研究成果の刊行に関する一覧表

書籍
雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
張田豊	遺伝的腎疾患（爪膝蓋骨症候群および*LMX1B*関連腎症）	腎と透析	In press		2016
Konomoto T, Imamura H, Orita M, Tanaka E, Moritake H, Sato Y, Fujimoto S, Harita Y et al.	Clinical and histological findings of autosomal dominant renal-limited disease with LMX1B mutation.	Nephrology	In press		2016
Yamamoto T, Tagawa A, Eguchi M, Ohashi N, Yasuda H, Harita Y, Hattori M, et al.	Glomerulopathy with distinctive fibrillar deposits but lacking glomerular deposition of type III collagen.	CEN Case Report,	In press		2016
Hattori M, Iwano M, Sako M, et al.	Transition of adolescent and young adult patients with childhood-onset chronic kidney disease from pediatric to adult renal services: a nationwide survey in Japan.	Clin Exp Nephrol.	In press		2016
Ogino D, Hashimoto T, Hattori M, et al.	Analysis of the genes responsible for steroid-resistant nephrotic syndrome and/or focal segmental glomerulosclerosis in Japanese patients by whole-exome sequencing analysis.	J Hum Genet.	61	137-41	2016
張田豊	特発性ネフローゼ症候群の発症機序(総説)	日本小児腎臓病学会誌	28	120-128	2015
Miyake N, Tsukaguchi H, Koshimizu E, Shono A, Matsunaga S, Shiina M, Mimura Y, Imamura S, Hirose T, Okudela K, Nozu K, Akioka Y, Hattori M, et al.	Biallelic Mutations in Nuclear Pore Complex Subunit NUP107 Cause Early-Childhood-Onset Steroid-Resistant Nephrotic Syndrome.	Am J Hum Genet.	97	555-66	2015
Hattori M, Sako M, Kaneko T, Ashida A, et al.	End-stage renal disease in Japanese children: a nationwide survey during 2006-2011.Resistant Nephrotic Syndrome.	Clin Exp Nephrol.	19	933-8	2015
Muso E, Mune M, Hirano T, Hattori M, et al.	Immediate therapeutic efficacy of low-density lipoprotein apheresis for drug-resistant nephrotic syndrome: evidence from the short-term results from the POLARIS Study.	Clin Exp Nephrol.	19	54-64	2015

IV.　研究成果の刊行物・別冊

腎と透析 2016 年増刊号

8 章その他の糸球体疾患

3.遺伝的腎疾患（爪膝蓋骨症候群および *LMX1B* 関連腎症）

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Key word: 爪膝蓋骨症候群、Nail-Patella 症候群、*LMX1B* 関連腎症、*LMX1B*

はじめに

爪膝蓋骨症候群(Nail-Patella 症候群)は爪形成不全、膝蓋骨の低形成、腸骨の角状突起、肘関節の異形成を 4 主徴とする遺伝性疾患である。約半数は蛋白尿や血尿を呈する腎症を発症、その一部は末期腎不全に進行し、腎予後が QOL に多大な影響を及ぼす。原因は *LMX1B* の遺伝子異常である。

LMX1B 変異はまた爪膝蓋骨症候群と同様の腎症を有するが爪、膝蓋骨、腸骨などの変化を伴わない孤発性腎症 (nail-patella-like renal disease(NPLRD))や家族性巣状糸球体硬化症(FSGS)の原因となる。そのため現時点で FSGS や原因不明の腎不全と診断されている症例の中で、*LMX1B* 変異を原因とする症例が一定数存在すると考えられ、このような腎症全体を *LMX1B* 関連腎症という一つの疾患概念として定義し直す必要がある。

本項では本邦で行われた *LMX1B* 関連腎症の実態調査の結果を含め、これらの疾患の特徴について整理する。

1、爪膝蓋骨症候群(Nail-Patella 症候群)

爪膝蓋骨症候群(Nail-Patella 症候群, MIM161200)は爪形成不全、膝蓋骨の低形成あるいは無形成、腸骨の角状突起(iliac horn)、肘関節の異形成を 4 主徴とする常染色体性優性遺伝性疾患である。

爪膝蓋骨症候群の症状は多彩であるが [1]、爪の異形成はほぼ 100%に認められ、程度は完全欠損から低形成まで様々である。三角状の爪半月が特徴である。爪の異常は生下時から認められることも多いが軽度であると気づかれにくい。膝蓋骨の形成不全は 9 割に認められ、理学所見上膝関節屈曲時の陥凹が認められる。機能的に問題となることは少ない。肘関節の異常も 9

