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Genetic Diagnosis in Consanguineous Families With Kidney Disease by Homozygosity Mapping Coupled With Whole-Exome Sequencing

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Abstract

Background—Accurate diagnosis of the primary cause of an individual's kidney disease can be essential for proper management. Some kidney diseases have overlapping histopathological features despite being caused by defects in different genes. In this report we describe two consanguineous Saudi Arabian families in which individuals presented with kidney failure and mixed clinical and histological features initially thought consistent with focal segmental glomerulosclerosis.

Study Design—Case series.

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Setting and participants—We studied members of two apparently unrelated families from Saudi Arabia with kidney disease.

Measurements—Whole-genome single-nucleotide polymorphism analysis followed by targeted isolation and sequencing of exons using genomic DNA samples from affected members of these families, followed by additional focused genotyping and sequence analysis.

Results—The two apparently unrelated families shared a region of homozygosity on chromosome 2q13. Exome sequence from the affected individuals lacked any sequence reads from the *NPHP1* gene, which is located within this homozygous region. Additional PCR based genotyping confirmed that affected individuals had *NPHP1* deletions, rather than defects in a known FSGS-associated gene.

Limitations—The methods used here may not result in a clear genetic diagnosis in many cases of apparent familial kidney disease.

Conclusions—This analysis demonstrates the power of new high-throughput genotyping and sequencing technologies to aid in the rapid genetic diagnosis of individuals with an inherited form of kidney disease. We believe it is likely that such tools may become useful clinical genetic tools and alter the manner in which diagnoses are made in nephrology.

Recent advances in genotyping and sequencing technologies are making previously intractable genetic analyses now possible ^{1, 2}. Utilizing such technologies to make precise genetic diagnoses may not only help distinguish between disease entities with mixed phenotypic and histopathologic patterns, but also permits one to draw conclusions from analyses performed on a small number of individuals. Technologies such as genome-wide genotyping of single-nucleotide polymorphisms (SNP) coupled with new massively parallel DNA sequencing can increase the efficiency, accuracy, and speed of diagnosis. These technologies are becoming more readily available in assisting in the accurate diagnosis of many genetic disorders including genetic disorders of the kidney ^{1, 2}.

Single-gene defects can cause kidney diseases of variable independent phenotypes, yet with some overlapping histopathological features. For example, focal segmental glomerulosclerosis (FSGS) is characterized by focal and segmental glomerular scarring in some, but not all, glomeruli of the kidneys. Several different genes have been identified that when mutated, cause FSGS. ^{3–910}. However, almost any chronic kidney injury, irrespective of cause, can lead to secondary glomerulosclerosis, which can be visualized histologically in kidney biopsies by segmental or global fibrosis of a number of glomeruli. The FSGS histologic phenotype can be familial, primary (idiopathic), and/or secondary to a multitude of pathological processes affecting the kidney, including tubulointerstitial diseases such as nephronophthisis ¹¹. For non-specific pathologic entities such as FSGS, the underlying primary processes may not be readily apparent. In such cases, especially if there is evidence of a familial inheritance pattern, genetic analysis may provide the simplest and most efficient method for making an accurate etiologic diagnosis.

Many disorders of the kidney can present as “fuzzy” collections of overlapping and non-specific phenotypes. Genetic analysis of disease-causing mutations in disorders that are caused by single-gene defects, as the case in many kidney disorders, is the most robust diagnostic approach for accurate diagnosis. Here we describe two consanguineous Saudi Arabian families in which individuals presented with kidney failure and clinical and histological features initially thought consistent with focal segmental glomerulosclerosis. We considered alternative diagnostic methods that are based on performing whole-genome genetic evaluation to explore the primary causes of disease in these families. This report emphasizes the importance of the use of these technologies on materials from patients and their families, particularly when meticulous clinical and histopathological information

cannot be obtained. We expect that high-throughput genetic tools will become a standard adjunct to traditional diagnostic methods.

