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A Novel *COL4A3* Mutation Causes Autosomal-Recessive Alport Syndrome in a Large Turkish Family

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Background: Alport syndrome (AS) is a genetically heterogeneous disorder that is characterized by hematuria, progressive renal failure typically resulting in end-stage renal disease, sensorineural hearing loss, and variable ocular abnormalities. Only 15% of cases with AS are autosomal recessive and are caused by mutations in the *COL4A3* or *COL4A4* genes, encoding type IV collagen. *Methods:* Clinical data in a large consanguineous family with four affected members were reviewed, and genomic DNA was extracted. For mapping, 15 microsatellite markers flanking *COL4A3*, *COL4A4*, and *COL4A5* in 16 family members were typed. For mutation screening, all coding exons of *COL4A3* were polymerase chain reaction- amplified and Sanger-sequenced from genomic DNA. *Results:* The disease locus was mapped to chromosome 2q36.3, where *COL4A3* and *COL4A4* reside. Sanger sequencing revealed a novel mis-sense mutation (c.2T>C; p.M1T) in exon 1 of *COL4A3*. The identified nucleotide change was not found in 100 healthy ethnicity-matched controls via Sanger sequencing. *Conclusions:* We present a large consanguineous Turkish family with AS that was found to have a *COL4A3* mutation as the cause of the disease. Although the relationship between the various genotypes and phenotypes in AS has not been fully elucidated, detailed clinical and molecular analyses are helpful for providing data to be used in genetic counseling. 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.

Introduction

Alport syndrome (AS) (Mendelian Inheritance in Man [MIM] 104200, 203780, and 301050) is a genetically heterogeneous disorder that is characterized by hematuria, progressive renal failure typically resulting in end-stage renal disease (ESRD), sensorineural hearing loss, and variable ocular abnormalities (Levy and Feingold, 2000). It affects 1 in 50,000 live births (Rana *et al.*, 2007). AS is caused by mutations in several genes encoding type IV collagen, which is a major structural component of the basement membrane (van der Loop *et al.*, 2000). X-linked inheritance is observed in ~85% of the cases, which is caused by mutations in *COL4A5* (MIM 303630) coding the α5-chain of type IV collagen (Feingold *et al.*, 1985; Barker *et al.*, 1990). About 15% of the cases show autosomal-recessive inheritance and are caused by homozygous or compound heterozygous mutations in *COL4A3* (MIM 120070) or *COL4A4* (MIM 120131) (Lemmink *et al.*, 1994; Mochizuki *et al.*, 1994). Autosomal-dominant inheritance is rare, and is usually caused by heterozygous *COL4A3* or *COL4A4* mutations (Jefferson *et al.*, 1997). The *COL4A3* and *COL4A4* genes code for type IV collagen α3- and α4-chains, respectively. To date, 52 *COL4A3* mutations have been determined as the cause of AS. These include mis-sense, nonsense, deletion, insertion, and splice-site changes, all of which are predicted to result in loss of a functional protein.

A clear-cut genotype–phenotype correlation for AS is not available. Therefore, it is important to identify new mutations and their associated phenotypes to predict the prognosis of the disease (Hoefele *et al.*, 2010). The aim of this study was to identify the causative mutations in a family with autosomal-recessive AS.

Materials and Methods

Subjects

A 29-year-old woman presented to our department with a history of hemodialysis starting at age 15 years and renal transplantation at age 25 years (V-5 in Fig. 1). She had mild sensorineural hearing loss and bilateral cataracts. The family history was remarkable for the presence of other individuals with similar health concerns and was suggestive of autosomal-recessive inheritance (Fig. 1). Three of her relatives also

