RESEARCH NOTE

Mutation analysis of *COL4A3* and *COL4A4* genes in a Chinese autosomal-dominant Alport syndrome family

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Abstract

Autosomal dominant Alport syndrome (ADAS) accounts for 5% of all cases of Alport syndrome (AS), a primary basement membrane disorder arising from mutations in genes encoding the type IV collagen protein family. Mutations in *COL4A3* and *COL4A4* genes were reported to be associated with ADAS. In this study, clinical data in a large consanguineous family with seven affected members were reviewed, and genomic DNA was extracted. For mutation screening, all exons of *COL4A3* and *COL4A4* genes were polymerase chain reaction-amplified and direct sequenced from genomic DNA, and the mutations were analyzed by comparing with members in this family, 100 ethnicity-matched controls and the sequence of *COL4A3* and *COL4A4* genes from GenBank. A novel mutation determining a nucleotide change was found, i.e. c.4195 A>T (p.Met1399Leu) at 44th exon of *COL4A4* gene, and this mutation showed heterozygous in all patients of this family. Also a novel intron mutation (c.4127+11 C>T) was observed at *COL4A4* gene. Thus the novel missense mutation c.4195 A>T (p.Met1399Leu) and the intron mutation (c.4127+11 C>T) at COL4A4 gene might be responsible for ADAS of this family. Our results broadened the spectrum of mutations in *COL4A4* and had important implications in the diagnosis, prognosis, and genetic counselling of ADAS.

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Introduction

Alport syndrome (AS) is a heterogeneous renal hereditary disease characterized by haematuria, proteinuria and progressive renal failure with structural defects of the glomerular basement membrane (GBM) and is often associated with sensorineural hearing loss and ocular lesions (Hertz 2009; Savige *et al.* 2015). A distinctive trait of AS is the predominant changes in the network α3/α4/α5 chains of type IV collagen in GBM. Any of the genes encoding these three chains could be involved in the pathogenesis of AS. Mutations in *COL4A5*, which encodes type IV collagen α5 chain are generally believed to cause X-linked AS (XLAS, OMIM: 301050), whereas mutations in *COL4A3* and *COL4A4* genes which encode type IV collagen α3 and α4 chains, respectively, can be associated with the autosomal-recessive AS (ARAS, OMIM: 203780) and autosomal-dominant AS (ADAS, OMIM: 104200).

While XLAS and ARAS are well known, ADAS is a form of AS that has been described more recently. In 1997, this form of AS was linked to the *COL4A3*/*COL4A4* locus in a large family from northern Ireland and three years later, the first report of a *COL4A3* heterozygous mutation segregating in a family with ADAS was observed (Jefferson *et al.* 1997; van der Loop *et al.* 2000). In 2004, Pescucci *et al.* (2004) investigated two ADAS families and confirmed that ADAS occurred due to mutations either in the *COL4A4* or *COL4A3* gene. Till date, only a few proven *COL4A4* or *COL4A3* mutations have been reported. Both female and male patients showed a high clinical variability, and a clear-cut genotype–phenotype correlation for ADAS is not available. Therefore, it is important to identify new mutations to clarify their clinical importance, to assess the prognosis of the disease, and to avoid renal biopsy for final diagnosis (Longo *et al.* 2002; Marcocci *et al.* 2009; Rosado *et al.* 2015).

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In the present study, a novel exonic variant c.4195 A>T (p.Met1399Leu) at 44th exon of *COL4A4* gene was found, and this mutation showed heterozygous in all the patients of a Chinese ADAS family. Also a novel intronic nucleotide change (c.4090+11 C>T) was observed in *COL4A4* gene. Our results broadened the spectrum of mutations in *COL4A4* and had important implications in the diagnosis, prognosis and genetic counselling of ADAS.

