

An exome sequencing study of 10 families with IgA nephropathy

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TITLE: AN EXOME SEQUENCING STUDY OF 10 FAMILIES WITH IGA NEPHROPATHY

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ABSTRACT

Background

IgA nephropathy is a heterogeneous disorder with a strong genetic component. The advent of whole exome sequencing (WES) has accelerated the discovery of genetic risk factors underlying familial disorders.

Objectives

We set out test whether damaging variants in known kidney disease genes explain a proportion of IgAN cases recruited in Ireland.

Methods

We performed WES in 10 Irish families with multiple affected members having kidney disease where at least one member had biopsy confirmed IgAN. Candidate variants were identified based on being shared between affected family members, minor allele frequency, function and predicted pathogenicity. Pathogenicity of variants was determined according to American College of Medical Genetics and Genomics guidelines.

Results

We detected candidate variants in three of ten families. We identified a likely pathogenic variant in *COL4A5* in one family and a variant of unknown significance (VUS) in *COL4A3* in another. Variants in *COL4A5* and *COL4A3* are known to cause Alport syndrome. In the third family, we identified a VUS in *LMX1B*, a gene associated with Nail-patella syndrome.

Conclusions

We identified a number of cases of familial IgAN where the families harbored variants in known kidney disease related genes indicating that potentially a number of cases of familial IgAN are mistaken for other familial kidney disorders. However, the majority of families studied did not carry a candidate variant in a known kidney disease causing gene indicating that there may be more than one underlying genetic mechanism present in these families.

1. INTRODUCTION

IgAN is a heterogeneous disorder defined by the presence of IgA deposits on immunofluorescence of kidney biopsies. It is the most common form of glomerulonephritis in the world and can occur *de novo* or associated with a multitude of different disease types (1). The condition varies greatly in progression and severity (2) ranging from individuals developing ESRD to cases of asymptomatic mesangial IgA deposits seen post-mortem (3). It affects people of all ages but most commonly in early adulthood (1). More males are affected than females, with a reported ratio of approximately 2:1 (4, 5).

There are many pieces of evidence which suggest that genetic factors play a major role in disease occurrence and progression. These include many reports over the last number of decades, of clustering in families and different prevalence of the disease across ethnicities living in similar environments (6, 7). Some of the reports of IgAN within families suggest a Mendelian pattern of inheritance (6), however, many of these families show incomplete penetrance, which fits with a multifactorial disease model and suggests that additional factors (either environmental or genetic) beyond a single causal mutational are needed to trigger the onset of the disease (6).

Despite more than 30 years of genetic analysis of IgAN, no clear causative gene has been identified. Linkage studies of families with IgAN have identified significantly associated loci including 3q24-23, 6q22-23 and 2q36 (8-10). In spite of a number of linked loci, in the majority of these families, the causal gene has remained elusive.

In the last decade, a handful of next-generation sequencing studies have been carried out to identify candidate genes for IgAN. One such study carried out whole exome sequencing (WES) in ten families with IgAN. They identified 6 candidate disease-causing variants in the genes *MYCT1*, *DEFA4*, *CARD8* and *ZNF543* (11). Another WES study identified a novel deleterious variant in the gene *SPRY2* that segregated with the disease in a large Sicilian family presenting with autosomal dominant IgAN. *SPRY2* is part of the MAPK/ERK pathway, however, the biological link between IgAN and MAPK/ERK pathway has yet to be explained (12).

A WES study by Cox *et al* examined rare variants segregating with IgAN in 16 families composed of 240 IgAN cases and 113 controls (13). They identified 23 genes with candidate

disease-causing variants that were functionally related to a large immune-related network of pathways. They hypothesized that IgAN disease status may be influenced by a variety of mutations influencing one over-arching immune-related network (13).

To further expand upon these studies, we conducted WES on 10 Irish families who presented with multiple affected family members having severe kidney disease (stage 3 or above) in whom at least one member had biopsy confirmed IgAN (see Table 1). In this study, we identified that a number of cases of familial IgAN are in fact likely mistaken for other familial kidney disorders. Our findings illustrate the role of molecular diagnosis in accurate disease classification.

2. MATERIALS AND METHODS

2.1 SELECTION OF FAMILIES

The Irish Kidney Gene Project is a national cross-sectional analysis of 1,809 patients who attended renal clinics and dialysis units in Ireland. Part of the project involved characterisation of family history among patients with kidney disease (14). The Irish Kidney Gene Project and several other data sources were accessed to identify all cases for inclusion in this study of familial IgAN.