Methods

Subjects and Clinical Data

We studied two consanguineous Saudi Arabian families (FAM012 and FAM001) with family histories of significant proteinuria, ESRD, and differing histological findings on kidney biopsy (Figure 1).

Three members of FAM012 have a history of chronic kidney disease (CKD) and significant proteinuria (> 1.5 gm/ 24 hrs), which was observed in the aunt (KFH-54) at an unconfirmed age. Two children (KFH-46 and KFH-47) from consanguineous parents also developed significant proteinuria (> 1.5 gm/ 24 hrs) at 9 years of age. Both children had edema, but there was no evidence of hypertension or diabetes. A kidney biopsy was performed on KFH-46, and light and electron microscopic images were obtained for our review. The biopsy showed global glomerulosclerosis in 3 of 12 glomeruli. It also showed segmental glomerulosclerosis in 4 of 12 glomeruli, without evidence of vascular disease (Figure 2a). Immunofluorescence studies revealed focal staining for C3 and IgM in the sclerosed glomerular segments (Figure 2b). Electron microscopic images demonstrated areas of extensive foot process effacement of visceral epithelial cells, microvillus change of the apical surface of these cells, and glomerular basement membrane wrinkling, changes consistent with focal segmental glomerulosclerosis (FSGS) (Figures 2c and 2d). KFH-46 was treated with prednisone for 6 months followed by cyclosporine A with no response and progressed to ESRD within a few years of initial diagnosis. Both KFH-46 and his maternal aunt (KFH-54) received cadaveric kidney transplants and, by report, both allografts continue to function.

Family FAM001 is of unrelated tribal ancestry to FAM012 (Figure 1). FAM001 has three siblings with ESRD, RKH-5, RKH-7 and RKH-8. RKH-8 is a female family member who presented at 21 years of age with kidney failure of unknown cause and a form of uveitis. Prior to her presentation with kidney failure, she was not known to have hypertension, hematuria, or proteinuria. A kidney biopsy was performed and light microscopic images were obtained for review. Sections showed nine glomeruli, seven of which were globally sclerosed, and two of which appeared unremarkable on light microscopy. Additional findings included marked interstitial fibrosis, patchy chronic inflammation and tubular atrophy, as well as marked medial hypertrophy of small arteries, consistent with vascular sclerosing disease. Electron micrographs were not available. Taken together, the changes described can be seen as a non-specific response to any type of chronic kidney damage, and are most commonly associated with chronic vascular disease or diabetes. RKH-7 is a female who presented at 15 years of age with mildly decreased kidney function and a strong family history of CKD. A kidney biopsy was obtained; sections showed 18 glomeruli, four of which were globally sclerosed. The remaining 14 revealed no significant pathologic changes on light microscopy. Additional findings seen on images provided included focal interstitial fibrosis, tubular atrophy, and marked vascular changes, potentially representing vascular sclerosing disease in the context of chronic hypertension or diabetes. Immunofluorescence studies were reportedly negative for C3 and C1q. Electron micrographs were not available for review. Based on this limited material received for review, the changes found are non-specific and do not show characteristic features of a distinct disease entity. Serum creatinine level was $217 \mu\text{mol/L}$ for RKH-7 and $283 \mu\text{mol/L}$ for RKH-8. RKH-5 in this family presented at 12 years of age with severe kidney failure, and no native kidney biopsy was obtained. RKH-5 received a kidney transplant and has been doing well to date. The transplant kidney biopsy showed normal glomeruli and no significant pathology.

The clinical parameters for the two families are shown on Table 1 and representative histopathology findings shown in Figure 2. From the history and pedigree structures, we suspected that the kidney disease in both of these two families was likely inherited as a recessive genetic trait. After screening for known nephrotic syndrome and FSGS genes, we performed genome-wide SNP genotyping as described below. Subjects were enrolled in this study after obtaining informed consent in accordance with a human subjects protocol approved by the King Faisal Specialist Hospital & Research Centre Institutional Ethics Research Committee and the Human Research Committee at the Brigham and Women's Hospital, Boston. We obtained clinical and family history information and reviewed medical records, as well as kidney biopsies and biopsy reports when available.

