COL4A3*, *COL4A4*, *COL4A5*, *DGKE* and *THBD*.

[a]Variant reported in Biobase for nonrenal disease. The splice site prediction scores were derived left to right from the MaxEnt, NNSPLICE and HSF prediction programs, respectively.

Cauc, Caucasian; *Ci*, *Ciona intestinalis;* DC, disease causing; Del, deleterious; *Dm, Drosophila melanogaster; Dr, Danio rerio;* dup, duplication; Euro, European; F, female; *Gg. Gallus gallus;* Hem, hemizygous; Het, heterozygous; Hisp, Hispanic; Hom, homozygous; M, male; N, no; NA, not applicable; PMP, polymorphism; Tol, tolerated; Turk, Turkish; Y, yes.

Table 3. Phenotypes of patients with pathogenic variants in 1 of 11 genes that if mutated cause AS, aHUS or TTP after multiplex PCR and NGS in 362 families (371 patients) with proteinuria and hematuria with an age of onset <25 years

Family	Sex	Age of onset (ESRD)	Proteinuria	Hematuria	Biopsy	Family history	Ethnicity (consanguinity)	Extrarenal treatment (response)	Biobase
COL4A3									
A2041	M	18 months	4 +(dipstick)	Microscopic	FSGS	None	Euro (N)	Azospermia, ACEi(UR)	[30] [31, 32]
A1916	F	6 years	6 g/day	Microscopic	MPGN type 1	None	Slavic (N)	HTN, HepB, SST, BAS, SS(PR), CS(NR), MMF(UR)	Novel
A2490	F	9 years	1 g/day	Microscopic	ND	None	Kazakh (N)	HTN, SS(CR)	Novel
A1479	F	4 years	1.08 g/day	Microscopic	ND	Mother	Turk (Y)	SS(CR)	Novel
A2358	M	10 years	4+	Microscopic	FSGS	2 siblings (deceased)	Asian (Y)	SR	Novel
A2609	M	10 years	UPC 4 mg/mg	Microscopic	ND	None	Euro (N)	SS(CR)	Novel
COL4A4									
B789	F	4 years	UPC 2.57 mg/mg	Microscopic	FSGS	None	Cauc (N)	–	[33]
COL4A5									
A5192	M	3 years (3 years)	5 g/day	Microscopic	FSGS, TubAt	None	Turk (Y)	SR, CP(NR)	Novel
A965	M	15 years	1.88 g/day	Macroscopic	Alport, BM lamellation	Mother	Euro (Y)	SR, CS(CR)	Novel
A3676	M	5 years	15.5 g/day	Y, type N/A	ND	None	Indian (Y)	Syncope, SR	[34]
A2917	M	2 years	2 g/day	Macroscopic	Non-specified	Mother	Slavic (N)	–	Novel
A3933	M	6 years	9.5 g/day	Microscopic	MCNS, MesP, TBM	Brother, paternal cousin, paternal grandma	Indian (N)	–	Novel
B711	M	15 years	2 g/day	Macroscopic	Alport, FSGS	Mother, father	Euro (N)	SR	[35]
A1963	M	14 years	7 g/day	Y, type N/A	ND	Uncle	Hisp (Y)	SR, CP(PR)	[11, 36]
B28	M	1 year	3 g/day	Microscopic	ND	None	Arabic (Y)	–	Novel
A4926	M	1 year	2 g/day	Microscopic	ND	None	Filipino (N)	Cough, Pnm, FR, capto, cerf/erythro, albmn trans	Novel
A169-21	M	7 years	UPC 1.37 mg/mg	Microscopic	Crescentic GN	Brother	Turk (Y)	SR, CP(NR)	Novel
A169-22	M	1 year	UPC 0.1 mg/mg	Microscopic	Crescentic GN	Brother	Turk (Y)	SR, TX	Novel
CFH									
A4035	F	13 years	UPC 2.57 mg/mg	Y, type N/A	FSGS, TMA	None	Euro (N)	HTN, SS(PR)	[37][a]
CFHR5									
A4967	M	7 years (7 years)	3 g/day	Microscopic	Active SLN	None	African (N)	HTN, SS(PR), ESRD	Novel
A2351	F	14 years	4 g/day	Microscopic	Diffuse MesP, TBM	None	Cauc (N)	HTN, SR, CP(UR)	[38, 39]
A3422	M	4 years	5 g/day	Microscopic	MPGN	None	Arabic (N)	–	Novel
CFI									
A2336	F	9 years	3.3 g/day	Microscopic	ND	Sister, father	Asian (N)	–	[40, 41]

The genes sequenced were *ADAMTS13*, *C3*, *CD46*, *CFH*, *CFHR5*, *CFI*, *COL4A3*, *COL4A4*, *COL4A5*, *DGKE* and *THBD*.

[a]Variant reported in Biobase for nonrenal disease.

ACEi, angiotensin-converting enzyme inhibitor; Albmn trans, albumin transfusions; BAS, bronchial asthma; BM, basement membrane; Capto, captopril; Cauc, Caucasian; Cerf/erythro, cefuroxime and erythromycin; CP, cyclophosphamide; CR, complete response; CS, cyclosporine; Euro, European; F, female; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; HepB, hepatitis B; Hisp, Hispanic; HTN, hypertension; M, male; MCNS, minimal change nephrotic syndrome; MesP, mesangial proliferation; MMF, mycophenolate mofetil; MPGN, membrane proliferative glomerulonephritis; N, no; ND, not done; NR, no response; Pnm, pneumonia; PR, partial response; SLN, sclerosing lobular nephritis; SR, steroid resistant; SS, steroid sensitive; SST, short stature; TBM, thin basement membrane; TMA, thrombotic microangiopathy; TubAt, tubular atrophy; Turk, Turkish; TX, transplant; type N/A, hematuria type unknown; UPC, urine protein:creatinine ratio; UR, unknown response; Y, yes.

