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Short Communication

Mutation c.359_363delGTATTinsATAC in the COL4A5 Causes alport syndrome in a Chinese family

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ABSTRACT

The X-linked form of Alport syndrome is associated with mutations in the *COL4A5* gene, which is located at Xq22.3 and encodes the α5 chain of type IV collagen. Here we clinically characterized a Chinese family with Alport Syndrome, but no ocular or hearing abnormalities have been observed in any patient in the family. Through Linkage analysis and direct DNA sequencing, a novel complex deletion/insertion mutation c.359_363delGTATTinsATAC in the *COL4A5* gene was identified in the family. The mutation was found in all affected family members, but was not present in the unaffected family individuals or the 200 controls. The predicted mutant protein in the family is a truncated protein consisting of only 153 residues. Our report for the first time revealed that the frameshift mutation in the type IV collagen chain α5 causes only renal disease, without extrarenal lesion. Our study broadens genotypic and phenotypic spectrum of *COL4A5* mutations associated with Alport syndrome.

1. Introduction

Alport syndrome (MIM #301050, AS) is a hereditary disorder of the basement membrane, resulting in progressive renal failure due to glomerulonephropathy, variable sensorineural hearing loss, and ocular anomalies (Gross et al., 2002). The pathogenesis of AS is related to the defect in the *COL4A3*, *COL4A4* and *COL4A5* genes encoding type IV collagen α-chain isoforms (α3, α4, and α5). These three genes are necessary for proper development of the glomerular basement membranes, which plays a crucial role in the purification of blood plasma in the kidney (Kalluri et al., 1997). AS is a genetically heterogeneous disease. About 85% of AS patients have X-linked AS(XLAS), caused by mutations in the *COL4A5* gene, which is located on chromosome X (Barker et al., 1990; Gross et al., 2002). *COL4A3* and *COL4A4* genes, located on chromosome 2, are involved in approximately 15% of autosomal recessive AS cases and the rarer autosomal dominant forms of AS (Jefferson et al., 1997; Mochizuki et al., 1994; van der Loop et al., 2000). XLAS males have severer phenotypes and usually progress to end-stage renal disease (ESRD), whereas the affected females, heterozygous for the *COL4A5* mutant gene, have more variable phenotypes, from microscopic hematuria to ESRD (Kashtan, 2000).

To date, 688 COL4A5 mutations have been identified according to the Human Gene Mutation Database (HGMD Professional 2012.1. Release date 30th March 2012) (http://www.hgmd.org/). Certain correlations between genotypes and phenotypes have been established in males (Bekheirnia et al., 2010; Gross et al., 2002; Jais et al., 2000). Patients with large deletions, nonsense or frameshift mutations demonstrated severer symptoms as compared to those with missense or splice site mutations. However, such genotype–phenotype correlations have not been found in females with variable phenotypes, even among family members (Jais et al., 2003).

In this study, we looked into a Chinese family with AS. Clinical examinations revealed that all affected members in the family displayed renal disorders, but no sensorineural hearing loss or variable ocular anomalies were found. Through genetic study, we identified a novel complex deletion/insertion mutation c.359_363delGTATTinsATAC in the *COL4A5* gene in the AS family. The effect of this mutation is the production of a truncated protein of 153 residues. Our data broaden the genotypic spectrum of *COL4A5* mutations associated with mild Alport syndrome.

Abbreviations: *COL4A5*, the α5 chain of type IV collagen; AS, Aport syndrome; XLAS, X-linked Alport syndrome; *COL4A3*, the α3 chain of type IV collagen; *COL4A4*, the α4 chain of type IV collagen; ESRD, end-stage renal disease; HGMD, the Human Gene Mutation Database; NC I, non-collagenous domain I.

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2. Materials and methods

2.1. Study subjects

A Chinese family with multiple individuals affected with Alport syndrome was enrolled in this study. They are from Zhejiang province of the People's Republic of China. Clinical diagnosis was performed for the participants, including a comprehensive review by a consultant ophthalmologist and an ENT surgeon. Ocular examination included visual acuity and examination for anterior lenticonus, cataract, or optic disc or retinal abnormalities. Examinations for sensorineural hearing loss were carried out using standard audiometry tests. Informed consent was obtained from the participants in accordance with the study protocols approved by the Ethics Committee of Huazhong University of Science and Technology.

2.2. Haplotype analysis

Genomic DNA was extracted from peripheral whole blood samples using Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Four polymorphic microsatellite markers close to *COL4A5* (MIM *303630), including DXS8084, DXS6797, DXS1059, and DXS1220 (Applied Biosystems, Inc., Foster City, CA), were selected to investigate if the main X-linked *COL4A5* gene may be the disease-causing gene in this family.