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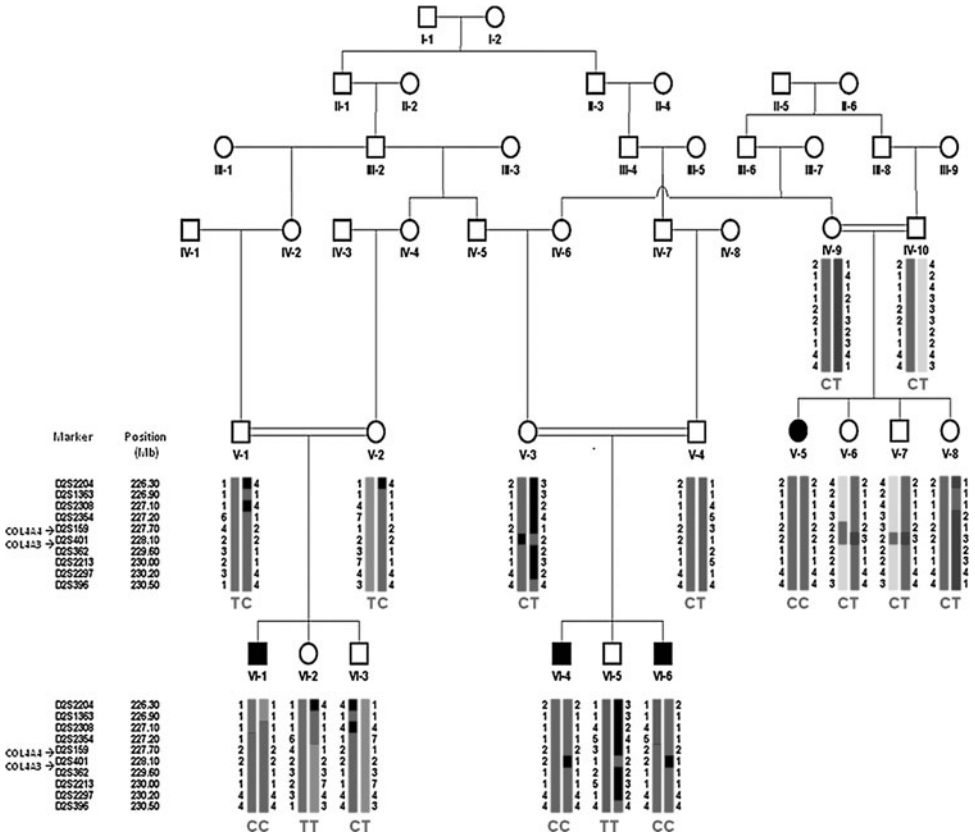

FIG. 1. Pedigree, haplotypes flanking the *COL4A3* and *COL4A4* genes, and *COL4A3* mutation results in the studied family. C and T are mutant and wild-type genotypes, respectively.

developed end-stage renal failure, and two of whom (VI-4 and VI-6) had already undergone renal transplantation. For each affected individual, a detailed clinical examination was obtained, including ophthalmologic and otologic evaluations, pure tone audiometry, and retroperitoneal ultrasonography besides routine blood and urine tests. Audiological investigations showed mild sensorineural hearing loss; eye exams showed various ocular abnormalities such as cataracts and anterior lenticonus (Table 1). Family members of affected individuals also received regular clinical evaluations to detect any symptoms that may be potentially associated with the disease or heterozygosity for the disorder. All unaffected individuals were clinically normal, and none of them had renal findings. This study was approved by the Ankara University Ethics Committee and by the Institutional Review Board at the University of Miami, and signed informed consent was obtained from each participant.

Genotyping and mutation analysis

Genomic DNA from affected (4) and unaffected (12) family members was extracted from peripheral blood by a phenol–chloroform method. Fifteen microsatellite markers (D2S401, D2S362, D2S2213, D2S2297, D2S396, D2S2204, D2S1363, D2S2308, D2S2354, D2S159, DXS8048, DXS8097, DXS6797, DXS1105, and DXS1210) from chromosomes 2 and X flanking the three known genes for AS (*COL4A3*, *COL4A4*, and *COL4A5*) were studied in all available samples from the family. Microsatellite (STR) markers tightly linked to the candidate genes for the disease were selected for linkage analysis from marshfieldclinic.org. The microsatellite markers were amplified by polymerase chain reaction (PCR), and the fragments were analyzed on an automated sequencer (ABI 3730xl).