Table 4. Pathogenic variants detected in 362 families (371 patients) with proteinuria and hematuria with an age of onset <25 years in 23 genes that cause nephrotic syndrome if mutated

Family	Exon (Zygo-sity)	Nucleotide change	Amino acid change	dbSNP (rs #)	Conserved to	Poly-phen 2	SIFT	Muta-tion taster	Sex	Ethnicity (consan-guinity)	gnOMAD general population	gnO-MAD closest ethnicity	Bio-base	Method
ACTN4														
A1055	14 (Het)	c. 1606C>A	P.Arg536Ser	–	Dr	0.933	Del	DC	M	Kurd (Y)	–	–	Novel	EXM
ARHGDIA														
A1432	5 (Hom)	c.518G>T	p.Gly173Val	–	Sc	1	Del	DC	F	Jewish (Y)	1/246016 (0 hom)	1/9840 (0 hom)	[42]	PCR
	5 (Hom)	c.518G>T	p.Gly173Val	–	Sc	1	Del	DC	M	Jewish (Y)	1/246016 (0 hom)	1/9840 (0 hom)	[42]	PCR
COQ2														
A103	3 (Het)	c.683A>G	p.Asn228Ser	121918232	Ce	0.918	Tol	DC	F	Euro (N)	32/276228 (0 hom)	0/111262 (0 hom)	[16, 43]	PCR
	5 (Het)	c.856C>T	p.Leu286Phe	776124921	Dm	0.997	Del	DC			2/245656 (0 hom)	2/111470 (0 hom)	[16]	PCR
CUBN														
A1213	19 (Hom)	c. 2613_2614del	p.Asp872Leu*3	386833777	NA	NA	NA	NA	M	Balkan (N)	6/276980 (0 hom)	0/126554 (0 hom)	[44]	EXM
	19 (Hom)	c. 2613_2614del	p.Asp872Leu*3	386833777	NA	NA	NA	NA	M	Balkan (N)	6/276980 (0 hom)	0/126554 (0 hom)	[44]	EXM
	19 (Hom)	c. 2613_2614del	p.Asp872Leu*3	386833777	NA	NA	NA	NA	M	Balkan (N)	6/276980 (0 hom)	0/126554 (0 hom)	[44]	EXM
INF2														
A675	2 (Het)	c.37G>A	p.Ala13Thr	201383094	Dr	0.982	Tol	DC	F	Euro (N)	85/240928 (1 hom)	40/106632 (0 hom)	[45][a]	PCR
LAMB2														
A1757	2 (Hom)	c.143A>C	p.Tyr48Ser	776905329	Dr	1	Del	DC	M	Hisp (N)	9/239510 (0 hom)	0/33384 (0 hom)	Novel	EXM
	2 (Hom)	c.143A>C	p.Tyr48Ser	776905329	Dr	1	Del	DC	F	Hisp (N)	9/239510 (0 hom)	0/33384 (0 hom)	Novel	EXM
A2356	7 (Hom)	c.736C>T	p.Arg246Trp	121912488	Ce	1	Del	DC	M	Asian (Y)	3/240414 (0 hom)	0/17184 (0 hom)	[16, 46]	PCR
A1613	10 (Hom)	c.1405 + 1G>A	Splice	780041521	NA	−100%	0%	−100%	M	Euro (N)	4/244056 (0 hom)	1/109858 (0 hom)	[16, 47]	PCR
LMX1B														
A200	4 (Het)	c.737G>A	p.Arg246Gln	–	Ce	1	Del	DC	F	Turk (Y)	–	–	[16, 48]	PCR
A2175	4 (Het)	c.737G>A	p.Arg246Gln	–	–	1	Del	DC	M	Euro (N)	–	–	[16, 48]	PCR
A3180	4 (Het)	c.737G>A	p.Arg246Gln	–	Ce	1	Del	DC	F	Euro (N)	–	–	[16, 48]	PCR
A4009	7 (Het)	c.929C>G	p.Thr310Arg	–	Gg	0.701	Del	DC	F	Arabic (Y)	–	–	Novel[a]	PCR
NPHS1														
A1803	2 (Hom)	c.139delG	p.Ala47Pro*81	386833882	NA	NA	NA	NA	M	Cauc (N)	2/241678 (0 hom)	1/107982 (0 hom)	Novel[a]	PCR
A3775	7 (Het)	c.840 + 1G>T	Splice	–	NA	−100%	−100%	−100%	F	Indian (N)	1/245036 (0 hom)	1/30778 (0 hom)	[49][a]	PCR
	21 (Het)	c.2903G>T	p.Gly968Val	771798618	Ce	1	Del	DC			1/236378 (0 hom)	0/30014 (0 hom)	Novel[a]	PCR
A3380	8 (Het)	c.928G>A	p.Asp310Asn	763972372	Dm	0.99	Del	DC	M	Asian (N)	3/241700 (0 hom)	3/17218 (0 hom)	[50, 51]	PCR
	21 (Het)	c.2816-3T>G	Splice	–	NA	−91.9%	−77.2%	−2.9%			–	–	[16, 51]	PCR
B115	12 (Hom)	c.1555C>T	p.Pro519Ser	–	Dr	0.984	Tol	PMP	F	Cauc (N)	–	–	[52]	EXM
A1500	20 (Hom)	c.2728T>C	p.Ser910Pro	–	Dr	0.959	Del	DC	F	Afr-Am (N)	–	–	[53, 54]	PCR
NPHS2														
A4681	1 (Hom)	c.1A>T	p.Met1*	–	NA	NA	NA	NA	F	Arabic (Y)	–	–	[16]	EXM
A4624	4 (Hom)	c.467dup	p.Leu156Phe*11	–	NA	NA	NA	NA	F	Arabic (Y)	–	–	[17, 51, 55, 56]	PCR
B188	7 (Hom)	c.855-856del	p.Arg286Thr*17	749740335	NA	NA	NA	NA	F	Hisp (Y)	18/275798 (0 hom)	0/34342 (0 hom)	[57, 58][a]	PCR
A1616	7 (Hom)	c.868G>A	p.Val290Met	200482683	Dr	0.998	Del	DC	F	Cauc (N)	33/276038 (0 hom)	32/126036 (0 hom)	[59–61]	PCR
A2239	8 (Hom)	c.926C>T	p.Ala309Val	–	Ce	0.742	Del	DC	M	Turk (N)	–	–	[62][a]	PCR
PLCE1														
A3233	10 (Hom)	c.3169C>T	p.Arg1057*	–	NA	NA	NA	NA	F	Arabic (Y)	1/245964 (0 hom)	0/111494 (0 hom)	[16]	PCR
A3617	11 (Hom)	c.3379_3380del	p.Asn1127*	–	NA	NA	NA	NA	F	Arabic (Y)	–	–	[16]	PCR
A3510	20 (Hom)	c.4600A>G	p.Lys1534Glu	–	Sc	0.998	Del	DC	F	Turk (Y)	–	–	[16]	PCR
A3869	26 (Hom)	c.5521A>G	p.Lys1841Glu	–	Sc	1	Del	DC	M	Arabic (Y)	–	–	[16]	PCR
SMARCAL1														
A3146	4 (Het)	c.49C>T	p.Arg17*	119473034	NA	NA	NA	NA	F	Euro (N)	2/246210 (0 hom)	1/111680 (0 hom)	[63][a]	PCR
	5 (Het)	c.836T>C	p.Phe279Ser	775057827	Ci	0.985	Tol	DC			28/277230 (0 hom)	23/126712 (0 hom)	[64][a]	PCR
A4162	12 (Hom)	c.1736C>A	p.Ser579*	–	NA	NA	NA	NA	F	Euro (N)	–	–	[16]	PCR
TRPC6														
A4685	2 (Het)	c.523C>T	p.Arg175Trp	–	Dr	1	Del	DC	F	Arabic (N)	–	–	[17]	EXM