DNA sequencing and Genotyping

DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Qiagen, www.qiagen.com) according to the manufacturer's protocol. PCR-amplified segments containing coding sequences and flanking splice sites of four known FSGS/nephrotic syndrome genes (*NPHS2* [podocin], *ACTN4* [actinin], *TRPC6*, and *INF2*) were resequenced using standard Sanger methodology on a capillary sequencer. This sequencing was done using DNA from six affected subjects and two unaffected subjects from FAM001 and FAM012; sequencing primers are available upon request.

At the Microarray Core at the Dana-Farber Cancer Institute, we performed genome-wide genotyping of single-nucleotide polymorphisms (SNPs) in both family 1 (FAM001) and family 12 (FAM012) using a commercial microarray designed to probe approximately 250,000 sites. DNA samples from 11 informative family members were genotyped. SNP data were analyzed to identify homozygous regions in the samples of affected individuals using a Hidden Markov Model with two hidden states. Details of this modeling are provided in Item S1 (available as supplementary online material).

Whole-exome capture and sequencing

Whole-exome capture and sequencing has been described in detail previously.⁽¹⁾ In brief, genomic DNA was captured using a commercial human exome array containing probes from most exons in the human genome. DNA was sheared, ligated to adaptors, and fractionated by agarose gel electrophoresis. Fragments of the desired size were excised and DNA was extracted, PCR amplified, and hybridized to the capture array. The array was washed and bound genomic DNA was eluted and amplified by ligation-mediated PCR. The resulting fragments were purified and subjected to massively parallel sequencing.

PCR Deletion Assay

Primers were designed from three genomic regions, one inside the deletion and two in the flanking regions. Three pairs of primers were designed for each region using the publicly available tool NCBI/Primer-Blast (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Primers and details of the PCR protocol are shown in Table S1 and Item S1.

Results

Candidate Nephrotic Syndrome/FSGS gene screening

Affected members of FAM001 and FAM012 were screened for the presence of mutations in genes known to cause nephrotic syndrome and/or FSGS. Using Sanger dideoxynucleotide DNA sequencing, we re-sequenced PCR-amplified segments containing coding sequences and flanking splice sites of 4 known FSGS/nephrotic syndrome genes (*NPHS2*, *ACTN4*, *TRPC6* and *INF2*) in eight subjects (six affected and 2 unaffected subjects from both family

FAM001 and family FAM012). Mutational analysis detected no mutations in the sequenced genes in these two families (data not shown).

Whole-genome SNP analysis and homozygosity mapping

We next performed high-resolution SNP genotyping in order to localize the genetic variants responsible for disease in these two families. We genotyped the three affected subjects and the two unaffected parents from the consanguineous family FAM012 (Figure 1a). In our initial analysis, we assumed recessive inheritance and searched for identical-by-descent homozygous regions shared among the affected family members. We identified a single common homozygous run of SNPs in the three affected subjects of this family (KFH-46, KFH-47, and the maternal aunt KFH-54). This homozygous run is located on chromosome 2q13 between the reference SNP 757139 (rs757139; genomic position 85,539,243 in build 36 of the human genome available at the National Center for Biotechnology Information [NCBI]) and rs1437432 (NCBI 36 genomic position 117,490,034). This region is approximately 31 Mb in size.

We also genotyped members of the second consanguineous family (FAM001). The three affected subjects (RKH-7, RKH-5 and RKH-8), the two unaffected parents, and one unaffected sibling were genotyped. A homozygous run was detected in the affected subjects RKH-7, RKH-5 and RKH-8 (Figure 1 b). This homozygous region is also located on chromosome 2q13 and falls between rs10496407 (NCBI 36 genomic position 106,786,575) and rs67212013 (NCBI 36 genomic position 133,608,192), and is approximately 27 Mb in size.