割近くに認められ、肘関節の伸展、回内、回外が制限される。腸骨の角状突起は 7-8 割に認められ、大きいと外表からも触知される。しばしば腎症を発症し、一部は末期腎不全に進行するため、本症候群の QOL に大きく影響する。

1998 年に爪膝蓋骨症候群の原因遺伝子として *LMX1B* が同定された。*LMX1B* は染色体 9q34 に位置する。*Lmx1b* 遺伝子ノックアウトマウスのホモ接合体欠失個体で爪の低形成、膝蓋骨の欠損、腎の異常などの類似の症状を呈する。

LMX1B はホメオドメインと一対の LIM ドメインを持つ、LIM ホメオドメイン蛋白の一つである。一般的には LIM ホメオドメイン蛋白は様々な器官形成やニューロンの分化などに関与する転写因子として機能する。ホメオドメインは DNA と結合し、LIM ドメインはタンパク質間相互作用に関与する。ヒト LMX1B は 372 個(選択的スプライシングにより 379 個)のアミノ酸よりなり、その mRNA は胎児、成人とも腎臓に強く発現している。さらに胎児期には LMX1B は膝蓋骨や手指の背側組織、特に母指側に強く見られ、爪膝蓋骨症候群の表現型の部位と一致する。

LMX1B には今までに 130 種類以上の変異が同定されており、すべてヘテロ接合体変異で発症する。親からの遺伝あるいは de novo 変異が原因である。シークエンス解析では約 20%の患者で変異が認められず、MLPA 法などによりそのうち一部には遺伝子の部分的な欠失が検出される症例や、プロモーター領域の変異や関連する別の遺伝子に起因する可能性が示唆される症例も存在する。しかしすべての患者に *LMX1B* を含めた遺伝子異常が認められる訳ではないため、異常がない場合でも診断を除外することはできない、すなわち *LMX1B* 遺伝子検査異常は爪膝蓋骨症候群の診断に必須ではない。

2, 爪膝蓋骨症候群(Nail-Patella 症候群)腎症

爪膝蓋骨症候群の約半数(報告により 25-62%)に腎症を合併する。通常は無症候性の蛋白尿、稀に血尿が見られるが、時に高度の蛋白尿により、ネフローゼ症候群を呈することもある。2-15%の症例で腎機能が進行性に悪化し末期腎不全になったと報告されている [2]。小児期に腎不全に至った症例も報告されている。

腎組織所見としては、光学顕微鏡レベルでは腎不全の程度に応じた所見であり、FSGS、増殖性糸球体腎炎など多様で特徴的な所見がない [2]。しかし電子顕微鏡で不規則に肥厚した糸

球体基底膜、その緻密層に認められる虫食い像(moth-eaten appearance)、タンニン酸染色やリンタングステン酸染色などの特殊染色により基底膜とメサンギウム基質にコラーゲン線維束が認められる(図 1)。また免疫染色では沈着するコラーゲン線維は III 型コラーゲンである。

本邦の *LMX1B* 関連腎症の実態調査により、腎症を有する爪膝蓋骨症候群患者の特徴として、爪膝蓋骨症候群の家族歴のない症例の診断年齢が高く、その診断が困難な場合が多いことが明らかになった。家族歴のある症例では7割が3歳以下で診断されていたが、家族歴のない症例(発端者を含む)では発症時年齢は全例4歳以上、診断時年齢は全例8歳以上であった。また出生後や一ヶ月検診などで上肢や肘の可動性に問題が有ったにもかかわらず、学校検尿での異常を契機に腎症が疑われた時点で初めて爪膝蓋骨症候群と診断された症例が複数存在した。逆に先に尿検査異常に気付かれ、蛋白尿や FSGS として治療を受けていたが、10 年以上経過して初めて爪の変化や膝蓋骨欠損などに気づかれ爪膝蓋骨症候群と診断された症例も複数存在した。

腎生検では三分の二の症例で特徴的な基底膜の変化(虫食い像あるいは不規則な肥厚)が認められたが残りの症例では FSGS、微小変化、Focal glomerular obsolescence と診断されており、病理所見だけで爪膝蓋骨症候群と診断することは困難である。治療としては免疫抑制治療(ステロイド、シクロフォスファミド)を受けた症例が1例、それ以外の症例では免疫抑制治療はされておらず、6例でアンギオテンシン阻害薬等が用いられていた。その他の 10 例では無治療で経過観察されていた。また本邦の腎症を有する爪膝蓋骨症候群のうち、約四分の一の症例が末期腎不全(CKD5)や高度腎機能低下(CKD4)を来しており、腎不全へと進行する割合は海外の報告と大きく異ならないと考えられる。