The genes sequenced were *ACTN4, ADCK4, ARHGAP24, ARHGDIA, CD2AP, COQ2, COQ6, CUBN, INF2, ITGA3, ITGB4, LAMB2, LMX1B, MYO1E, NPHS1, NPHS2, PDSS2, PLCE1, PTPRO, SMARCAL1, TRPC6* and *WT1*.

[a]Solved in this study using multiplex PCR. The splice site prediction scores were derived left to right from the MaxEnt, NNSPLICE and HSF prediction programs, respectively.

Afr-Am, African American; Cauc, Caucasian; Ce, *Caenorhabditis elegans*; Ci, *Ciona intestinalis*; DC, disease causing; Dr, *Danio rerio*; Del, deleterious; Dm, *Drosophila melanogaster*; dup, duplication; Euro, European; F, female; EXM, homozygosity mapping and whole exome sequencing; Gg, *Gallus gallus*; Het, heterozygous; Hisp, hispanic; Hom, Homozygous; M, male; N, no; NA, not applicable; PCR, Fluidigm multiplex PCR + NGS; PMP, polymorphism; Sc, *Saccharomyces cerevisiae*; Tol, tolerated; Turk, Turkish; Y, yes.

Table 5. Phenotypes of patients with pathogenic variants in 1 of 23 genes that if mutated cause nephrotic syndrome after multiplex PCR and NGS in 362 families (371 patients) with proteinuria and hematuria with an age of onset <25 years