Table 1. Phenotypic Characteristics of the Patients

	V-5	*VI-1*	*VI-4*	*VI-6*
Gender	Female	Male	Male	Male
Hemodialysis starting at age	15	13	14	14
Age at diagnosis	11	10	9	11
End-stage renal failure	+	+	+	+
Renal transplantation	+	+	+	–
Audiometric results (left/right)	53/57	55/59	50/48	52/55
Ophthalmic findings	Bilateral anterior subcapsular cataract	Bilateral anterior subcapsular cataract	Bilateral anterior lenticonus and bilateral anterior subcapsular cataract	Bilateral anterior subcapsular cataract

The coding 52 exons of *COL4A3* (NM_000091.4) were PCR-amplified using primer sets designed by primer 3.0. The list of primers is available on request. PCRs were run in a 25-mL volume applying a touch-down protocol and annealing temperatures between 65°C and 57°C. PCR products were visualized on agarose gels and cleaned over Sephadex columns, and BigDye reactions were performed following the manufacturer's recommendations (Applied Biosystems, Inc.). A DNA Sequencer (ABI 3730xl) was used to detect mutations. Results were visualized with Sequencher 4.7 software (Gene Codes Corporation).

Results

Results of mapping studies showed a haplotype flanking *COL4A3* that cosegregates in the entire family as an autosomal-recessive trait (Fig. 1).

DNA sequencing showed a novel homozygous mutation c.2T>C resulting in p.M1T in the *COL4A3* gene (Fig. 2). This variant segregated in the entire family as a completely penetrant autosomal-recessive trait. The identified nucleotide change was not found in 100 healthy ethnicity-matched controls via Sanger sequencing.

Discussion

In 1927, Alport described a syndrome that bears his name. Passwell *et al.* (1981) described an autosomal-recessive form of the disease in a 1-year-old girl who presented with failure to thrive, nephritis, and deafness and was born to first-cousin parents. Subsequently, Mochizuki *et al.* (1994) reported four unrelated families with autosomal-recessive AS. In 1997, Colville *et al.* and Rhys *et al.* reported on the ocular manifestations of autosomal-recessive AS. They found that anterior lenticonus, dot-and-fleck retinopathy, and recurrent corneal erosions are seen in patients with autosomal-recessive AS more frequently (or severe) than those observed in age-matched controls. In 1994, Mochizuki *et al.* identified a homozygous deletion of five nucleotides in exon 5 of *COL4A3*, resulting in a truncated protein. Subsequently, Lemmink *et al.* (1994) identified compound heterozygous mutations in *COL4A3* in a patient with autosomal-recessive AS. To date, 71 *COL4A3* variants that include mis-sense, nonsense, deletion, insertion, and splice-site changes have been determined, 52 of which are related with autosomal-recessive or autosomal-dominant AS. The other *COL4A3* mutations have been found to be related with hematuria, focal segmental glomerulosclerosis, microhematuria, and proteinuria, and one mutation has been found to be related with chronic obstructive pulmonary disease (Lemmink *et al.*, 1994; Ding *et al.*, 1995; Knebelmann *et al.*, 1995; Van Der Loop *et al.*, 2000; Heidet *et al.*, 2001; Badenas *et al.*, 2002; Longo *et al.*, 2002; Tazon *et al.*, 2003; Pescucci *et al.*, 2004; Wang *et al.*, 2004; Nagel *et al.*, 2005; Longo *et al.*, 2006; Hou *et al.*, 2007; Slajpah *et al.*, 2007; Voskarides *et al.*, 2007; Hou *et al.*, 2008; Kim *et al.*, 2008, Hoefele *et al.*, 2010; Zhang *et al.*, 2011).

Autosomal-recessive AS can be caused by homozygous or compound heterozygous mutations in *COL4A3* (120070) or *COL4A4* (120131), both of which map to chromosome 2q (Finielz *et al.*, 1998). In a very recent study, including 17 unrelated Chinese patients with autosomal-recessive AS, it was determined that the *COL4A3* and *COL4A4* mutations are present in 82% and 18% patients, respectively (Zhang *et al.*, 2012).

