A Novel COL4A5 Mutation Identified in a Chinese Han Family Using Exome Sequencing

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Received 14 May 2014; Accepted 20 June 2014; Published 6 July 2014

1. Introduction

Alport syndrome (AS) is a monogenic disease of the basement membrane (BM), resulting in progressive renal failure due to glomerulonephropathy, variable sensorineural hearing loss, and ocular anomalies. It is caused by mutations in the collagen type IV alpha-3 gene (COL4A3), the collagen type IV alpha-4 gene (COL4A4), and the collagen type IV alpha-5 gene (COL4A5), which encodes type IV collagen α3, α4, and α5 chains, respectively. To explore the disease-related gene in a four-generation Chinese Han pedigree of AS, exome sequencing was conducted on the proband, and a novel deletion mutation c.499delC (p.Pro167Gln fs*36) in the COL4A5 gene was identified. This mutation, absent in 1,000 genomes project, HapMap, dbSNP132, YH1 databases, and 100 normal controls, cosegregated with patients in the family. Neither sensorineural hearing loss nor typical COL4A5-related ocular abnormalities (dot-and-fleck retinopathy, anterior lenticonus, and the rare posterior polymorphous corneal dystrophy) were present in patients of this family. The phenotypes of patients in this AS family were characterized by early onset-age and rapidly developing into end-stage renal disease (ESRD). Our discovery broadens the mutation spectrum in the COL4A5 gene associated with AS, which may also shed new light on genetic counseling for AS.
hematuria (BFH) or thin basement membrane nephropathy (TBMN) to end-stage renal disease (ESRD) resulting from various mutations, though the COL4A5-related BFH and TBMN were considered to be the milder subtypes of AS [5–7].

The purpose of our study is to explore the disease-related gene in a four-generation Chinese Han pedigree of AS. Exome sequencing is a powerful and cost-effective tool for uncovering the genetic basis of diseases [8, 9]. Conventional mutation screening by Sanger sequencing is time-consuming and expensive due to genetic heterogeneity of AS and large size of those three genes (COL4A3, COL4A4, and COL4A5). Therefore, we detected the proband of the family using exome sequencing to identify the gene responsible for this disease. A novel deletion mutation c.499delC (p.Pro167Gln fs*36) in the COL4A5 gene was identified, and it cosegregated with the disease in the family. Our data broaden the genotypic spectrum of COL4A5 mutations associated with AS.

2. Materials and Methods

2.1. Subjects. A pedigree consisting of 10 individuals across 4 generations of Chinese Han family was enrolled in this study (Figure 1). Peripheral blood samples were collected from 6 members of this family, including 4 patients. Peripheral blood samples were also collected from 100 unrelated ethnically matched normal controls (male/female: 50/50, age 40.6 ± 8.4 years). All participants underwent clinical evaluation, audiological and typical COL4A5-related ophthalmological examinations (dot-and-fleck retinopathy, anterior lenticonus, and the rare posterior polymorphous corneal dystrophy). The protocol of this study was approved by the Ethics Committee of the Third Xiangya Hospital, Central South University, and all participants signed informed consent.

2.2. Clinical Data. All family members underwent urinalysis and renal function evaluation. Members with no more than trace amount of hematuria or proteinuria and normal renal ultrasound examination were considered normal [10]. Kidney biopsy was performed for the proband. Global and segmental sclerosis and mesangial expansion were identified by light microscopy. Electron microscopy revealed irregular thickening and splitting of the glomerular basement membranes (GBMs). Immunofluorescence and electron microscopy detected no immunoglobulin A (Ig A) deposits. None of the family members showed any evidence of auditory, typical COL4A5-related ophthalmological (dot-and-fleck retinopathy, anterior lenticonus, and posterior polymorphous corneal dystrophy), or platelet abnormalities or leiomyomatosis.

2.3. Exome Capture. Genomic DNA was isolated from peripheral blood leukocytes by standard phenol-chloroform extraction method [11]. Three micrograms (μg) of genomic DNA was used to construct the exome library. Genomic DNA of the proband was sheared by sonication and hybridized to the Nimblegen SeqCap EZ Library for enrichment, according to the manufacturer’s protocol. Enriched exome fragments were sequenced on the HiSeq 2000 platform (Illumina, San Diego, CA, USA) to get paired-end reads with read length of 90 bp. A mean exome coverage of 81.65× was obtained to accurately call variants at 99.41% of the targeted exome [12, 13].