Family	Sex	Age of onset (ESRD)	Proteinuria	Hematuria	Biopsy	Family history	Ethnicity (consanguinity)	Extrarenal treatment (response)	Biobase
ACTN4									
A1055	M	10 years	UPC 4 mg/mg	Y, type N/A	FSGS	None	Kurd (Y)	SS(CR), CS(CR)	Novel
ARHGDIA									
A1432	F	2 years (3 years)	ACR 0.85 mg/mg	Y, type N/A	DMS	None	Jewish (Y)	DF, SST, ID, CS(NR), TX	[42]
	M	1 year (1 year)	17.3 g/day	Y, type N/A	ND	None	Jewish (Y)	SST, TX	[42]
COQ2									
A103	F	1 year	4 g/day	Y, type N/A	FSGS	None	Euro (N)	SR, CS(PR)	[16, 43]
CUBN									
A1213	M	12	ACR 0.28 mg/mg	Microscopic	ND	None	Balkan (N)	Hypocalcemia	[44]
	M	5 years	ACR 0.26 mg/mg	Microscopic	ND	None	Balkan (N)	–	[44]
	M	<1× month	ACR 0.9 mg/mg	Microscopic	ND	None	Balkan (N)	–	[44]
INF2									
A675	F	16 years	UPC 7.5 mg/mg	Y, type N/A	FSGS	None	Euro (N)	Steroids(UR)	[45][a]
LAMB2									
A1757	M	13 years	2.8 g/day	Y, type N/A	FSGS	Aunt	Hisp (N)	HTN, SR, Cellcept(NR)	Novel
	F	13 years	Y	Y, type N/A	FSGS	Aunt	Hisp (N)	–	Novel
A2356	M	1 year	4+	Microscopic	Finnish type	2 Brothers	Asian (Y)	Blindness	[16, 46]
A1613	M	<1 month	9.8 g/day	Y, type N/A	Dilated tubules, Microcysts	None	Euro (N)	FTT	[16, 47]
LMX1B									
A200	F	8 years (9 years)	1.5 g/day	Y, type N/A	FSGS	Mother	Turk (Y)	SR, HemDi	[16, 48]
A2175	M	4 years (43 years)	3.1 g/L	Y, N/A	FSGS	None	Euro (N)	Factor XII Deficiency, SR, CS(PR), Acei(UR), HemDi, TX	[16, 48]
A3180	F	18 years (43 years)	UPC 2 mg/mg	Y, type N/A	FSGS	None	Euro (N)	HemDi, TX	[16, 48]
A4009	F	<1 month	UPC 4 mg/mg	Y, type N/A	MCNS	None	Arabic (Y)	SR, CS(NR)	Novel[a]
NPHS1									
A1803	M	<1 month	UPC 38 mg/mg	Microscopic	MCNS	None	Cauc (N)	CS(NR)	Novel[a]
A3775	F	1 year	0.95 g/day	Y, type N/A	Diffuse MesP	None	Indian (N)	SS(CR)	[49][a]
A3380	M	<1 month	Y	Y, type N/A	ND	None	Asian (N)	–	[50, 51]
B115	F	<1 month	50 mg/mg	Y, type N/A	ND	None	Cauc (N)	Hypothyroidism	[52]
A1500	F	1 year	UPC 20 mg/mg	Y, type N/A	MCNS	None	Afr-Am (N)	HTN	[53, 54]
NPHS2									
A4681	F	7 years	UPC 8 mg/mg	Y, type N/A	FSGS	None	Arabic (Y)	SR	[16]
A4624	F	1 year	UPC 3.9 mg/mg	Y, type N/A	MPGN	None	Arabic (Y)	HTN, SR, CP(NR), CS(NR)	[17, 51, 55, 56]
B188	F	3 years (15 years)	3+	Macroscopic	MCNS	None	Hisp (Y)	TX	[57, 58][a]
A1616	F	5 months	2.2 g/day	Y, type N/A	ND	None	Cauc (N)	Presentation post infection	[59–61]
A2239	M	<18	5.1 g/day	Microscopic	FSGS	Cousin	Turk (N)	–	[62][a]
PLCE1									
A3233	F	2	UPC 8.4 mg/mg	Y, type N/A	ND	None	Arabic (Y)	HTN, SR, CP(NR)	[16]
A3617	F	7 months	UPC 12 mg/mg	Y, type N/A	FSGS	Cousin, aunt	Arabic (Y)	–	[16]
A3510	F	1 year	UPC 2.7 mg/mg	Microscopic	ND	None	Turk (Y)	HTN	[16]
A3869	M	7 months	UPC 4.5 mg/mg	Y, type N/A	FSGS	None	Arabic (Y)	–	[16]
SMARCAL1									
A3146	F	9 years	5 g/day	Y, type N/A	FSGS	None	Euro (N)	HTN, ID	[63][a]
A4162	F	4 years	UPC 7.5 mg/mg	Y, type N/A	FSGS	None	Euro (N)	Celiac, VSD, PDA, SR, CS(UR)	[16]
TRPC6									
A4685	F	17 years	UPC 9.8 mg/mg	Y, type N/A	FSGS	None	Arabic (N)	HTN, Allergies, CS(NR)	[17]

The genes sequenced were *ACTN4, ADCK4, ARHGAP24, ARHGDIA, CD2AP, COQ2, COQ6, CUBN, INF2, ITGA3, ITGB4, LAMB2, LMX1B, MYO1E, NPHS1, NPHS2, PDSS2, PLCE1, PTPRO, SMARCAL1, TRPC6* and *WT1*.

[a]Solved in this study using multiplex PCR.

ACEi, angiotensin-converting enzyme inhibitor; ACR, albumin:creatinine ratio; Afr-Am, African American; Cauc, Caucasian; CP, cyclophosphamide; CR, complete response; CS, cyclosporine; DF, deafness; DMS, diffuse mesangial sclerosis; Euro, European; F, female; FSGS, focal segmental glomerulosclerosis; FTT, failure to thrive; HemDi, hemodialysis; Hisp, Hispanic; HTN, hypertension; ID, intellectual disability; M, male; MCNS, minimal change nephrotic syndrome; MesP, mesangial proliferation; MPGN, membrane proliferative glomerulonephritis; N, no; ND, not done; NR, no response; PDA, persistent ductus arteriosus; PR, partial response; SR, steroid resistant; SS, steroid sensitive; SST, short stature; Turkish; TX, transplant; type N/A, hematuria type unknown; UPC, urine protein:creatinine ratio; UR, unknown response; VSD, ventricular septal defect; Y, yes.

with Denys–Drash syndrome, Frasier syndrome or Wilms tumor phenotypes during patient cohort selection prior to beginning the study. Yet in the three previous cohort studies, mutations in *WT1* only explained 4.8%, 5.8% and 2.6% of solved NS cases, respectively [16, 17, 90]. In addition, our cohort differed from previous NS cohorts in two major ways: first by the additional required inclusion criteria of the presence of hematuria, and second by the fact that the previous NS cohort studies only screened patients with SRNS, while our cohort included both steroid-sensitive and steroid-resistant NS patients. In addition, one of the previous studies actively excluded patients diagnosed with AS from their cohort [17]. Despite our broad inclusion criteria of proteinuria plus hematuria, we were still able to molecularly solve 14.1% of patients for monogenic forms of AS, aHUS or SRNS, further highlighting the power of using genetic screening to obtain a molecular etiology of disease.