2.3. Mutational analysis

The proband DNA sample was used for initial mutational analysis. All exons and intron–exon junctions of *COL4A5* (Genbank No:NM_000495) were amplified by PCR and mutational analysis was carried out using direct DNA sequence analysis with primers described previously (Tang et al., 2008). PCR products were run on a 1.5% agarose gel, purified using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA), and sequenced with both forward and reverse primers. DNA sequencing was carried out using the BigDye Terminator Cycle Sequencing v3.1 kit (ABI, Foster City, CA). To determine whether the mutation co-segregated with the disease in the family and whether the mutation was absent in 200 normal controls, the exon 6 of *COL4A5* in all family members and the 200 controls were amplified by PCR and sequenced with both forward and reverse primers.

Table 1
The clinical manifestation of patients in the family with mutations in *COL4A5*. *The data are according to the clinical documents of these three patients before they died.

ID	Gender	Age of ESRD	Haematuria	Eyes lesions	Hearing loss
I2*	F	54	+	−	−
II2*	M	30	+	−	−
II4	F	−	+	−	−
II6*	F	42	+	−	−
II8	F	−	+	−	−
III1	F	−	+	−	−
III3	F	−	+	−	−

3. Results

3.1. Pedigree analysis

The pedigree consists of three generations including 18 members (8 males and 10 females) (Fig. 1). The proband (III 1), the cousin (III 3), and two paternal aunts (II 4,II 8) have mild hematuria. The father (II 2), the paternal grandmother(I 2), and one of her paternal aunt (II 6) of the proband reached the ESRD stage at 30 , 54 and 42 year-old respectively, and they all died of ESRD. No ocular or hearing abnormalities have been observed in any member of the family (Table 1 and Fig. 1).

3.1.1. Haplotype analysis

Haplotype analysis revealed the same haplotype in all affected family members. This result suggests that the disease-causing gene in the family may be the *COL4A5* gene (Fig. 1).

3.2. Mutation analysis

To identify the causative mutation in the family, we sequenced the entire coding region and exon–intron boundaries of the *COL4A5* gene. A complex deletion/insertion mutation (c.359_363delGTATTinsATAC) in exon 6 was identified in the proband (Fig. 2). It resulted in a frame-shift in codon 120 and produced a truncated protein with only 153 amino acids including an aberrant 33 residues. Direct DNA sequence analysis of the members of the family showed that the mutation co-segregated with disease, and was not present in unaffected family members or 200 normal control individuals. This suggests that this

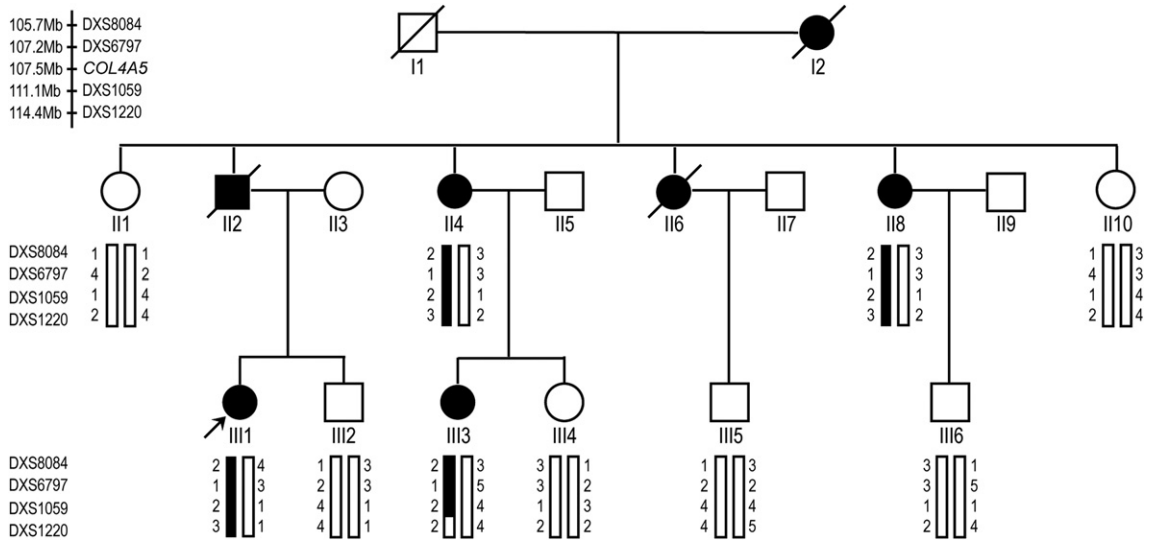

Fig. 1. Pedigree structure of the Chinese Alport syndrome family and results from Haplotype analysis for markers on chromosome Xq23. Blackened and open bars indicate disease and normal haplotypes, respectively. Filled squares or filled circles represent individuals affected with AS. Normal individuals are shown with empty squares (males) and circles (females). Haplotype analysis showed that all patients in the family inherited affected alleles from I2, whereas all unaffected members inherited alleles contain normal *COL4A5*.