When these homozygous regions identified in the affected members of the two unrelated families were compared with each other, they were found to overlap. The common region of homozygosity is a region between rs6754115 (NCBI 36 genomic position 109,328,776) and rs17464100 (NCBI 36 genomic position 111,284,252). Assuming the two families share a common genetic etiology, this reduced the critical homozygous interval to ~ 2 Mb that is shared in the affected subjects from both two families. The ~ 2 Mb homozygous run contains a number of known genes including *SEPT10* (septin 10 isoform 2); *RGPD1*, *RGPD5*, and *RGPD6* (RANBP2-like and GRIP domain-containing isoforms); *LIMS3* (LIM and senescent cell antigen-like domains 3); *MALL* (T-cell differentiation protein-like mal); isoform 3 of *NPHP1* (nephrocystin 1); *BUB1* (budding uninhibited by benzimidazoles 1); *SH3RF3* (SH3 domain-containing ring finger 3), and a number of pseudo genes (Figure 3).

Coupling of Whole-Exome Capture to Massively Parallel Sequencing

We next performed whole-exome capture followed by massively parallel sequencing to identify the possible genetic defects associated with clinical and pathological findings in one affected individual from these families. New sequencing technologies are allowing reasonably cost-effective approaches to sequencing vastly greater amounts of DNA than possible by traditional Sanger sequencing. We used one so-called next-generation methodology that relies on hybridization-based “capture” of a subset of genomic DNA from an individual using a special type of microarray, followed by preparation of this captured DNA, and finally a sequencing technology that detects single bases as they are incorporated into DNA strands. The protocols used for whole-exome capture and sequencing are described by Choi *et al*¹ and are outlined in brief in the methods section and in Item S1. We captured and sequenced genomic DNA from affected subject RKH-5 in FAM001. The resulting sequence data were filtered and aligned to the reference human genome (hg18). A total of 36,792,066 reads of 72 basepairs were generated and 99.4% aligned to the human reference sequence; 41% of the total bases mapped to the targeted bases with mean coverage of 32x.

In this individual, we found a total of 8147 exomic SNP variations from the human genome reference sequence; 3384 exomic SNPs were homozygous and 4863 SNPs were heterozygous. A total of 183 exomic copy number polymorphisms (CNPs, or insertions/deletions) were also detected in this sample; 34 of these CNPs were homozygous and 149 were heterozygous. After filtering the exomic SNPs against build 130 of the Database of Single Nucleotide Polymorphisms (dbSNP-130; www.ncbi.nlm.nih.gov/projects/SNP) we found a total of 808 SNPs that are novel in RKH-5; 31 were homozygous and 777 were heterozygous (Table 2). When we filtered against dbSNP-130 and restricted our search in the largest homozygous run (106,764,546-133,600,658 of chromosome 2) in FAM001 we found a total of 2 homozygous SNPs, 1 homozygous CNP, and as expected zero heterozygous SNPs and CNPs.

We expected to find a homozygous disease-causing mutation within the ~ 2 Mb homozygous run that is shared between the two consanguineous families. Consequently, we looked specifically for likely mutations within this segment. As part of a related study, we also performed whole-exome capture and sequencing with DNA from subject KFH-41, a member of a family that did not share this same run of homozygous SNPs. We used data from this second Saudi DNA sample as the basis for initial comparisons.

When we compared the exome sequence data from KFH-41 with the results for RKH-5, we observed a major structural variation. We noted that in the DNA sample from RKH-5, exome capture and sequencing failed (i.e. no sequences from this region identified) in twenty-one consecutive exons within the minimal homozygous run. By contrast, these exons were captured and sequenced in Subject KFH-41 to a mean coverage per-exon of 75× (Figure 4). The failure to capture and sequence this region in RKH-5 was consistent with an observation that we made in the genotypic data for the two families. SNP detection efficiency between rs7575835 (genomic position 110,191,850) and rs7575835 (genomic position 110,337,635) was either very weak or absent, consistent with possible homozygous deletion of the region (data not shown). The lack of sequence coverage in the same region in the exome data in RKH-5 indicates the existence of a structural variant in the form of a homozygous deletion. The structural homozygous deletion found in the two families encompasses 21 consecutive exons: all twenty exons of *NPHP1* and the first exon of the adjacent gene *MALL*.