3, Nail-Patella-Like Renal Disease (NPLRD)

爪膝蓋骨症候群と同様の腎症を有するが爪、膝蓋骨、腸骨などの変化を伴わない孤発性腎症が存在し、nail-patella-like renal disease(NPLRD) と呼ばれている(MIM 256020)。これらの症例では蛋白尿などの検尿所見、ネフローゼ症候群、時には腎不全を呈し、特に電子顕微鏡所見として糸球体基底膜の変化が認められるものの、爪、膝蓋骨、腸骨などの腎外症状を呈さない [2]。私達は本邦の典型的な NPLRD 症例において *LMX1B* R246Q 変異を同定した

[3]。この変異はDNA結合部位の変異であり、LMX1Bの転写活性を部分的に阻害する。この症例では腎外症状は全くないものの糸球体基底膜の典型的な虫食い像が見られること、特殊染色により基底膜にIII型コラーゲンの沈着を認めたことがNPLRDの診断の決め手となった[3]。

4, *LMX1B* R246変異によるFSGS

NPLRDの原因となるR246変異はFSGS患者においても同定されている。2013年にフランスのグループが家族性FSGS症例に対して網羅的遺伝子解析行い、*LMX1B*のR246Q (2家系)およびR246P (1家系) 変異をその原因として報告した[4]。一部の症例では末期腎不全に至っていた。これらの家系では爪や膝蓋骨などの腎外症状を有していないことはNPLRDと共通していたが、本邦のNPLRD症例では認められた爪膝蓋骨症候群の腎症に特徴的な基底膜の変化はフランスから報告された三家系では一切認められなかった。これらの事実からR246変異は爪や骨格系に変化をおこさずに孤発性腎症の原因となりうること、しかも爪膝蓋骨症候群において特徴的といわれる基底膜変化は必ずしも伴わないことが明らかになった。最近本邦においても家族性FSGSとして遺伝子検査を施行されて変異が明らかとなった家族例(1家系3症例)が報告されてた[5]。この家系では診断年齢は1-11歳と小児期であり、全員小児期に血尿蛋白尿として発症していた。爪、膝蓋骨、腸骨、肘関節などの腎外症状はなく、その他中枢神経等の合併症も有していない。この家系のうち腎生検が行われた一例では基底膜の部分的な菲薄化が認められたものの、特徴的な基底膜変化(虫食い像など)は認められず、やはり必ずしも基底膜変化が*LMX1B*による腎症の発症に必須ではないことを裏付けている。さらに最近R249Q変異が家族性の腎機能不全家系の遺伝子解析でも見いだされており、その家系においても爪や骨格系に変化は認められなかったとされる[6]。

5, *LMX1B*関連腎症

これらの知見からこれまでFSGSや原因不明の腎不全と診断されている症例の中で、*LMX1B*変異を原因とする症例が一定数存在することが想定される。腎症を中心としてみると、腎外症状を有しているかどうかに関わらず、このような腎症全体を*LMX1B*関連腎症という一つの疾患概念として定義し直す必要がある[3, 7]。慢性的な腎障害　(血尿、蛋白尿、あるいは腎機能障害) を来す症例の診療においては、爪や関節症状などに注意

をして観察する事が重要であり、また腎外症状がない場合にも *LMX1B* 遺伝子のヘテロ接合体変異、あるいは腎糸球体基底膜の特徴的電顕所見(腎生検病理において、腎糸球体基底膜の肥厚と虫食い像" moth-eaten appearance"を認め、さらにリンタングステン酸染色あるいはタンニン酸染色により基底膜内に線維成分が染色される)が存在する場合には *LMX1B* 関連腎症として慢性腎不全へ進行する可能性もふまえた長期的なフォローアップが必要である。

おわりに

LMX1B の変異は爪膝蓋骨症候群に伴う腎症、R246 変異による FSGS/MCNS、病理学的に特徴的な象を示す NPLRD など様々な臨床像を呈する。これらの中には検尿異常のみを呈する軽微な腎症や原因不明の腎不全とされていた症例も含まれる。遺伝子検査が身近になった現在、原因遺伝子である *LMX1B* を中心としてこれらの多様な疾患群を定義し直すことにより *LMX1B* 関連腎症の特徴や長期予後、特異的な治療が明らかになる事が期待される。