Two of the previous NS cohort studies excluded consanguineous cases from their cohorts [17, 90]. The third previous SRNS cohort study had an overall consanguinity rate of 20.9% and solved 59.5% of consanguineous cases and 25% of nonconsanguineous cases [16]. In our study with an overall consanguinity rate of 15.5%, we detected a causative mutation in 20 of the 56 (35.7%) consanguineous families and we detected a causative mutation in 31 of the 306 (10.1%) nonconsanguineous families. This approximately matches previous reported rates of identification of causative monogenic variants in SRNS genes [16].

Study limitations

In this study we did not sequence *CFB*, *CFHR1* and *CFHR3*, which have previously been described to cause aHUS when mutated [91, 92]. Currently 50 genes have been identified to cause SRNS when mutated [93]. Lastly, only patients who underwent WES were sequenced for all monogenic forms of NS. The majority of patients were sequenced for mutations in NS-causing genes using multiplex PCR limited to 23 genes. Therefore only 23 SRNS-causing genes were systematically screened in all patients. This contributed to the low rate at which a causative mutation was identified (Supplementary data, Tables S1 and S2).

Future directions

Although we used a minor allele frequency cutoff of 0.1% for dominant alleles, we detected heterozygous, pathogenic mutations in two patients in which the minor allele frequency was >0.1%. In patient A2336, we detected a heterozygous *CFI* mutation with a minor allele frequency of 0.9% in the general population. This exact allele, however, had been published twice [40, 41]. The first study identified this allele in 1 of 45 patients screened for mutations in aHUS-causing genes [40]. The second report of this allele was in a patient with sporadic aHUS not secondary to human immunodeficiency virus (HIV) or shiga toxin etiology [41]. In both reports, no additional phenotypic details were reported, but given the convergence of evidence from multiple research groups, we believe that the c.1558 + 5 G > T splice site mutation may cause aHUS.

In patient A2351, we detected a heterozygous mutation in *CFHR5* with a minor allele frequency of 0.2% in the general population. As was the case with the *CFI* allele in patient A2336, the *CFHR5* allele in patient A2351 was also published twice previously [38, 39]. It was first reported in a 7-year-old presenting with proteinuria, hematuria and loss of corticomedullary differentiation on ultrasound [39]. A biopsy 9 months later showed mesangial hypercellularity, CFHR5 deposits and a thickened glomerular basement membrane [39]. Like our patient, this patient carried the same *CFHR5* allele heterozygously, but the healthy mother and sister were also heterozygous carriers, suggesting incomplete penetrance for this allele [39]. The patient had significantly reduced serum CFHR5 when compared with healthy controls [39]. In the second report of this allele, an 11-year-old male presented with gross hematuria, edema, proteinuria and hypoalbuminemia [38]. The authors reported dense deposits on the glomerular basement membrane and Bowman's capsule and ophthalmic drusen [38]. This second patient also carried the *CFHR5* allele heterozygously [38]. Given the above evidence, we believe the *CFHR5* allele in patient A2351 can cause aHUS. We detected AS-causing mutations in some patients without a documented familial history of renal disease or copresentation with ocular abnormalities. Future investigations should focus on early detection of ocular abnormalities with more careful follow-up by health care providers in these patients.

CONCLUSIONS

To our knowledge, this is the first study undertaken to determine the prevalence of mutations in AS-, aHUS- and NS-causing genes in a pediatric cohort with inclusion criteria of proteinuria and hematuria, which are common and practically relevant findings in any pediatric nephrology clinic. We detected disease-causing mutations in 14.1% of families sequenced, highlighting the utility of using genetics to obtain a definitive molecular etiology of disease. Clinicians should continue to utilize DNA sequencing technologies to further clinical practice, especially in cases of monogenic aHUS, where recently developed therapies such as eculizumab can be used to chronically treat the disease. Early angiotensin blockade can delay the onset of ESRD in patients with AS and an early molecular genetic etiology of disease is critical to helping delay a severe decline of renal function in pediatric patients [94].

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

D.S., A.D., J.A.L., A.J.M., S.L., W.T., J.K.W., I.F., J.R., M.A., H.Y.G., R.S., E.W., T.H., S.A., T.J.-S., A.T.v.d.V., M.N., S.S., D.A.B. and F.H. generated total genome linkage data, performed exome capture with massively parallel sequencing and performed whole exome evaluation and mutation analysis. D.S., A.D., J.A.L., A.J.M., S.L., W.T., J.K.W. and F.H. recruited patients and gathered detailed clinical information for the study. All authors critically reviewed the article. F.H. conceived of and directed the project and wrote the article.

CONFLICT OF INTEREST STATEMENT

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Neither this manuscript nor substantial parts of it are under consideration for publication elsewhere. Twenty-two families analyzed in this study were independently and previously published, either as index families in papers describing novel SRNS-causing genes or in our previous high-throughput exon sequencing paper on monogenic forms of SRNS [16]; however, none of the families included in this study had previously been analyzed comprehensively for AS- or aHUS-causing mutations.