This analysis prompted us to examine the sequencing coverage obtained for several additional known kidney disease genes. Table 3 shows the sequence coverage for 27 candidate or known FSGS, interstitial nephritis, and NPHP genes in this exome data. These FSGS and NPHP genes had average sequence coverage of 38× in RKH-5 exome and 42× in KFH-46 exome.

Detection of NPHP1/MALL deletion by PCR

We designed a PCR assay to detect and confirm homozygous deletion of the *NPHP1/MALL* locus by means of absence of the expected amplicon. Three primer pairs were designed to amplify three consecutive segments in the region 5' of the deletion, three primer pairs were made for three consecutive segments within the deleted region, and three primers were made for three consecutive loci on the 3' flanking region (see Figure S1 for the approximate locations of the primers and Table S1 for primer sequences). We used these primers to PCR-amplify genomic DNA from RKH-5 and KFH-41. For sample RKH-5, PCR gave the expected amplicons for the regions flanking the presumptive deletion, but amplification failed with all targets within the deletion. As expected, all primers gave the expected amplicon in subject KFH-41. This analysis demonstrated a homozygous deletion of the *NPHP1/MALL* locus in RKH-5.

We then carried this PCR analysis on all clinically affected and unaffected subjects from FAM001 and FAM012. The PCR deletion assay showed that the six affected from FAM001 (RKH-5, RKH-7, and RKH-8) and from FAM012 (KFH-46, KFH-47, and KFH-54) carry this *NPHP1/MALL* deletion while their unaffected parents and siblings do not, with the exception of subject 67 in FAM001 who has the deletion and is apparently healthy and without overt kidney disease at the time of the analysis. The same PCR analysis was carried out in eight sporadic patients with FSGS and probands from four families with FSGS. None of these individuals carried the homozygous deletion.

Discussion

In this report, we describe two unrelated consanguineous Saudi Arabian families (FAM001 and FAM012) that presented with histopathological findings initially thought consistent with FSGS. Because analysis of known FSGS genes failed to identify likely causal genetic variants, we considered the possibility that FSGS could be a secondary form of injury in these patients and that the list of possible underlying processes was quite long and diverse. We therefore used whole-genome SNP genotyping and exome sequencing in order to identify the causative gene alterations. This analysis facilitated a rapid genetic diagnosis of an *NPHP1* deletion. We first identified a shared homozygous run in the affected members of the two families. We then found, by analysis of sequence data, that these individuals harbored homozygous structural deletions of the nephronophthisis gene *NPHP1*. This report demonstrates the use of this strategy to make a speedy and accurate genetic diagnosis of patients with unclear histopathological features, albeit consistent with FSGS. It is important to note that as these technologies become increasingly able to generate genome-wide sequence data, increasing attention must be placed on data analysis.

The initial genotyping we performed did not identify any regions of homozygosity containing any known FSGS genes. Genotyping members of a second family indicated the involvement of a homozygous segment containing a small number of genes, including *NPHP1*. *NPHP1* locus deletion causes nephronophthisis, a recessive disorder characterized by medullary cysts and progressive kidney failure. We performed exome capture and sequencing on affected subject RKH-5 from FAM001 since this step was more cost effective and time efficient than sequencing the whole genome or sequencing individual genes in the homozygous run. Given the knowledge we extracted from the genotyping data we anticipated a homozygous disease-causing mutation within the ~ 2 Mb homozygous run that is shared between the two unrelated consanguineous families. In our analysis, a total of 36,792,066 72 bp reads were generated and 99.4% aligned to the human reference sequence and the targeted bases (the exome) constituted ~ 41% of all bases read, consistent with previous reports^{1, 2}. We also compared the exome data in this region for RKH-5 to that of another individual (KFH-41) who was unaffected. The mean exome sequencing coverage (i.e. the number of sequence reads covering a particular exon sequence) for both RKH-5 and KFH-41 was 32×, while in the deletion locus there was zero coverage in RKH-5 but a mean coverage of 75× in KFH-41.