All patients in this study provided informed written consent. The study protocol and design were subject to appropriate Ethics Committee approval at Beaumont Hospital, Dublin, Ireland (research ethics committee reference: 12/75).

Families presenting with IgAN were identified through a clinical analysis carried out by Fennelly et al (15). Patients were selected on the basis that they had a clinical picture compatible with IgAN predominantly. Specifically patients with advanced liver disease that is associated with IgAN or patients with other disease that can show IgAN deposition such as Lupus or membranous nephropathy were excluded. In this study, 8,033 kidney biopsies performed at Beaumont Hospital over the period 1986–2016 were reviewed and identified 1,283 cases of IgAN. Of these cases, 23 (1.8%) were found to have a family history of kidney disease and were classed as familial IgAN. These 23 cases represented 14 different families. The families were then contacted and blood/saliva was collected on any available,

consented family member by the staff at Beaumont Hospital. DNA from these patients was obtained at Beaumont Hospital from these collected blood or saliva samples and processed through the Rare Kidney Disease Biobank at St. James Hospital, Dublin, Ireland. We then chose families who had at least one family member with biopsy proven IgAN and one other family member with either biopsy-proven IgAN or ESRD as previously described (15) and selected families that had at least two affected members with DNA available. This left us with ten families for the exome sequencing analysis. All unaffected individuals that had gene sequencing were labelled unaffected on the basis of negative dipstick for significant blood or proteinuria. All families recruited were Irish and were reported as non-consanguineous.

Ten families were selected for exome sequencing, labelled F104, F141, F229, F315, F74, F404, F433, F654, F224, and F91. In family F141, we sequenced three affected members. In family F104, we sequenced one affected member and in the remaining eight families, we sequenced two affected members (F229, F315, F74, F404, F433, F654, F224, and F91). Families were selected for exome sequencing on the basis of meeting the aforementioned phenotype criteria and also having sufficient DNA to carry out exome sequencing and test for segregation, as required.

2.2 WHOLE EXOME SEQUENCING ANALYSIS

We exome sequenced the 20 individuals from 10 families to 100X coverage using either an Ion Proton platform (n=6, families = F229, F315 and F91) or an Illumina Hiseq platform (n=14, families = F141, F74, F104, F224, F404, F433 and F654). Six individuals were sequenced using the SureSelect XT Human All Exon V6+UTRs Kit (members from F141, F74 and F104), eight were sequenced using the SureSelect XT Human All Exon V6 Kit (members from F224, F404, F433 and F654), two were sequenced with the Ion TargetSeq Exome (both individuals in F91) and the remaining 4 were sequenced using the Ion Ampliseq exome RDY kit (families F229 and F315).

2.3 ANNOTATING VARIANTS

Following alignment and quality control of exome data (see Supplementary Methods for details), VCF files were annotated using Annovar(16). Candidate variants were defined as

variants that were shared between affected family members, functional, rare and predicted deleterious (see Supplementary Methods for details).

Candidate variants and where relevant, the associated genes were cross-referenced with the Online Mendelian Inheritance in Man catalog (OMIM), Clinvar (version 20150330), gnomad, Missense Tolerance Ratio (MTR) Gene Viewer and RVIS (Residual Variation Intolerance Score) (17-21).

We incorporated clinical data using OMIM and Clinvar to see if any of the candidate variants were thought to be involved in kidney disease or had been previously been reported as pathogenic. We then re-examined the pedigree information to select variants that fit the disease model. Finally, we used the American College of Medical Genetics and Genomics (ACMG) guidelines to annotate whether or not the variant was considered pathogenic, likely pathogenic, uncertain significance, likely benign or benign (22).

We used Sanger sequencing to confirm candidate variants and to test for segregation (see Supplementary Methods).

2.4 SEARCHING FOR VARIANTS ACROSS FAMILIES

Families in which we did not identify a candidate variant in a gene previously linked with renal disease were assessed for candidate variants found in genes shared across these families. Genes with a candidate variant found in more than one family were selected. Artefacts of sequencing were identified through examination of the bam files using the Integrative Genomics Viewer (IGV) (23).