REFERENCES

1. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol* 2016; 12: 133–146
2. Smith JM, Stablein DM, Munoz R *et al.* Contributions of the transplant registry: the 2006 annual report of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS). *Pediatr Transplant* 2007; 11: 366–373
3. Kashtan CE, Segal Y. Genetic disorders of glomerular basement membranes. *Nephron Clin Pract* 2011; 118: c9–c18
4. Lemmink HH, MochlzukJ T, van den Heuvel LPWJ *et al.* Mutations in the type IV collagen alpha 3 (COL4A3) gene in autosomal recessive Alport syndrome. *Hum Mol Genet* 1994; 3: 1269–1273
5. Mochizuki T, Lemmink HH, Mariyama M *et al.* Identification of mutations in the alpha 3(IV) and alpha 4(IV) collagen genes in autosomal recessive Alport syndrome. *Nat Genet* 1994; 8: 77–81
6. Antignac C, Knebelmann B, Drouot L *et al.* Deletions in the COL4A5 collagen gene in X-linked Alport syndrome. Characterization of the pathological transcripts in nonrenal cells and correlation with disease expression. *J Clin Invest* 1994; 93: 1195–1207
7. Savige J, Gregory M, Gross O *et al.* Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. *J Am Soc Nephrol* 2013; 24: 364–375
8. Kruegel J, Rubel D, Gross O. Alport syndrome—insights from basic and clinical research. *Nat Rev Nephrol* 2013; 9: 170–178
9. Pieri M, Stefanou C, Zaravinos A *et al.* Evidence for activation of the unfolded protein response in collagen IV nephropathies. *J Am Soc Nephrol* 2014; 25: 260–275
10. Giglio S, Provenzano A, Mazzinghi B *et al.* Heterogeneous genetic alterations in sporadic nephrotic syndrome associate with resistance to immunosuppression. *J Am Soc Nephrol* 2015; 26: 230–236
11. Moriniere V, Dahan K, Hilbert P *et al.* Improving mutation screening in familial hematuric nephropathies through next generation sequencing. *J Am Soc Nephrol* 2014; 25: 2740–2751
12. Lemaire M, Fremeaux-Bacchi V, Schaefer F *et al.* Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet* 2013; 45: 531–536
13. Neumann HP, Salzmann M, Bohnert-Iwan B *et al.* Haemolytic uraemic syndrome and mutations of the factor H gene: a registry-based study of German speaking countries. *J Med Genet* 2003; 40: 676–681
14. Noris M, Caprioli J, Bresin E *et al.* Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol* 2010; 5: 1844–1859
15. Westra D, Vernon KA, Volokhina EB *et al.* Atypical hemolytic uremic syndrome and genetic aberrations in the complement factor H-related 5 gene. *J Hum Genet* 2012; 57: 459–464
16. Sadowski CE, Lovric S, Ashraf S *et al.* A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 2015; 26: 1279–1289
17. Wang F, Zhang Y, Mao J *et al.* Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol* 2017; 32: 1181–1192
18. KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int Suppl* 2012; 2: 1–274
19. Halbritter J, Diaz K, Chaki M *et al.* High-throughput mutation analysis in patients with a nephronophthisis-associated ciliopathy applying multiplexed barcoded array-based PCR amplification and next-generation sequencing. *J Med Genet* 2012; 49: 756–767
20. Kruglyak L, Daly MJ, Reeve-Daly MP *et al.* Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996; 58: 1347–1363
21. Strauch K, Fimmers R, Kurz T *et al.* Parametric and nonparametric multipoint linkage analysis with imprinting and two-locus-trait models: application to mite sensitization. *Am J Hum Genet* 2000; 66: 1945–1957
22. Gudbjartsson DF, Jonasson K, Frigge ML *et al.* Allegro, a new computer program for multipoint linkage analysis. *Nat Genet* 2000; 25: 12–13
23. Hildebrandt F, Heeringa SF, Ruschendorf F *et al.* A systematic approach to mapping recessive disease genes in individuals from outbred populations. *PLoS Genet* 2009; 5: e1000353
24. Sayer JA, Otto EA, O'Toole JF *et al.* The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat Genet* 2006; 38: 674–681
25. Boyden LM, Choi M, Choate KA *et al.* Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. *Nature* 2012; 482: 98–102
26. Tory K, Menyhard DK, Woerner S *et al.* Mutation-dependent recessive inheritance of NPHS2-associated steroid-resistant nephrotic syndrome. *Nat Genet* 2014; 46: 299–304
27. Adzhubei IA, Schmidt S, Peshkin L *et al.* A method and server for predicting damaging missense mutations. *Nat Methods* 2010; 7: 248–249
28. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009; 4: 1073–1081
29. Schwarz JM, Cooper DN, Schuelke M *et al.* MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014; 11: 361–362
30. Weber S, Strasser K, Rath S *et al.* Identification of 47 novel mutations in patients with Alport syndrome and thin basement membrane nephropathy. *Pediatr Nephrol* 2016; 31: 941–955
31. Chatterjee R, Hoffman M, Cliften P *et al.* Targeted exome sequencing integrated with clinicopathological information reveals novel and rare mutations in atypical, suspected and unknown cases of Alport syndrome or proteinuria. *PLoS One* 2013; 8: e76360

