

LAMA2 and LOXL4 Are Candidate FSGS Genes

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Abstract

Background: Focal and segmental glomerulosclerosis (FSGS) is a histologic pattern of injury that characterizes a wide spectrum of diseases. Many genetic causes have been identified in FSGS but even in families with comprehensive testing, a significant proportion remain unexplained.

Results: In one FSGS family with 6 affected relatives, we performed linkage analysis and whole exome sequencing (WES). Linkage analysis narrowed the disease gene(s) to within 3% of the genome. Whole exome sequencing revealed 5 heterozygous rare variants, which were sequenced in 11 relatives where DNA was available. Two of these variants, in *LAMA2* and *LOXL4*, remained as candidates after segregation analysis and encode extracellular matrix proteins of the glomerulus. Renal biopsies showed classic segmental sclerosis/hyalinosis lesion on a background of mild mesangial hypercellularity. Examination of basement membranes with electron microscopy showed regions of dense mesangial matrix in one individual and wider glomerular basement membrane (GBM) thickness in two individuals compared to historic control averages.

Conclusions: Based on our findings, we postulate that the additive effect of digenic inheritance of heterozygous variants in *LAMA2* and *LOXL4* leads to adult-onset FSGS. Limitations to our study includes the absence of functional characterization to support pathogenicity. Alternatively, identification of additional FSGS cases with suspected deleterious variants in *LAMA2* and *LOXL4* will provide more evidence for disease causality. Thus, our report will be of interest to clinicians and genetic groups as sequencing in renal disease becomes more widespread. Overall, our results provide an example of more complicated genetic inheritance patterns that underlie glomerular disorders in unexplained families.

Background

Defects in basement membrane proteins of the kidney such as type IV collagen and LAM β 2 are reported to cause focal and segmental glomerulosclerosis (FSGS), a histologic lesion with varied causes (1, 2). We describe a family with FSGS, where our detailed genetic analysis identified an association with segregating heterozygous variants in *LAMA2* and *LOXL4*, whose encoded proteins serve roles in basement membrane assembly and function.

Results

The proband, 6238, presented with proteinuria in his early 20s and a kidney biopsy at age 41 demonstrated FSGS (**Figures 1 and 2**). His brothers (7825, 6237) were shown to have FSGS in the 4th and 5th decades of life. The proband and his elder brother, 7825, developed end-stage kidney disease (ESKD) in the 5th decade of life while the youngest brother, 6237, has stage 3b A3 CKD at age 59. One sister, 6464, developed proteinuria and impaired kidney function at the time of last follow-up at age 54. Her daughter, 6463, who was found to have FSGS at age 23, had ~3.3 g/d of proteinuria and an eGFR of 48 mL/min/1.73m² at age 30. Two of the proband's sisters, 7014 and 7015, were reported to have

proteinuria and no renal biopsies by the 5th decade of life. The proband's mother was reported to have FSGS, developing ESKD at age 68. The proband's son, 7824, was described to have proteinuria.

Genotype data from 11 individuals of this European descent pedigree was analyzed for multipoint linkage analysis, which included a set of 11,335 SNPs across autosomes. The segregation of the disease in the pedigree was not compatible with X-linked disease and therefore only autosomal linkage analysis was performed under a fully penetrant dominant model, with the affection status of 5 individuals as affected, 3 individuals as unaffected and 3 as unknown. The maximum LOD score was 0.9028, and it was observed at 388 markers across 11 different chromosomes (chromosome 1, 2, 3, 4, 6, 7, 8, 10, 12, 13 and 18), narrowing the candidate gene(s) to only 3% (388 SNPs/11,335 SNPs) of the genome (**Supplementary Figure 1 and 2**). Copy number variants (CNV) were also analyzed but there was no co-segregation of any particular CNV in all affected individuals of the family.