Subjects and methods

Patients

The index patient was a 21-year-old male (III5, figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/) who was admitted to the hospital with a history of facial swelling for the past one month and had cough because of cold from half a month. The urinalysis revealed glomerular haematuria, and the phase contrast microscope showed the proportion of poikilocyte that was more than 60%. The 24-h urine protein excretion was 5.0 g, serum creatinine was 146 μmol/L and blood urea nitrogen was 8.64 mmol/L. B-ultrasonic showed diffuse renal injury. In addition, he was found to have moderate bilateral neurosensorial hearing loss. A renal biopsy revealed typical AS alteration with irregular thickening, thinning and splitting in the GBM (figure 2a in electronic supplementary material). Collagen IV immunofluorescence analysis exhibited negative collagen 3(IV) and 4(IV) immunostaining in Bowman's capsular basement membrane and the tubular basement membrane (figure 2b in electronic supplementary material).

His 45-year-old uncle and 26-year-old sister (II7 and III3, figure 1 in electronic supplementary material) presented glomerular haematuria and proteinuria, and were in the state of renal compensatory. They also had mild sensorineural hearing loss. It was reported that the father (II3) had undergone dialysis since the age of 60 and died of kidney failure at the age of 62. Detailed information of the family are shown in table 1 in electronic supplementary material. There is no history of consanguineous marriage in this family and pedigree analysis of this family showed the inheritance pattern was autosomal-dominant.

The study protocol was approved by the local Ethics Committee and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Mutation analysis

Genomic DNA was extracted using a QIAamp DNA Blood Maxi kit (Qiagen, Hilden, Germany) according to the manufacturers' protocol. To amplify the different exons of the *COL4A3* and *COL4A4* genes, a standard PCR

Table 1. Mutations of *COL4A3*/*COL4A4* genes found in ADAS family.

Gene	Site	gDNA change	rs numbers	Nucleotide change	Amino acid] change	Mutation type	Reference
COL4A3	Exon 21	g.104260 C>T	rs772153778	c.1195 C>T	A399L	Polymorphism	Slajpah *et al.* (2007)
	Exon 21	g.104288 G>A	rs536930700	c.1223 G>A	R408H	Polymorphism	Papazachariou *et al.* (2014)
COL4A4	Exon 33	g.118444 C>T	rs1800517	c.3011 C>T	L1004P	Polymorphism	Boye *et al.* (1998)
	Exon 44	**g.147491 A>T**	**rs149117087**	**c.4195 A>T**	**M1399L**	**Missense mutation**	**Novel**
	Exon 44	g.147503 T>C	rs375289	c.4207 T>C	S1403P	Polymorphism	Boye *et al.* (1998)
	Intron 44	**g.147524 C>T**	–	**c.4090+11 C>T**	–	**Intron mutation**	**Novel**
	Exon 47	g.161281 A>G	rs199517662	c.4548 A>G	V1516V	Polymorphism	Slajpah *et al.* (2007)
	Exon 48	g.162094 C>T	rs2228557	c.4932 C>T	F1644F	Polymorphism	Longo *et al.* (2002)

The novel mutation determining a nucleotide change c.4195 A>T (p.Met1399Leu) at 44th exon of *COL4A4* gene, and a novel intron mutation (c.4127+11 C>T) observed in *COL4A4* gene are in bold.

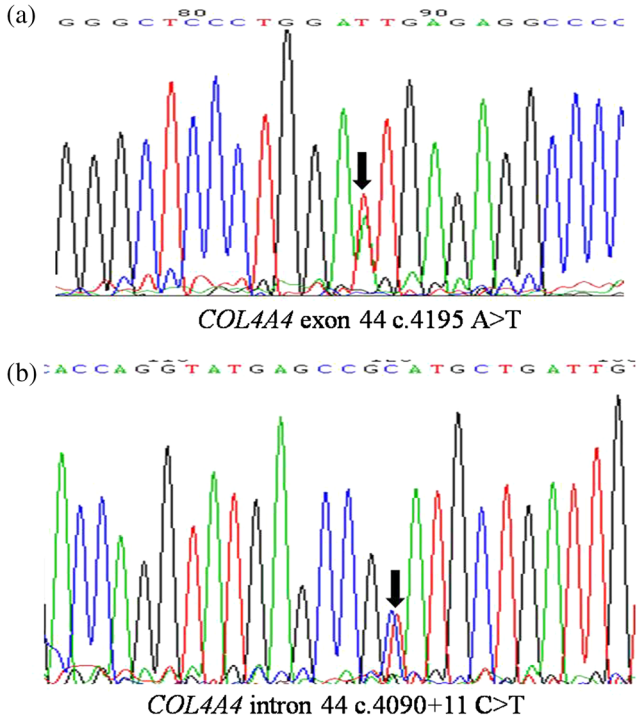

Figure 1. Sequencing results of the c.4195 A>T and c.4127+11 C>T mutation of *COL4A4* gene. (a) c.4195 A>T mutation at 44th exon of *COL4A4* gene. (b) c.4090+11 C>T mutation in the intron 44 of *COL4A4* gene.