3. RESULTS

We performed WES on 20 individuals across 10 families (see Table 1). The average age in the affected individuals (n=30) across these 10 families was 41 (standard deviation = 15.8 years), with 22 males and 8 females (See Supplementary table 1 for further details on affected family members). The average age in the unaffected individuals (n=26) across these 10 families was 58 (standard deviation = 11.6 years), with 8 males and 17 females. In 7 families (F224, F91, F404, F433, F654, F229 and F315), we did not identify candidate variants in genes known to cause kidney disease, or that were found across multiple families in

biologically relevant genes (see Supplementary table 2 for list of qualifying variants found in these families). In three families (F141, F74 and F104), we identified candidate mutations that were found in genes known to cause kidney disease (see Table 2).

3.1. EXOME SEQUENCING: FAMILY 141

Family 141 (F141) is a multi-generational family with multiple affected members presenting with blood and proteinuria. In one member a kidney biopsy confirmed IgA deposition in the mesangium. There was clear male to male transmission suggesting an autosomal dominant mode of inheritance (see Figure 1). The proband (IV.1) in this family was diagnosed (via biopsy) with IgAN and mild arteriosclerosis/arteriolar sclerosis (see Figure 2). The proband's father (III.6) was diagnosed with focal proliferative glomerular nephropathy, thin glomerular basement membrane and mild arteriolar sclerosis. The proband's grandmother (II.1) was reported to have "scarred kidneys" but the reason for this scarring was not reported. The proband's sister (IV.2) had signs of early stage chronic kidney disease with both blood and proteins present in the urine. The proband's paternal uncle (III.5) was also found to have kidney disease with urinalysis showing presence of protein and haemolysed blood and upon biopsy showed signs of thin basement membrane nephropathy (see Figure 2). The proband's aunt (III.3) also presented with 2+ blood in the urinalysis. A cousin (IV.3) was reported as affected, but exact details on this individual are unknown.

Three of the affected individuals were selected for WES (see Figure 1) – the proband (IV.1), the sister (IV.2) and the affected uncle (III.5). In F141, we identified a likely pathogenic variant (according to ACMG standards) in the gene *COL4A5*, a gene known to cause X-linked Alport syndrome (24). This variant was present in IV2 and III5 but not in the proband. This variant, rs104886228:G>A, was found on chromosome X in the 38th exon of *COL4A5*. This nucleotide change was a nonsynonymous single nucleotide variation (SNV) predicted to result in an amino-acid change (glycine to serine) at position 1,143 of the *COL4A5* protein p.(Gly1143Ser). We interpreted this variant according to ACMG criteria as 'likely pathogenic' (see Table 3).

We went on to genotype the variant in 9 family members (six affected and three unaffected) to test for segregation. The 'likely pathogenic' ('A') allele was found in to segregate with affected status along the proband's' father's side of the family (see Figure 1 and

Supplementary Figure 1). As this variant is on the X chromosome, the father cannot pass the mutation to the proband indicating that there may be two very similar diseases segregating in one family. Notably, there was one healthy female carrier (III.1) who was found to have a clear urinalysis (urinalysis carried out at age 44). Due to potential X-inactivation (25, 26) and the segregation pattern of the variant, we did not consider cosegregation data as evidence towards ACMG. This pedigree appears to demonstrate sporadic IgA nephropathy within a family with Alport mutations. This case demonstrates the difficulty of studying familial kidney disease where two kidney disease patterns occur within the one family or where a family member is a phenocopy with a different disease.

3.2 EXOME SEQUENCING: FAMILY 104

Family 104 (F104) presented with two affected brothers with a recessive inheritance pattern (see Figure 3). The first affected brother (II.1) presented with hematuria and proteinuria at the age of 29 and was diagnosed with IgAN upon biopsy. He progressed to end stage kidney disease undergoing transplant approximately 6 years after his IgAN diagnosis. The second brother (II.3) was also diagnosed with IgAN. No other family members reported kidney disease.

In F104, a variant of unknown significance (VUS, according to ACMG guidelines), rs200302125:T>C, was identified in the non-collagenous domain of *COL4A3* (see Table 2 and Figure 3). Here, a nonsynonymous SNV was predicted to result in the replacement of a leucine with a proline at amino acid position 1474 p.(Leu1474Pro). *COL4A3* is associated with autosomal dominant and recessive forms of Alport syndrome as well as autosomal dominant benign familial hematuria (27-32).

The minor allele frequency was high for a disease-causing heterozygote variant (0.12% MAF in 1000G_All and 0.2% in gnomad). Several computational tools predicted the variant to be damaging (SIFT_pred=D, Polyphen2_HDIV_pred=D, MutationTaster_pred=D, CADD_phred = 15.76). The variant met a number of criteria for ACMG, as laid out in Table 4. However, the evidence was not sufficient to call this variant as pathogenic, and so at the time of writing, this variant comes under the VUS category.