Chronic kidney damage, such as nephronophthisis, may result in secondary glomerulosclerosis. This is identified by variable histological staining methods under light microscopy as segmental or global scarring of the glomeruli. Determining the underlying cause of glomerulosclerosis from the biopsy findings may be a difficult task, especially when a significant chronic kidney disease has developed, as was the case in affected members of families FAM001 and FAM012. The histological findings of FSGS may be recognized, even in the absence of features of the primary injury ultimately responsible for the injury. This may have occurred in at least one of the cases described in this report (KFH-46). In patients with primary FSGS or minimal change disease, electron microscopy

examination typically shows diffuse podocyte foot-process effacement, along with other signs of podocyte injury, including cytoplasmic vacuolization and microvillus change. These patterns were consistent with the findings seen in affected subject KFH-46.

Adding to the complexity of the diagnosis is that when glomerulosclerosis is secondary to other forms of kidney damage, signs of podocyte injury may be focal and segmental and most likely to be seen in glomeruli undergoing senescence. However, the degree of foot-process effacement can be variable and thus cannot be used reliably to distinguish primary from secondary FSGS^{14,15}. Moreover, In some hereditary glomerular diseases, particularly the autosomal dominant and later-onset forms of familial FSGS, such as that caused by *ACTN4*, *TRPC6*, and *INF2* mutations, the podocyte injury may be less severe and often shows a focal and segmental distribution, thus making the distinction from secondary FSGS difficult^{6, 16, 9}.

Ten nephronophthisis genes (NPHPs) have been described to date^{17,18}. Due to the propensity of recurrent deletion in the region containing *NPHP1*, this gene is responsible for the vast majority (~85%) of the purely renal form of nephronophthisis. *NPHP1* encodes a protein (nephrocystin), which interacts and form a complex with at least 3 other NPHP proteins, nephrocystin 3 (*NPHP3*), nephrocystin 4 (*NPHP4*) and inversin (*INVS*). It also interacts with other proteins involved in cell-cell and cell-matrix signaling such as filamin, tensin, and tubulin, suggesting that nephrocystins have a role in the integrity and architecture of renal tubular epithelial cells¹⁷. The most frequent mutation observed in familial juvenile nephronophthisis is a large homozygous deletion encompassing the whole *NPHP1* gene on chromosome 2q13. The *NPHP1* deletion was characterized by Saunier *et al*¹⁹. This study concluded that these deletions are likely to be a result of homologous recombination between direct repeats. The *NPHP1* gene is flanked by two large inverted repeats of ~ 330 kb, the distal one interrupted by a ~ 45 kb sequence, which is in turn directly repeated upstream of *NPHP1*. The deletion breakpoints were localized within the 45 kb repeats, which resulted from unequal recombination event between two homologous non-allelic copies of 45 kb repeats¹⁹. The unequal recombination between the repeats occurs either by chromosome misalignment, followed by an unequal crossover, or by the formation of a loop structure on a single chromosome, resulting in breakpoints that remove the *NPHP1* locus¹⁹.

Previous haplotype analyses by Konrad *et al*²⁰ in *NPHP1* families strongly suggested that the deletions detected in those families were independent and not due to a founder effect. The findings in our genotyping experiment, however, indicate that the deletion in these two Saudi families was an event that occurred in a common ancestor and that the deletion and surrounding chromosomal region was passed through many generations to the affected individuals. The SNP array contained 36 SNPs from within the homozygous run of ~ 2 Mb (between rs6754115 at genomic position 109,328,776 and rs17464100 at genomic position 111,284,252 of NCBI 36) which were found to be homozygous in affected subjects from the two families. This suggests the possibility that this deletion may have a non-trivial frequency in the Saudi regions where these families originate.