programme was used and conditions were adapted to each exon. All PCR products were purified and bidirectionally sequenced using ABI BigDye Terminator ver. 3.0 reaction cycle on ABI 3730XL DNA sequencer (Applied Biosystems, Foster City, USA).

Results

DNA sequencing of *COL4A3* and *COL4A4* genes in the index patient revealed the presence of eight nucleotide changes, including one exonic change c.4195 A>T, Met1399Leu at 44th exon of *COL4A4* gene (table 1; figure 1), which showed heterozygous in all patients (II7, III3, III5, IV2; figure 1 in electronic supplementary material) of this family, but it was absent in the healthy members of the family.

We also found one new intronic change c.4090+11 C>T at 44th intron of *COL4A4* gene (table 1; figure 1), which was only present in the proband. None of these variants were detected among 100 healthy individuals. Further, six polymorphisms were identified, including c.1195 C>T, c.1223 G>A in *COL4A3* gene, and c.3011 C>T, c.4207 T>C, c.4548 A>G, c.4932 C>T in *COL4A4* gene, they were also found in the control group, and all of them were previously reported in other populations (table 1).

Discussion

Till date, ~25–50% mutations of AS have been reported to be glycine substitution (Ciccarese *et al.* 2001; Nabais Sa *et al.* 2015). Glycine is considered as the only amino acid to fit into the centre of the triple helix structure of type IV collagen, whose substitution mutation can interrupt the characteristic triple Gly–X–Y repeats in collagenous domain of the α3(IV) chain (Kharrat *et al.* 2006; Rosado *et al.* 2014). The missense mutation (Met1399Leu) identified in the present study was the first to report pathogenic methionine substitution. All the patients in this family were heterozygous for Met1399Leu 123 mutation, however, the proband's mother who had no obvious clinical manifestation, and other healthy members of the family, so as the 100 healthy individuals were detected to be absent in this mutation. Further, we checked the conservation status of this exonic variant, namely, Clustal X software in 10 species including *Homo sapiens*, *Pan troglodytes*, *Cercocebus atys*, *Macaca nemestrina*, *Bubalus bubalis*, *Ovis aries musimon*, *Canis lupus familiaris*, *Oryctolagus cuniculus*, *Mus musculus* and *Rattus norvegicus*. Results showed that the novel exonic variant c.4195 A>T (p.Met1399Leu) was conservative in primates (figure 3a in electronic supplementary material), suggesting the possible pathogenecity of this exonic variant in human beings. Also, *in silico* analysis was done, namely, SIFT and Polyphen. Results showed that the exonic variant was predicted to affect protein functions (figure 3b in electronic supplementary material). From this, we deduced that there was a high probability that the mutation Met1399Leu was the causative mutation of ADAS in this family, which was different from the common glycine replace mutation.

Another novel change, c.4090+11 C>T, in the intron 44 of *COL4A4* gene was found only in the proband of this ADAS family. Interestingly, the proband's condition was the most severe in the family and progressed to renal failure, we speculated that this mutation might be associated with different severity of clinical manifestations. Because it was not a splice site mutation, the association between the mutation and the severity of the illness was not clear.

Also, some papers reported that ADAS might be associated with other genes except *COL4A3* and *COL4A4* genes (Rosado *et al.* 2015; Savige *et al.* 2015). In this family, II7, III3, III5 and IV2, who presented the same heterozygous mutation, had different severity of clinical manifestations, proteinuria and haematuria, it is possibly due to combined effect with other genes to some extent. Still definite information about association between phenotype and genotype are needed in this family.

In conclusion, we described a novel nucleotide change c.4195 A>T (p.Met1399Leu) in the *COL4A4* gene that might be responsible for a Chinese ADAS family.

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