3.3. EXOME SEQUENCING: FAMILY 74

The third family, family 74 (F74), presented with an autosomal dominant inheritance pattern with four affected individuals across two generations (see Figure 4). The proband (II.11) presented with biopsy confirmed IgAN and diabetic nephropathy which progressed to end stage kidney disease and required the patient to undergo transplant. The proband's nephew (III.1) presented with biopsy confirmed IgAN and mild arteriosclerosis/arteriolarsclerosis (see Figure 5). The second nephew (III.2) of the proband self-reported as healthy, however, urinalysis showed haemolysed blood. The proband's brother (II.1) presented with CKD and on renal biopsy IgA deposits were found in the glomerular mesangium (see Figure 5).

Two individuals in F74 were selected for WES (II.1 and III.1). We identified a candidate variant, rs373336352:G>A, in the gene *LMX1B*, a gene associated with Nail-patella syndrome (see Table 2 and Figure 4) (33). This variant, was a nonsynonymous SNV which was predicted to cause an amino acid change from glycine to serine, p.(Gly339Ser). Although this variant was also found to segregate with the disease in affected family members, it had mixed results for computational evidence supporting a deleterious effect on the gene or gene product (CADD Phred score of 10.5, Polyphen2_HDIV_pred = D, MutationTaster=D, SIFT_pred = T) and had a higher MAF than would be expected for a pathogenic variant. As such, it was classified as VUS (see Table 5). It was not possible to obtain DNA samples for unaffected family members in F74 and therefore only affected family members were sequenced.

4. DISCUSSION

We analysed exome sequence data from ten Irish families presenting with IgAN, filtering for rare, functional, exonic or splicing, predicted damaging variants that were shared between affected members of the family. We detected an ACMG-satisfying disease causing mutation in one of the ten families, with VUS found in kidney-disease related genes in another two families.

IgAN is the most common form of glomerulonephritis worldwide and likely has multiple different forms, from monogenic to multifactorial. Despite strong evidence from familial segregation, to date a clearly monogenic form has yet to be fully characterised. Given the relative frequency of IgAN, it is also clear that IgA deposits may be present on kidney biopsies with a second clear renal pathological diagnosis. This points to the difficulty in analysing kidney pathology where IgA deposition may be present but other disease mechanisms are the predominate lesion affecting kidney structure. In the families presented in this study, there was clear evidence of IgA deposition thus making the criteria for a diagnosis of IgAN in extended families with advanced kidney disease.

In our analysis, we did not identify any potentially causal qualifying variants in 7 of the families. We made a clear molecular diagnosis in one case and in two further cases, we identified variants of uncertain significance. In family F141, a likely-pathogenic mutation was found in the *COL4A5* gene (notably, this variant did not meet the criteria originally set out in this study as it was not present in the proband). Mutations in this gene are commonly associated with Alport syndrome. This variant and a mutation resulting in a different amino acid change at the same codon had previously been identified as pathogenic in families with Alport syndrome (34, 35). In F141, the father's biopsy report diagnosis was focal proliferative glomerular nephropathy with mild arteriolar sclerosis and the uncles' biopsy showed thin basement membrane nephropathy (see Figure 2). In comparison, the proband's biopsy report showed IgAN.

This finding indicates that the father (and associated carriers of the *COL4A5* variant) have a similar, yet different, cause of kidney disease compared to the proband, who has clear features of IgAN and does not carry the *COL4A5* variant. Deafness, a clinical phenotype often found in individuals with Alport syndrome, was not found in this family (36). This highlights the range and diversity in the phenotypes associated with type 4 collagen mutations. The proband in this family had clear evidence of IgA nephropathy with multiple affected family members with overt kidney disease which was likely resulting from collagen mediated disease. This family illustrates the difficulty in diagnosing familial kidney disease as there was clear evidence of two distinct pathogenic processes coexisting within the one family (IgAN and collagen basement membrane disease).