Whole exome sequencing was performed in 6237, 6238 and 6463, identifying 5 heterozygous rare variants (minor allele frequency or MAF <0.01) in the linked regions (**Figure 1**). Sanger sequencing of the 5 variants in 11 individuals where DNA was available identified 2 of these to be segregating in all affected individuals, while the other 3 did not (**Figure 1**). This included the variants in *LAMA2* (chr6, NM_000426.3:c.380A>G(p.T127A)) and *LOXL4* (chr10, NM_000221.1:c.1684_1686del(p.E562del)), which affected amino acid residues that were found to be highly conserved across species (**Figure 1**). Both were predicted to be deleterious by *in silico* programs (**Supplementary Table 1 and 2**).

LAMA2 contributes to laminin networks and localizes to the mesangium while *LOXL4* catalyzes cross linking of collagens and is expressed in glomeruli and tubules. Detailed examination of electron microscopy of basement membranes was undertaken in 4 individuals: 6237, 6238, 6463 and 7825. In 6237, regions of dense mesangial matrix were observed (**Figure 2**) but this was not observed in other biopsies of the same individual or the 3 other individuals. The glomerular basement membrane (GBM) thickness was also compared. The average GBM widths (\pm SEM) were: 574.3 nm \pm 11.8 (6237; male), 345.7 nm \pm 8.1 (6238; male), 375.6 nm \pm 8.5 (7825; male), 495.9 \pm 7.2 (all individuals) (**Figure 2**). Two of these patients, 6237 and 7825, had wider GBMs than historic control averages (male 373 \pm 42 nm, n=59 male kidney donors; and 326 \pm 45 nm, n=59 female kidney donors) (3).

Discussion And Conclusions

Our comprehensive genetic analysis in this FSGS family consisting of linkage analysis narrowed candidates to 3% (388 SNPs/11,335 SNPs) of the genome. Whole exome sequencing subsequently identified segregating rare variants in *LAMA2* and *LOXL4* as candidate disease genes. Laminins are found in an intricate lattice of proteins that compose extracellular matrices of organs. *LAMA2* encodes the laminin alpha-2 subunit. In the glomerulus, it heterotrimerizes with laminin beta-1 or 2 (*LAMB1*, *LAMB2*) and laminin gamma-1 (*LAMC1*), called laminin 211 or 221, to form the mesangial extracellular matrix (4, 5). The variant *LAMA2* T127A exists in the laminin N-terminal (LN) domain, which is responsible for trimer-trimer interaction of laminin polymer formation involved in the initiation of

basement membrane assembly (6). Certain variants in *LAMA2* have reported to cause *LAMA2*-muscular dystrophy, an autosomal recessive disorder caused by loss of laminin-211 in skeletal muscle (7). None of the affected family members had evidence of *LAMA2*-muscular dystrophy.

LOXL4 encodes an amine oxidase enzyme that is copper dependent and hypothesized to catalyze the cross linking of collagens and elastins (8). It is expressed in both glomeruli and tubules (<https://www.proteinatlas.org/ENSG00000138131-LOXL4/tissue/kidney>; <https://gtexportal.org/home/gene/LOXL4>). The single amino acid deletion occurs at the C-terminus of the protein, which is highly conserved.

Limitations to our study includes the absence of functional characterization to support pathogenicity, which is challenging for matrix proteins. Alternatively, identification of additional FSGS cases with suspected deleterious variants in *LAMA2* and *LOXL4* will provide more evidence for disease causality. Thus, our report will be of interest to clinicians and genetic groups as sequencing in renal disease becomes more widespread. Another limitation is our use of whole exome sequencing, which only evaluates coding region but not intronic or intergenic sequence.