32. Gast C, Pengelly RJ, Lyon M *et al.* Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2016; 31: 961–970
33. Nabais Sa MJ, Storey H, Flinter F *et al.* Collagen type IV-related nephropathies in Portugal: pathogenic COL4A3 and COL4A4 mutations and clinical characterization of 25 families. *Clin Genet* 2015; 88: 456–461
34. Plant KE, Green PM, Vetrie D *et al.* Detection of mutations in COL4A5 in patients with Alport syndrome. *Hum Mutat* 1999; 13: 124–132
35. Hashimura Y, Nozu K, Kaito H *et al.* Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV alpha5 chain. *Kidney Int* 2014; 85: 1208–1213
36. Mencarelli MA, Heidet L, Storey H *et al.* Evidence of digenic inheritance in Alport syndrome. *J Med Genet* 2015; 52: 163–174
37. Hoffman JD, Cooke Bailey JN, D'Aoust L *et al.* Rare complement factor H variant associated with age-related macular degeneration in the Amish. *Invest Ophthalmol Vis Sci* 2014; 55: 4455–4460
38. Vernon KA, Goicoechea de Jorge E, Hall AE *et al.* Acute presentation and persistent glomerulonephritis following streptococcal infection in a patient with heterozygous complement factor H-related protein 5 deficiency. *Am J Kidney Dis* 2012; 60: 121–125
39. Figueres ML, Fremeaux-Bacchi V, Rabant M *et al.* Heterogeneous histologic and clinical evolution in 3 cases of dense deposit disease with long-term follow-up. *Hum Pathol* 2014; 45: 2326–2333
40. Geerdink LM, Westra D, van Wijk JA *et al.* Atypical hemolytic uremic syndrome in children: complement mutations and clinical characteristics. *Pediatr Nephrol* 2012; 27: 1283–1291
41. Caprioli J, Noris M, Brioschi S *et al.* Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood* 2006; 108: 1267–1279
42. Gee HY, Saisawat P, Ashraf S *et al.* ARHGDIA mutations cause nephrotic syndrome via defective RHO GTPase signaling. *J Clin Invest* 2013; 123: 3243–3253
43. Diomedi-Camassei F, Di Giandomenico S, Santorelli FM *et al.* COQ2 nephropathy: a newly described inherited mitochondriopathy with primary renal involvement. *J Am Soc Nephrol* 2007; 18: 2773–2780
44. Tanner SM, Sturm AC, Baack EC *et al.* Inherited cobalamin malabsorption. Mutations in three genes reveal functional and ethnic patterns. *Orphanet J Rare Dis* 2012; 7: 56
45. Brown EJ, Schlondorff JS, Becker DJ *et al.* Mutations in the formin gene INF2 cause focal segmental glomerulosclerosis. *Nat Genet* 2010; 42: 72–76
46. Zenker M, Aigner T, Wendler O *et al.* Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet* 2004; 13: 2625–2632
47. Bredrup C, Matejas V, Barrow M *et al.* Ophthalmological aspects of Pierson syndrome. *Am J Ophthalmol* 2008; 146: 602–611
48. Isojima T, Harita Y, Furuyama M *et al.* LMX1B mutation with residual transcriptional activity as a cause of isolated glomerulopathy. *Nephrol Dial Transplant* 2014; 29: 81–88
49. Ovunc B, Ashraf S, Vega-Warner V *et al.* Mutation analysis of NPHS1 in a worldwide cohort of congenital nephrotic syndrome patients. *Nephron Clin Pract* 2012; 120: c139–c146
50. Fu R, Gou MF, Ma WH *et al.* Novel NPHS1 splice site mutations in a Chinese child with congenital nephrotic syndrome. *Genet Mol Res* 2015; 14: 433–439
51. Klaassen I, Ozgoren B, Sadowski CE *et al.* Response to cyclosporine in steroid-resistant nephrotic syndrome: discontinuation is possible. *Pediatr Nephrol* 2015; 30: 1477–1483
52. Heeringa SF, Vlangos CN, Chernin G *et al.* Thirteen novel NPHS1 mutations in a large cohort of children with congenital nephrotic syndrome. *Nephrol Dial Transplant* 2008; 23: 3527–3533
53. Schoeb DS, Chernin G, Heeringa SF *et al.* Nineteen novel NPHS1 mutations in a worldwide cohort of patients with congenital nephrotic syndrome (CNS). *Nephrol Dial Transplant* 2010; 25: 2970–2976
54. Sabi KA, Noto-Kadou-Kaza B, Gnionsahe DA *et al.* [Changing c.106delG (p.Ala36fs*6) loss syndrome associated with congenital Finnish type: first case diagnosed in Togo]. *Nephrol Ther* 2013; 9: 494–496
55. Caridi G, Bertelli R, Carrea A *et al.* Prevalence, genetics, and clinical features of patients carrying podocin mutations in steroid-resistant nonfamilial focal segmental glomerulosclerosis. *J Am Soc Nephrol* 2001; 12: 2742–2746
56. Schultheiss M, Ruf RG, Mucha BE *et al.* No evidence for genotype/phenotype correlation in NPHS1 and NPHS2 mutations. *Pediatr Nephrol* 2004; 19: 1340–1348
57. Boute N, Gribouval O, Roselli S *et al.* NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 2000; 24: 349–354
58. Correction to 'NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome'. *Nat Genet* 2000; 25: 125
59. Karle SM, Uetz B, Ronner V *et al.* Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 2002; 13: 388–393
60. Lu L, Sun XM, Yin Y *et al.* The amino acid mutations of the podocin in proteinuria: a meta-analysis. *Ren Fail* 2015; 37: 1329–1337
61. Sylva Skálová MP, Vondrák K, Chernin G. Plasmapheresis-induced clinical improvement in a patient with steroid-resistant nephrotic syndrome due to podocin (NPHS2) gene mutation. *Acta Medica (Hradec Kralove)* 2010; 53: 3
62. Bouchireb K, Boyer O, Gribouval O *et al.* NPHS2 mutations in steroid-resistant nephrotic syndrome: a mutation update and the associated phenotypic spectrum. *Hum Mutat* 2014; 35: 178–186
63. Boerkoel CF, Takashima H, John J *et al.* Mutant chromatin remodeling protein SMARCAL1 causes Schimke immuno-osseous dysplasia. *Nat Genet* 2002; 30: 215–220
64. Lucke T, Billing H, Sloan EA *et al.* Schimke-immuno-osseous dysplasia: new mutation with weak genotype-phenotype correlation in siblings. *Am J Med Genet A* 2005; 135: 202–205
65. Ebarasi L, Ashraf S, Bierzynska A *et al.* Defects of CRB2 cause steroid-resistant nephrotic syndrome. *Am J Hum Genet* 2015; 96: 153–161
66. Levy GG, Nichols WC, Lian EC *et al.* Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001; 413: 488–494
67. Fremeaux-Bacchi V, Miller EC, Liszewski MK *et al.* Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood* 2008; 112: 4948–4952
68. Noris M, Brioschi S, Caprioli J *et al.* Familial haemolytic uraemic syndrome and an MCP mutation. *Lancet* 2003; 362: 1542–1547
69. Edelsten AD, Tuck S. Familial haemolytic uraemic syndrome. *Arch Dis Child* 1978; 53: 255–256
70. Gale DP, de Jorge EG, Cook HT *et al.* Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* 2010; 376: 794–801
71. Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J *et al.* Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. *J Med Genet* 2004; 41: e84
72. Delvaeye M, Noris M, De Vriese A *et al.* Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; 361: 345–357
73. Kaplan JM, Kim SH, North KN *et al.* Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 2000; 24: 251–256
74. Ashraf S, Gee HY, Woerner S *et al.* ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. *J Clin Invest* 2013; 123: 5179–5189
75. Akilesh S, Suleiman H, Yu H *et al.* Arhgap24 inactivates Rac1 in mouse podocytes, and a mutant form is associated with familial focal segmental glomerulosclerosis. *J Clin Invest* 2011; 121: 4127–4137
76. Gupta IR, Baldwin C, Auguste D *et al.* ARHGDIA: a novel gene implicated in nephrotic syndrome. *J Med Genet* 2013; 50: 330–338
77. Lowik MM, Groenen PJ, Pronk I *et al.* Focal segmental glomerulosclerosis in a patient homozygous for a CD2AP mutation. *Kidney Int* 2007; 72: 1198–1203
78. Heeringa SF, Chernin G, Chaki M *et al.* COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. *J Clin Invest* 2011; 121: 2013–2024
79. Ovunc B, Otto EA, Vega-Warner V *et al.* Exome sequencing reveals cubilin mutation as a single-gene cause of proteinuria. *J Am Soc Nephrol* 2011; 22: 1815–1820
80. Has C, Sparta G, Kiritsi D *et al.* Integrin alpha3 mutations with kidney, lung, and skin disease. *N Engl J Med* 2012; 366: 1508–1514