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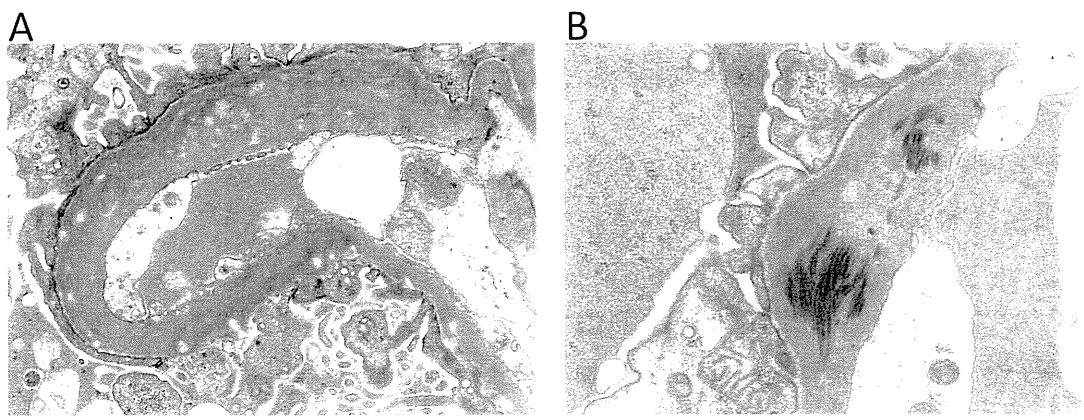
図1　*LMX1B*異常による糸球体基底膜変化

A 電子顕微鏡所見(基底膜内の虫食い像)、B タンニン酸染色による基底膜内の膠原線維。(文献3より引用)



Original Article

Clinical and histological findings of autosomal dominant renal-limited disease with *LMX1B* mutation

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nep.12666

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Short running title: Renal-limited disease with *LMX1B* mutation

ABSTRACT

Aim: Mutations of *LMX1B* cause nail-patella syndrome, a rare autosomal dominant disorder. Recently, *LMX1B* R246Q heterozygous mutations were recognised in nephropathy without extrarenal manifestation. The aim of this study was to clarify characteristics of nephropathy caused by R246Q mutation.

Methods: Whole exome sequencing was performed on a large family with nonsyndromic autosomal dominant nephropathy without extrarenal manifestation. Clinical and histological findings of patients with *LMX1B* mutation were investigated.

Results: *LMX1B* R246Q heterozygous mutation was identified in five patients over three generations. Proteinuria or haematoproteinuria was recognised by urinary screening from all patients in childhood. Proteinuria gradually increased to nephrotic levels and renal function decreased in adolescence. Two patients progressed to end-stage renal disease in adulthood. Renal histology demonstrated minimal change in childhood and focal segmental glomerulosclerosis in adulthood. Using electron microscopy, focal collagen deposition could be detected in glomeruli even when a "moth-eaten appearance" was not apparent in the glomerular basement membrane. In addition, podocin expression in glomerular podocytes was significantly decreased, even in the early stages of disease progression.

Conclusion: Comprehensive genetic analyses and collagen or tannic acid staining may be useful for diagnosis of *LMX1B*-associated nephropathy. While renal prognosis of R246Q may be worse than that of typical NPS nephropathy, signs of podocytopathy can be detected during the infantile period; thus, childhood urinary screening may facilitate early detection.

Keyword: autosomal dominant, LMX1B, nail-patella syndrome, podocin, whole exome sequencing

Introduction

LIM homeobox transcription factor 1 beta (LMX1B) plays crucial roles during embryonic development. Mutations in *LMX1B* gene lead to nail-patella syndrome (NPS), a rare autosomal dominant disorder associated with iliac horn formation and dysplasia of patella, nails and elbows. While skeletal and nail abnormalities have been observed in a majority of NPS patients, renal involvement only occurs in approximately 30–50% of patients with NPS.[1-3] The natural history of NPS nephropathy has not been described in detail. Urinary abnormalities often manifest as asymptomatic proteinuria, but can progress to nephrotic syndrome or nephritis and occasionally end stage renal disease (ESRD). Proteinuria may present at any age from birth onwards and may be intermittent, and some NPS patients presented infantile and childhood nephrotic syndrome.[1, 4] Progression of NPS nephropathy to ESRD is usually slow, however in a few cases rapid exacerbation to ESRD at a young age had been reported. [1, 3] Additionally, the severity of nephropathy was reported extremely variable both within and between families.[2] The renal pathology of NPS is characterised by irregular thickening of the glomerular basement membrane (GBM) with type III collagen fibrils and an electron-lucent area, often referred to as a "moth-eaten appearance".[2, 3, 5]