Based on our findings, we narrow candidates to 3% of the genome and identify *LAMA2* and *LOXL4* as the candidate genes in a family with FSGS. We postulate that the additive effect of digenic inheritance of heterozygous variants in *LAMA2* and *LOXL4* leads to adult-onset disease in the affected relatives. We further postulate that the absence of clinically significant extra-kidney features including muscular dystrophy is as a result of the impact of the variant (heterozygous missense for *LAMA2*, heterozygous single base pair deletion in *LOXL4*), which should lead to translated protein rather than complete deficiency that can be seen in autosomal recessive disorders like *LAMA2*-muscular dystrophy. Overall, our results provide an example of more complicated genetic inheritance patterns that underlie glomerular disorders in unexplained families.

Declarations

Ethics approval and consent to participate

Study participants have given their written informed consent and the study protocol was approved by the Toronto General Hospital's committee on human research (98-U013).

Consent to Publish

Consent for publication is included in our consent form approved by the Toronto General Hospital's committee on human research (98-U013).

Availability of Data and Materials

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request but restrictions to protect identity of

patients.

Competing Interests

None

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Authors' Contributions

All authors have read and approved the manuscript. SH investigated candidate genes, reviewed literature and generated data presented in Figure 1. PV and TY analyzed exome sequencing and PV aided in development of Figures and generated data in Supplementary Tables 1/2. MAQ reviewed and summarized clinical reports for letter. RJ reviewed kidney biopsies and contributed to Figure 2. ADP provided guidance in genetic analysis and made editing contributions to letter. BD and RL performed detailed analysis on measurements of extracellular matrix on EM images and generated Figure 2. YP recruited proband, retrieved pathology and collected/stored all DNA samples while also making editing contributions to letter. MB recruited family members, collected samples, funded, supervised all aspects of the work and wrote letter.

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Figures

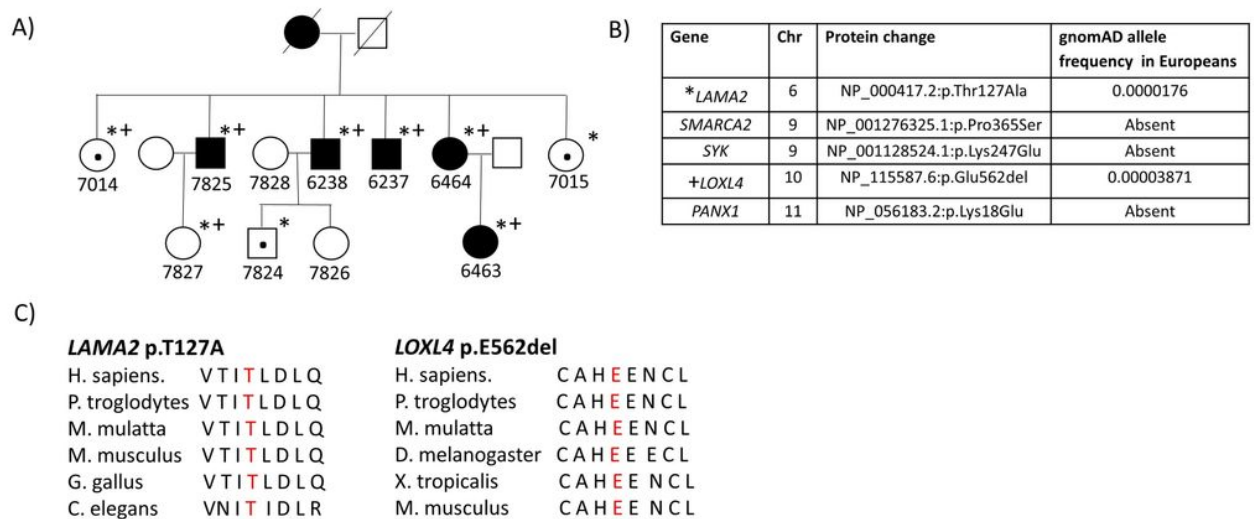


Figure 1

Digenic Inheritance of Rare Variants in *LAMA2* and *LOXL4* in a Family with Autosomal Dominant FSGS. Individuals with dot indicates microalbuminuria and unclear affection status. (A) Exome Sequencing

was performed in 3 affected members (6238, 6237, 6463) of family FSGS 15. (B) Five heterozygous rare variants were identified and sequenced in each relative, with LAMA2 (*) and LOXL4 (+) segregating in affected individuals. (C) These variants affect highly conserved residues across species and are predicted to be deleterious by prediction programs. gnomAD v.2.1.1 accessed May 3, 2020.