ESRD is a significant cause of morbidity in Saudi Arabia and worldwide. The main data source on kidney disease incidence in Saudi Arabia is available on ESRD and published annually by the Saudi Center for Organ Transplantation (SCOT: <http://www.scot.org.sa/>). The latest annual report from SCOT recorded 11,168 ESRD patients on dialysis; 2,350 of these presented with ESRD in the year 2008 alone. The reported average ESRD incidence rate is 150 patients per million per year in the period from 1999 to 2008. In 2008 alone there were 1300 mortalities amongst ESRD patients (12% of the total reported ESRD cases). These numbers reveals the incident rates for ESRD, while the incidence of CKD remains

largely undetermined. Further studies are necessary to determine the extent to which *NPHP1* deletions contribute to kidney disease in this region.

In cases of suspected inherited kidney disease, genome-wide genetic analysis such as that described here has the potential to become a clinical tool. While it is possible that the correct diagnosis of nephronophthisis could have been made by kidney biopsy, it is no longer clear that examination of kidney tissue is still the simplest, fastest, or most accurate way to make a correct etiologic diagnosis. Establishment of diagnosis based on genetic analysis on DNA samples from peripheral blood has the advantage of providing ease and accuracy in addition to being less intrusive. The utilization of high throughput analyses such as that presented in this report demonstrates that correct etiologic diagnoses can be made non-invasively even when clinical and histopathologic data are inconclusive or incomplete. We anticipate that analyses of this type will become a part of clinical diagnostic practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Homozygosity Mapping in Two Consanguineous Families. Pedigrees for a) family 12 (FAM012) and b) family 1 (FAM001) are shown. Squares indicate males and circles females. Pedigree identifier is shown in the bottom of the figure. Arrow heads point to affected subjects who share the homozygous runs that are indicated in red bar on the chromosome view in the right side for each pedigree.

Figure 2.

Kidney biopsy findings in an affected member of FAM012 (KFH-46). a) Masson's trichrome stain representation of a glomerulus showing glomerular tuft segmental sclerosis in the superior half; segments in the inferior half display mesangium and capillary walls and lumens with conserved architecture. b) Direct immunofluorescence showing focal staining for C3 and IgM in the sclerosed glomerular segments. c) and d) Electron micrographs revealing areas of extensive foot process effacement (red arrowheads) and glomerular basement membrane wrinkling, changes indicating FSGS.

Figure 3.

Homozygous run shared in FAM012 and FAM001. The homozygous run localizes between rs6754115 (genomic position 109,328,776) and rs17464100 (genomic position 111,284,252) and the critical homozygous interval is ~ 2 Mb.

Figure 4.

Exome capture and sequence data from the *NPHP1/MALL* locus. In RKH-5 (affected), there is zero sequencing coverage inside the deletion, while in KFH-41 (unaffected) there was an average coverage of 74× (black arrowheads). Both RKH-5 and KFH-41 had sequencing coverage in the region outside the deletion (red arrowheads). This figure was generated using Integrated Genomic View: <http://www.broadinstitute.gov/igv/>.

Table 1

Clinical characteristics in FAM001 and FAM012

Case #	Gender	Age at Diagnosis (Current)	History of Proteinuria *	Kidney Biopsy findings	SCr	kidney cysts?	Current Status
FAM012	KFH-46	M	9(16)	> 1.5 gm/ 24 hr	FSGS	not known	Post kidney tx
	KFH-47	F	9(10)	> 1.5 gm/ 24 hr	not known	no	ESRD, on Dialysis
	KFH-48	F	unaffected	-ve	NA	NA	NA
	KFH-49	M	unaffected	-ve	NA	NA	NA
	KFH-50	M	unaffected	-ve	NA	NA	NA
	KFH-51	F	unaffected	-ve	NA	NA	NA
	KFH-52	F	unaffected	-ve	NA	NA	NA
	KFH-53	F	unaffected	-ve	NA	NA	NA
	KFH-54	F	unknown	> 1.5 gm/ 24 hr	not known	NA	Post kidney tx
	KFH-54	F	unknown	> 1.5 gm/ 24 hr	not known	NA	Post kidney tx
FAM001	RKH-5	F	12 (22)	not known	NA	149 µmol/L	post kidney tx
	RKH-7	F	15 (19)	not known	non-specific	217 µmol/L	ESRD, on Dialysis
	RKH-8	F	21 (34)	not known	non-specific	283 µmol/L	ESRD, on Dialysis
	RKH-16	M	unknown	unknown	NA	NA	NA
	RKH-17	F	unaffected	-ve (dip stick)	NA	NA	NA
	RKH-63	M	unaffected	-ve (dip stick)	NA	NA	NA
	RKH-64	M	unaffected	-ve (dip stick)	NA	NA	NA
	RKH-65	M	unaffected	-ve (dip stick)	NA	NA	NA
	RKH-67	M	unaffected	30 (dip stick)	NA	NA	NA
	RKH-67	M	unaffected	30 (dip stick)	NA	NA	NA