2.4. Read Mapping and Variant Analysis. The sequence reads were aligned to human genome reference obtained from UCSC database (http://genome.ucsc.edu/), version hg19 (build 37.1), using the program SOAP aligner. Single nucleotide polymorphisms (SNPs) were called using SOAPsnp set with the default parameters after the duplicated reads (produced mainly in the PCR step) were deleted [14]. Short insertions or deletions (indels) altering coding sequence or splicing sites were also identified by GATK. We filtered candidate SNPs with the following criterion: SNP quality ≥20, sequencing depth ≥4, the estimated copy number ≤2, and the distance between two SNPs >5 (the quality score is a Phred score, generated by the program SOAPsnpl0.3, and quality score 20 represents 99% accuracy of a base call) [6]. Candidate mutations were filtered against databases including the single nucleotide polymorphism database (dbSNP132, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary .cgi/), 1,000 genomes data (1,000 genomes release_20100804), HapMap (2010-08_phase II + III) and YanHuang1 (YH1) project, and synonymous substitutions. Potential disease-causing variants were evaluated by SIFT prediction (http://sift.jcvi.org/). Sanger sequencing was employed to verify the identified potential disease-causing variants with ABI3500 sequencer (Applied Biosystems, Foster City, CA, USA). Sequences of the primers were as follows: 5’-TGAATCTTCCAGATCTTTTCTGG-3’ and 5’-GAGGGA-TTGTGTAAATCTTCTGG-3’.

3. Results

We performed exome sequencing of the proband (III: 1, Figure 1) in a Chinese Han family with AS. We generated 8.14 billion bases of 90-bp paired-end read sequence for the patient. Among the 8.14 billion bases, 7.88 billion (96.81%) passed the quality assessment, 7.37 billion (93.53%) aligned
Figure 2: Sequencing analysis of COL4A5 c.499delC (p.Pro167Gln/fs*36) mutation. The arrow shows site of the novel c.499delC (p.Pro167Gln/fs*36) deletion mutation in the COL4A5 gene. (a) Heterozygous mutation carrier (III: 1). (b) Hemizygous mutation carrier (III: 3).

Figure 3: Conservation analysis of COL4A5 p.Pro167 amino acid residue.

4. Discussion

AS is a clinically and genetically heterogeneous disease, and severity of this disease is usually equal between males and females in the autosomal recessive form (autosomal recessive AS, ARAS), while greater in males with X-linked form (X-linked AS, XLAS). XLAS is caused by mutations in the COL4A5 gene with an approximately prevalence of 1/10,000 [5], and it accounts for 40%–45% of female patients with AS [16]. Female patients with XLAS have a variable and generally mild clinical course with 12% reaching ESRD by the age of 40 years and about 30% by the age of 60 years in European cohorts [17]. While male patients are more severe than females with 70% of affected males developing into ESRD before the age of 30 years (juvenile form), the remaining 30% are progressing toward ESRD after the age of 30 years (rare adult form) [18]. Furthermore, hearing loss and ocular abnormality happened in 90% and 35% of male patients, respectively [10].