In family F104, we identified a VUS in *COL4A3*. This gene has previously been shown to cause autosomal recessive and dominant Alport syndrome as well as autosomal dominant benign familial haematuria (28). This variant was recently reported in a family diagnosed with focal segmental glomerular sclerosis indicating that the spectrum of disease caused by mutations in *COL4A3* may be wider than previously thought (37). However, the variant had a minor allele frequency that is higher than what we would expect for a causal heterozygous pathogenic variant. That being said, kidney disease is relatively common in any population. According to a 2010 study, the age-standardized global prevalence of CKD in adults (minimum age of 20) is 10.4% in men and 11.8% in women (38). Therefore, it is possible that variants with relatively high minor allele frequencies, may in fact be causal, or contributing risk. Also, kidney disease has an extremely broad age of onset, becoming more common as age increases (39). This means that variants causing kidney disease may not be under the same selective pressure as variants causing disease with earlier ages of onset as the mean age for end stage kidney disease in patients with IgAN is past the average age for child bearing (mean age recently reported as 42.8 (40)). Therefore, variants causing the disease could be at relatively high frequencies in the general population. However, without further evidence we cannot class the mutation found in this family as pathogenic and so whether this variant is causal or an incidental finding has yet to be determined.

In the third family, F74, we identified a variant in the gene *LMX1B*, known to cause autosomal dominant Nail-patella syndrome. This syndrome is characterised by dysplastic nails, absent or hypoplastic patellae and, in a number of cases, nephropathy resembling glomerulonephritis. *LMX1B* binds the enhancers of *COL4A4* (41). In mice with *LMX1B* knock-out mutations (-/-), there is a strong decrease in the alpha-3 and alpha-4 chains of type IV collagen (41). Similar to that of Alport syndrome, the dysregulation of the alpha-3 and alpha-4 chains of type IV collagen results in glomerulopathy. It is thought that haploinsufficiency is the pathogenic mechanism of Nail-patella syndrome, with the disease following an autosomal dominant inheritance pattern (33). A study examining individuals with nail patella-like renal disease (NPLRD) reported a number of families who carried a mutation in the homeodomain of *LMX1B* indicating that individuals with mutations in this gene do not always present with the full spectrum of Nail-patella syndrome phenotypes and can cause renal specific disease (42). However, it should be kept in mind that the variant in

LMX1B was found at a higher frequency than expected, was not found in the homeodomain region (43) and had conflicting computational evidence for pathogenicity and so whether this variant is causal or an incidental finding remains uncertain. Investigations of the prevalence of variants of unknown significance in genes known to cause kidney disease is warranted to help us understand the likelihood of these variants occurring.

In the remaining seven families, there was just one gene, *PIK3C2B*, that contained qualifying variants in more than one family (see Supplementary Table 2). We identified one family (F91) that carried a frameshift deletion and one family (F433) containing a nonsynonymous variant in *PIK3C2B*. This gene has no known links to kidney disease and according to RVIS, is in the 77.93% most intolerant genes (i.e. very tolerant to mutations) and so this may be an incidental finding or potentially is a risk factor as opposed to a causal variant – adding to an overall polygenic load. We did not identify any other genes with qualifying variants that were shared across these families. This raises a number of potential scenarios. Potentially, the disease in these families is caused by non-genetic (or at least not exclusively genetic) factors. IgAN is correlated with a number of environmental factors in particular infections. For example HIV, staphylococcus mucosal infections, Chlamydia, malaria and Lyme disease have all been associated with the occurrence of IgAN (44-47).

Also, some forms IgAN may be polygenic. As IgAN is a relatively heterogeneous disorder, it is possible that different families have a different underlying genetic causes in genes yet to be linked to kidney disease. Notably, a number of the families carried variants in genes (*TRAF2* and *NFKB2* – see supplementary table 2) associated with TRAF6 mediated NF-kB activation. TRAF6 mediated NF-kB activation is involved in the production of galactose-deficient IgA1 which is needed for the formation of immune complexes found in individuals with IgAN (48). Potentially, variants in the pathway and other pathways associated with the production of galactose-deficient IgA1 may be risk factors for the development of IgAN. Further analysis of larger cohorts of families presenting with IgAN will be needed to decipher this important question.

This study had a number of limitations. Firstly, the absence of a control group of Irish ancestry would have allowed us to better interpret the candidate variants presented in this study in the context of the given population. Further work is required to decipher the

frequency of these variants in the Irish population. Another limitation of this study was that different sequencing platforms (Ion Torrent and Illumina) were used across the families. The resulting variants were refined using visual inspection with IgV and followed up with Sanger sequencing to reduce the number of potential artefacts. However, the use of different sequencing platforms may have resulted in false negatives, where for example variants could be identified in one family was not observed in other families due to the differences in sequencing methodology. Also, due to the relatively small pool of families (n=10), we were underpowered to carry out polygenic or burden analyses.