Recently, *LMX1B* R246Q heterozygous mutation was identified in a patient

with nail-patella-like renal disease (NPLRD), which displays typical renal pathology of NPS despite a lack of skeletal or nail abnormalities.[6] The same mutation was also identified in two large families exhibiting hereditary focal segmental glomerulosclerosis (FSGS) without extrarenal manifestation.[7] To date, clinical findings and kidney histology related to renal-limited LMX1B nephropathy remain unclear, as there are a limited number of affected patients and chronological assessment of urinary abnormalities and/or kidney function in these individuals is lacking.

Using whole exome sequencing (WES), we identified heterozygous *LMX1B* R246Q mutation in a large family with nonsyndromic autosomal dominant nephropathy without extrarenal involvement. Clinical and pathological findings revealed observable changes in characteristics, even during early stages and throughout varying stages of disease progression.

Methods

Study participants

The present study involved a family exhibiting an apparent autosomal dominant inheritance pattern of nephropathy. Clinical evaluation of patients included physical examination, urinalysis, renal function and renal histology, when appropriate. All study

procedures were reviewed and approved by the Research Ethics Committee of the Faculty of Medicine at the University of Miyazaki, with written informed consent obtained from either patients or their parents.

Whole exome sequencing

Genomic DNA (gDNA) was extracted from blood samples using Gentra® Puregene® Blood Kit (Qiagen, Venlo, Netherlands). Patient gDNA was enriched for WES using SureSelect Human All Exome V5 (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer's protocol. Prepared libraries were sequenced in pair-end mode using a HiSeq2500 (Illumina, San Diego, CA, USA). Raw data were converted to FASTQ format by bcl2fastq Conversion Software (Illumina). NovoalignMPI software version 3.02.06 (Novocraft, Selangor, Malaysia) was used to perform read mapping and base quality score recalibration of FASTQ files. For this step, human reference genome sequence hg19/GRCh37 and single nucleotide variation (SNV) information from Single Nucleotide Polymorphism Database build 138 were downloaded from the UCSC genome browser[8] and merged. Aligned reads were sorted by Novosort software (Novocraft). Polymerase chain reaction (PCR) and optical duplication were removed by MarkDuplicates of the Picard Tools package (http://picard.sourceforge.net). Genome

Analysis Toolkit (GATK) version 3.1-1 [9] was used to perform local realignment (GATK IndelRealigner) and variant call (GATK HaplotypeCaller) using an in-house workflow management tool.[10] Called SNVs and short insertion/deletion loci (indels) were selected as candidate mutations using two criteria: all patients were not reference-homozygote and all unaffected individuals were reference-homozygote. Selected SNVs and indels were annotated using ANNOVAR software.[11] Finally, variants meeting the following criteria were selected as "deleterious": 1) mutations leading to gain or loss of a stop codon, nonsynonymous mutation, or splice site according to gene information of GENCODE version 19;[12] 2) allele frequencies deviating from the Complete Genomics 46 dataset of unrelated individuals,[13] 1000 Genome April 2012 dataset[14] and Human Genetic Variation Database (Japanese 1,208 exome dataset) version 1.41 (http://www.genome.med.kyoto-u.ac.jp/SnpDB) equal to or less than 0.5%; 3) variations not included in segmental duplication.[15, 16] In addition, pathogenicity of variants was analysed using *in silico* predictions from Polyphen2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), Sorting Intolerant From Tolerant (SIFT) (http://sift.jcvi.org) and PROVEAN (http://provean.jcvi.org/index.php).

Target sequencing

Sanger sequencing was performed on the proband. Mutation analysis was carried out by Sanger sequencing of both strands of all exons from *WT1, ACTN4, CD2AP, TRPC6* and *INF2*, using exon-flanking primers. The entire coding region and exon-intron boundaries of genes were PCR-amplified from gDNA using KOD-Plus (Toyobo, Osaka, Japan). Products were subsequently purified and subjected to direct sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) and an automated DNA sequencer. Candidate variants identified by WES, including *LMX1B,* were validated by Sanger sequencing. Primers used for *LMX1B* PCR amplification were previously described.[17] PCR-amplified products corresponding to individual candidates were purified and subjected to direct sequencing.