* Proteinuria at diagnosis using any of these analyses [spot urine with protein-creatinine ratio, 24 hr protein in urine, spot urine with albumin-creatinine ratio, or dip-stick].

Abbreviations and definitions: NA, not applicable; SCr, serum creatinine; tx, transplant; ESRD, end-stage renal disease; -ve, negative

Table 2

Summary Statistics for whole-exome sequencing from RKH-5

Polymorphism Type	No.
Total exomic SNPs	8147
Homozygous SNPs	3284
Heterozygous SNPs	4863
Total Exomic CNPs	183
Homozygous CNPs	34
Heterozygous CNPs	149
Total filtered* exomic SNPs	808
Homozygous SNPs	31
Heterozygous SNPs	777

* Filtered using build 130 of the Database of Single Nucleotide Polymorphisms (dbSNP-130; www.ncbi.nlm.nih.gov/projects/SNP)

SNP= Single-Nucleotide Polymorphism; CNP= Copy Number Polymorphism

Table 3

Exome sequencing coverage in RKH-5 and KFH-41

Chromosome	Genomic Locus		Gene	Coverage	
	Starts	Ends		RKH-5	KFH-41
Genes Associated With FSGS/Nephrotic Syndrome					
1	177786297	177811707	NPHS2	35×	35×
1	202390571	202402088	RENIN	12×	28×
2	216985382	217056019	SMARCAL1	33×	44×
3	49133551	49145603	LAMB2	8×	17×
4	77298918	77354059	SCARB2	71×	71×
4	84404001	84424988	COQ2	0	0
6	47553484	47702955	CD2AP	90×	71×
6	107580454	107887472	PDSS2	64×	58×
9	128416569	128503132	LMX1B	11×	21×
10	95743736	96078138	PLCE1	0	0
11	100827505	100959869	TRPC6	69×	64×
11	32266639	32467137	WT1	23×	29×
14	104226988	104256992	INF2	0	0
16	20251874	20271538	UMOD	17×	30×
17	71229111	71265494	ITGB4	11×	22×
19	43830167	43904358	ACTN4	20×	38×
19	41008114	41034579	NPHS1	11×	19×
22	35007272	35113927	MYH9	19×	35×
NPHP Genes					
1	5845456	5975118	NPHP4	0	0
2	110238202	110319928	NPHP1	0	74×
3	133882143	133923966	NPHP3	77×	67×
3	122971299	123036616	IQCB1 (NPHP5)	80×	64×
9	101901331	102103247	INVS (NPHP2)	65×	61×
12	86966920	87060124	CEP290 (NPHP6)	0	0
16	4322225	4329599	GLIS2 (NPHP7)	6×	14×
16	52191318	52295272	RPGRIPL1 (NPHP8)	101×	84×

Chromosome	Genomic Locus		Gene	Coverage	
	Starts	Ends		RKH-5	KFH-41
17	24079958	24093911	NEK8 (NPHP9)	16×	28×

Note: RKH-5 is an affected individual from FAM001; KFH-41 is an unaffected individual from another family.
Abbreviations: FSGS, focal segmental glomerulosclerosis; NPHP, nephronophthisis.