81. Kambham N, Tanji N, Seigle RL *et al.* Congenital focal segmental glomerulosclerosis associated with beta4 integrin mutation and epidermolysis bullosa. *Am J Kidney Dis* 2000; 36: 190–196
82. Dreyer SD, Zhou G, Baldini A *et al.* Mutations in LMX1B cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. *Nat Genet* 1998; 19: 47–50
83. Mele C, Iatropoulos P, Donadelli R *et al.* MYO1E mutations and childhood familial focal segmental glomerulosclerosis. *N Engl J Med* 2011; 365: 295–306
84. Kestila M, Lenkkeri U, Mannikko M *et al.* Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1998; 1: 575–582
85. Lopez LC, Schuelke M, Quinzii CM *et al.* Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. *Am J Hum Genet* 2006; 79: 1125–1129
86. Hinkes B, Wiggins RC, Gbadegesin R *et al.* Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. *Nat Genet* 2006; 38: 1397–1405
87. Ozaltin F, Ibsirlioglu T, Taskiran EZ *et al.* Disruption of PTPRO causes childhood-onset nephrotic syndrome. *Am J Hum Genet* 2011; 89: 139–147
88. Winn MP, Conlon PJ, Lynn KL *et al.* A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science* 2005; 308: 1801–1804
89. Jeanpierre C, Denamur E, Henry I *et al.* Identification of constitutional WT1 mutations, in patients with isolated diffuse mesangial sclerosis, and analysis of genotype/phenotype correlations by use of a computerized mutation database. *Am J Hum Genet* 1998; 62: 824–833
90. Wang Y, Dang X, He Q *et al.* Mutation spectrum of genes associated with steroid-resistant nephrotic syndrome in Chinese children. *Gene* 2017; 625: 15–20
91. Goicoechea de Jorge E, Harris CL, Esparza-Gordillo J *et al.* Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc Natl Acad Sci USA* 2007; 104: 240–245
92. Zipfel PF, Edey M, Heinen S *et al.* Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. *PLoS Genet* 2007; 3: e41
93. Lovric S, Ashraf S, Tan W *et al.* Genetic testing in steroid-resistant nephrotic syndrome: when and how? *Nephrol Dial Transplant* 2016; 31: 1802–1813
94. Povey S, Al Aqeel AI, Cambon-Thomsen A *et al.* Practical guidelines addressing ethical issues pertaining to the curation of human locus-specific variation databases (LSDBs). *Hum Mutat* 2010; 31: 1179–1184

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Genetic variants in the *LAMA5* gene in pediatric nephrotic syndrome

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ABSTRACT

Background. Nephrotic syndrome (NS), a chronic kidney disease, is characterized by significant loss of protein in the urine causing hypoalbuminemia and edema. In general, ∼15% of childhood-onset cases do not respond to steroid therapy and are classified as steroid-resistant NS (SRNS). In ∼30% of cases with SRNS, a causative mutation can be detected in one of 44 monogenic SRNS genes. The gene *LAMA5* encodes laminin-α5, an essential component of the glomerular basement membrane. Mice with a hypomorphic mutation in the orthologous gene *Lama5* develop proteinuria and hematuria.

Methods. To identify additional monogenic causes of NS, we performed whole exome sequencing in 300 families with pediatric NS. In consanguineous families we applied homozygosity mapping to identify genomic candidate loci for the underlying recessive mutation.

Results. In three families, in whom mutations in known NS genes were excluded, but in whom a recessive, monogenic cause of NS