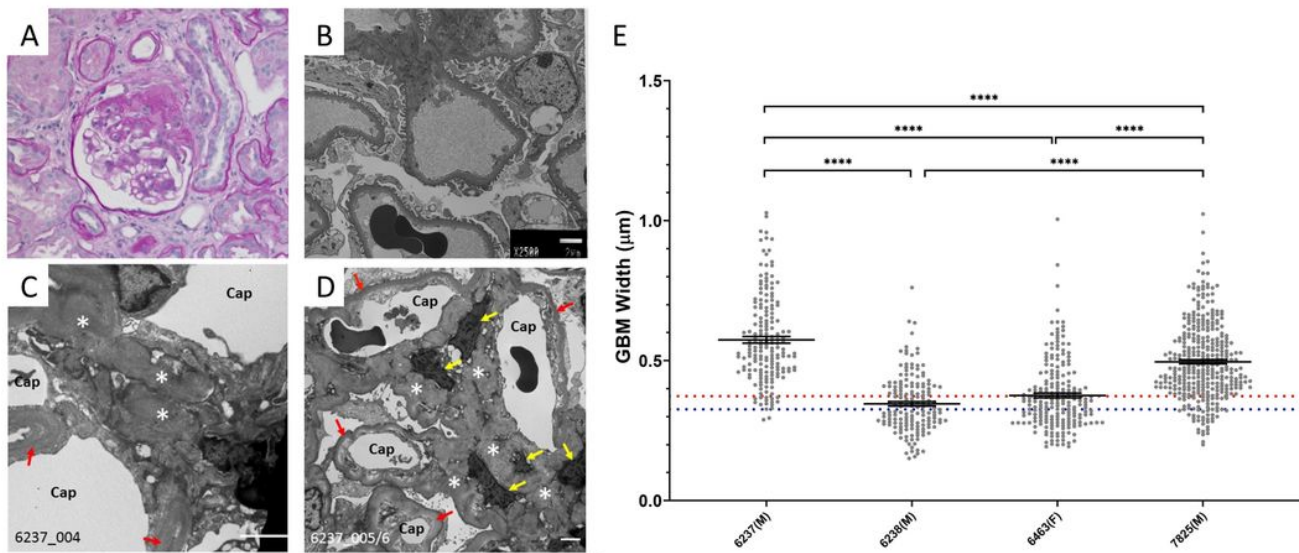


Figure 2

Kidney biopsies from proband, 6238, and sibling 6237. (A) 6238: The first biopsy showed classic segmental sclerosis/hyalinosis lesion on a background of mild mesangial hypercellularity (PAS, 20x). (B) 6238: Ultrastructural examination showed mild podocyte foot process effacement and normal glomerular basement membranes (GBM)(2500x). (C and D) 6237: no significant GBM alterations (red arrows) are seen but there are regions of dense extracellular matrix (*), postulated to be mesangial, which appear to enclose cells to the point where only the nucleus is visible (yellow arrows). (E) Comparison of nested averages (mean±SEM) for each individual: 6237(M): 574.3nm±11.8; 6238(M): 345.7nm±8.1; 6463(F): 375.6nm±8.5; 7825(M): 495.9nm±7.2; all individuals, except 6238(M) & 6463(F), are significantly different from each other (p<0.0001). Dotted lines are the average GBM thickness for males (red; 373±42 nm) and females (blue; 326±45 nm) according to Steffes et al. Lab Invest. 1983 Jul;49(1):82-6.

Supplementary Files

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