Renal histological analyses

Immunohistochemical analyses were performed using either frozen or paraffin-embedded sections of kidney tissue. Type I and type III collagens were stained using goat polyclonal antibodies (SouthernBiotech Birmingham, AL, USA). Tissue for electron microscopy was fixed in glutaraldehyde solution and collagen was stained by tannic acid. Immunohistochemical analysis of podocyte protein expression was

performed using mouse monoclonal antibodies against human podocin, as previously described.[6] Expression of podocin was compared with age-matched samples from patients with minimal change disease (MCD), a childhood nephrotic syndrome with typical proteinuria.

Results

Identification of *LMX1B* mutation in a family with nonsyndromic hereditary nephropathy

A large family exhibiting nephrotic range proteinuria without extrarenal manifestation attracted significant attention, as it consists of five affected patients distributed over three generations and two unaffected members (Fig. 1A, Table 1). The proband (III-1) had chance proteinuria and microscopic haematuria identified by urinary screenings at one year of age. Proband's mother (II-2) exhibited proteinuria and haematuria in a school urinary screening performed at six years of age. Renal function of the mother decreased gradually and progressed to ESRD; subsequently, haemodialysis was commenced at 38 years of age. Proband's grandfather (I-1) progressed to ESRD of unknown cause at 40 years of age. Proband's aunt (II-4) and aunt's daughters (III-2 and III-3) had proteinuria detected by chance in urinary

screening performed at age thirteen, eleven and one, respectively.

To identify the causal variant, WES was performed on the entire family (five patients and two unaffected family members). Disease-causing candidate mutations were assessed by criteria described in the Methods section. Eleven variants in eleven genes, including *TMEM51*, *LACTBL1*, *UBR3*, *TRIM42*, *GIN1*, *SH3TC2*, *IDO2*, *FLI4*, *ZIC5*, *GP1BA*, and *LMX1B*, were selected as disease-causing candidate mutations. Amongst these genes, variants of *TMEM51, GLI4, GP1BA* and *LMX1B* were predicted as disease-causing mutations based on *in silico* modelling (supplementary table 1). *TMEM51* gene encodes transmembrane protein 51, whose function is largely unknown. However, this protein is not expressed in kidneys and, furthermore, no disorders were found to be associated with the *TMEM51* gene. *GLI4* gene, which encodes the GLI family zinc finger 4 protein solely expressed in skin, also has no known associated genetic disorders. *GP1BA* is known to be the causal gene for platelet-type von Willebrand disease (VWD), one subtype of this bleeding disorder, which only became apparent on bleeding history.[18] None of our patients presented with obvious bleeding features; therefore, *LMX1B* represented the greatest potential for causing nonsyndromic familial kidney disease. Sanger sequencing confirmed a heterozygous G-to-A mutation in exon 4 of *LMX1B* (c.737G>A, p.R246Q) in all affected patients, but not in the two

unaffected individuals (Fig. 1B). Nonsyndromic autosomal dominant FSGS genes, including *WT1, ACTN4, CD2AP, TRPC6* and *INF2*, were analysed by Sanger sequencing in the proband, as exonic mutations were not observed.

Clinical characterisation of the affected patients

Clinical data are summarised in Table 1 and Figure 2. All patients, except grandfather (I-1), first presented with chance proteinuria or haematoproteinuria by urinary screening in childhood and none of these patients presented with obvious extrarenal clinical features, such as dysplasia of patella (Fig. 1Ba), nails and elbows, or iliac horn formation (Fig. 1Bb), or glaucoma. Haematuria was observed in all patients at last follow up. Renal histological examination of the proband (III-1) confirmed MCD at two years of age, which was treated with angiotensin-converting enzyme inhibitor (ACEI). Her proteinuria gradually increased; hence, a second kidney biopsy was performed at ten years of age, with results identical to those from the first biopsy (Fig. 3Aa, b). Proband's treatment was changed to angiotensin II receptor blocker (ARB); her proteinuria increased to nephrotic level at last follow up.

During the second pregnancy of proband's aunt (II-4) at 30 years of age, she developed nephrotic-range proteinuria, haematuria and hypertension. She was treated