In our family, four patients presented with heterogeneous clinical phenotypes of glomerulopathy, while none of them showed any clinical features of either sensorineural hearing loss or typical COL4A5-related ocular abnormalities. A COL4A5 c.499delC (p.Pro167Gln/fs*36) mutation in exon 9, cosegregating with the disease, was identified. The deletion mutation leads to a truncated protein and is absent in 1,000 genomes project, HapMap, YanHuang1 (YH1) project, and dbSNP. The mutation is located in the Gly-X-Y repeats. The p.Pro167 is a highly conserved amino acid residue among different species from chicken to human, suggesting its structural and functional importance (Figure 3). This mutation was predicted to affect the protein features and be disease causing (predicted by http://www.mutationtaster.org/). SIFT prediction also showed a damaging effect with a confidence score of 0.858 (http://sift.bii.a-star.edu.sg/www/SIFT_indels2.html).
The COL4A5 gene is located at Xq22 and contains 51 exons, encoding type IV collagen α5 chain [6]. Type IV collagen α5 chain contains 1,685 amino acid residues, which consist of a 26-residue signal peptide, a 1,430-residue collagenous domain starting with a 14-residue noncollagenous sequence, a Gly-X-Y-repeat sequence interrupted at 22 locations, and a 229-residue carboxyl-terminal NC1 domain [19]. To date, 688 COL4A5 mutations have been identified according to the Human Gene Mutation Database (http://www.hgmd.org/), including missense, nonsense, deletion, splicing mutation, and complex rearrangements [17, 20], without identification of any mutation hot spot. Genotype-phenotype correlations between COL4A5 mutations and XLAS have been extensively described. For genotype-phenotype correlation purposes, typical XLAS is classified into three types: (1) severe type with ESRD at ~20 years (juvenile-onset ESRD), 80% of hearing loss, and 40% of ocular lesions, caused by large rearrangements, premature stop, frameshift, donor splice, and mutations in the NCI domain; (2) moderate-severe type with ESRD at ~26 years, caused by non-Gly-X-Y-missense, Gly-X-Y mutations in 21–47 exons; (3) moderate type with ESRD at ~30 years (late-onset ESRD), 70% of hearing loss and <30% ocular lesions, caused by Gly-X-Y mutations in 1–20 exons [21, 22]. Four patients of our family showed no clinical features of either sensorineural hearing loss or typical COL4A5-related ocular abnormalities. Though our family is not large, a moderate type of XLAS is considered due to the mutation located in COL4A5 exon 9 and the late-onset ESRD (ESRD at 36 years, II: 1; Table 1). More severe clinical phenotypes and earlier onset-age were observed in male patient of this family (III: 3), consistent with previous reports [6].

Mutations in genes encoding α chain of type IV collagen could lead to dysfunction of BM and then lead to the development of human disease in the eye, kidney, ear, and so forth [1]. Once the α5 chain is missing, the formation of the normal α3α4α5 (IV) protomer is disrupted in BM of glomerulus, ear, eye, and lung, which could lead to structural and functional defects [23]. This is supported by the immunohistochemical finding of frequent loss of α3, α4, and α5 signals in the GBM of XLAS patients [24, 25]. The cause of clinical heterogeneity of XLAS, such as difference in age of disease onset, disease severity, and disease progression, may be multifactorial, including random X chromosome inactivation, ethnic background, and environment factors.

Animal models with genetic deficiency may provide probabilities to reveal the pathogenesis and treatment of AS [26]. Two Col4a5 truncation mutations have been identified in dogs (Samoyed and Navasota dogs) with clinical features of proteinuria and progressive kidney disease leading to terminal failure [27]. Intriguingly, a deletion in Col4a5 resulting in disruption of the Gly-X-Y repeats, similar to human p.Pro167Gln fs*36 mutation, was observed in a family of mixed-breed dogs with an inherited nephropathy that exhibits the clinical, immunohistochemical, pathological, and ultrastructural features of human XLAS, and the truncated peptide chain may probably prevent extracellular assembly in type IV collagen networks [28]. Further studies on the Col4a5 genetic-deficient AS animal models will provide new insight into mechanism research, diagnosis, and target therapy of AS in human.

5. Conclusions

In our study, we identified a novel deletion mutation c.499delC (p.Pro167Gln fs*36) in the COL4A5 gene, which may be responsible for AS in this family. Our study showed that exome sequencing is a fast, sensitive, and relatively low-cost method to identify gene(s) responsible for AS. The discovery broadens the genotypic spectrum of COL4A5 mutations associated with AS and has implications for genetic diagnosis, therapy, and genetic counseling of this family.

Conflict of Interests

The authors declare that there is no conflict of interests in this paper.

Acknowledgments

The authors thank the participating individuals for their cooperation and their efforts in collecting the genetic information and DNA specimens. This work was supported by
National Natural Science Foundation of China (81101339, 81010176); Natural Science Foundation of Hunan Province, China (10JJ4020, 10JJ5029); Construction Fund for Key Subjects of the Third Xiangya Hospital, Central South University, China; Postgraduates Innovative Pilot Scheme of Hunan Province (7138000008), China; the Fundamental Research Funds for the Central Universities of Central South University (2014zzts360); and Students Innovative Pilot Scheme of Central South University (YC12417), China.

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