Our results confirm the heterogeneous nature of IgAN and provides evidence that while a proportion of families who present with IgAN may carry a disease causing variant in a known kidney disease related gene, the majority do not. With the continued discovery of disease genes, it is possible that families with a currently unknown genetic cause for their disorder may be receive a genetic diagnosis in the near future. Further analysis of large, carefully phenotyped families with IgAN should provide insights into disease mechanisms of IgAN.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

STATEMENT OF ETHICS

Subjects have given their written informed consent. The study protocol has been approved by the Beaumont Hospital Ethics committee (research ethics committee reference: 12/75).

AUTHOR CONTRIBUTIONS

NKF, CK, DC and SLM recruited patients, compiled pedigrees, gathered patient phenotype data and collected blood/saliva samples. CPS, GLC and PJC designed the analyses. CPS carried out DNA sample preparation and data analysis. GLC and PJC supervised the study. CPS wrote the manuscript with consultation and guidance from GLC, PJC and NKF. NKF, AMD and BD imaged and analysed biopsies. All authors reviewed and gave input to the final manuscript.

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FIGURE LEGENDS

Figure 1. Pedigree of family F141

Circles indicate females, squares indicate males, diamond indicates uncertain sex, arrow indicates proband, diagonal line through a shape indicates a deceased individual, filled shapes indicates affected status, unfilled shaped indicate an unaffected individual. Individuals with A or G letter under their shape indicates their genotype at the COL4A5 variant. The proband (IV1), father (III.6) and uncle (III.5) underwent biopsies.

Figure 2. Light and electron micrographs showing appearances seen in IgAN and Thin basement membrane nephropathy of family 141.

Image A: Hematoxylin & Eosin (H&E) stained light micrograph (20X) showing diffuse mesangial matrix expansion and hypercellularity from kidney cortex of individual IV1 from F141. Image B: Electron micrograph (5000X) showing electron dense deposits in the mesangium (solid arrows) and a thin basement membrane on the glomerular capillary loop (dashed arrow) from individual IV1. Image C: Hematoxylin & Eosin (H&E) stained light micrograph (20X) showing a normal glomerulus in individual III.5. Image D: Electron micrograph (10000X) showing a thin glomerular basement membrane in individual III.5 (dashed arrow). No electron dense deposits were found in the mesangium of III.5. In individual IV1, immunofluorescence showed global and diffuse granular mesangial positivity for IgA in all glomeruli (data not shown), whilst no evidence of immune complex deposition was seen in individual 111.5. The findings in the above images, taken together with the immunofluorescence results, demonstrate features typical of IgAN and thin basement membrane nephropathy (TBMN) for IV1 and TBMN for 111.5. See Supplementary figure 4 for colour version of images.

Figure 3. Pedigree of family F104

Circles indicate females, squares indicate males, diamond indicates uncertain sex, arrow indicates proband, filled shapes indicates affected status, unfilled shaped indicate an unaffected

individual. Individuals with T or C letter under their shape indicates their genotype at the COL4A3 variant. Individual II.1 underwent biopsy.

Figure 4. Pedigree of family F74

Circles indicate females, squares indicate males, diamond indicates uncertain sex, arrow indicates proband, filled shapes indicates affected status, unfilled shaped indicate an unaffected individual. Individuals with A or G letter under their shape indicates their genotype at the LMX1B variant. Individuals II.1, III.1 and II.11 underwent biopsy.

Figure 5. Light and electron micrographs showing appearances seen in IgAN of family 74

Image A: Hematoxylin & Eosin (H&E) stained light micrograph (20X) showing areas of mesangial hypercellularity and mesangial matrix expansion (arrows) from kidney cortex of individual II.11. The inset shows one of these areas at higher magnification (40x). Images and E: Hematoxylin & Eosin (H&E) stained light micrographs (20X) showing diffuse mesangial matrix hypercellularity and expansion from kidney cortex of individuals II.1 and III.1, respectively. Image B, D and E: Electron micrographs (15000X) showing electron dense deposits in the mesangium (arrows) and also capillary loops (image B). The diamonds show the glomerular capillary loop spaces. Immunofluorescence for all patients demonstrated global and diffuse granular mesangial positivity for IgA (data not shown). The findings in the above images, taken together with the immunofluorescence results, demonstrate the features typical of IgAN. See Supplementary figure 5 for colour version of images.