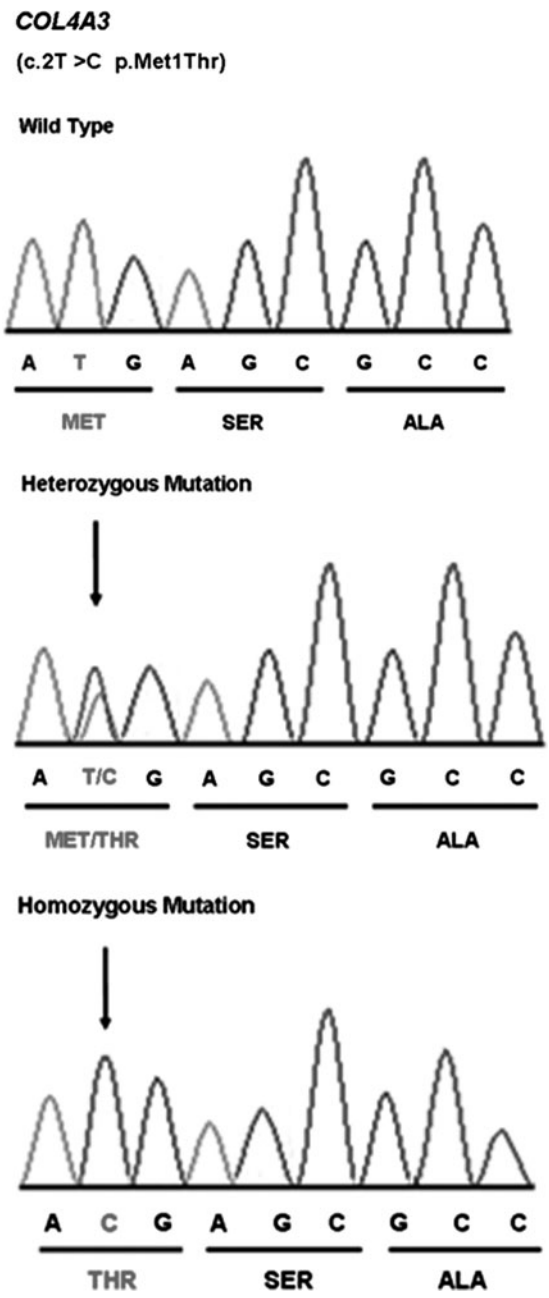

FIG. 2. Electropherograms showing the c.2T>C mutation resulting in p.Met1Thr in *COL4A3*.

The c.2T>C mutation identified in this study affects the initiation codon of the *COL4A3* gene and is likely, therefore, to affect the initiation of translation from *COL4A3* mRNA. Methionine at codon 1 is completely conserved in all species, as expected. When there is a mutation in the initiation codon, it is possible that an alternative AUG within the transcript may be used as an aberrant translation initiation site. There is an ATG triplet (which can function as an initiation start site) in exon 11, at nucleotide positions 625–627, 621 bp downstream from the authentic initiation codon. It is possible that this downstream ATG acts as an initiation codon. Even if that ATG was used as an initiation codon, though the resulting truncated protein would lack 208 amino acids from the N-terminus of

the precursor protein, including the entire signal peptide spanning from amino acid residues 1 to 26. This sequence motif is thought to play a critical role in the targeting of proteins, and it is therefore unlikely that the mutant protein will be functional.

In this study, the ages of patients at first clinical presentation were similar and ranged from 9 to 11 years. On average, each individual required hemodialysis within 3.75 years after their first clinical diagnosis. End-stage renal failure occurred earlier when compared with some of the previous reports. According to the previous studies, patients with autosomal-recessive AS develop ESRD at variable ages. Mochizuki *et al.* (1994) reported a case with deletion in the *COL4A3* gene resulting in ESRD by age 9. Finielz *et al.* (1998) reported four families with an insertion in the *COL4A3* gene; two of the cases resulted in ESRD by ages 26 and 28. In another family in the same study that had the same mutation, end-stage renal failure occurred earlier (ages from 14 to 18 years). In 2001, 60 patients affected with AS were reported, 73% of whom reached ESRD. Age at end-stage renal failure was 21.8 years on average, ranging from 10 to 44 years (Heidet *et al.*, 2001). Based on the limited variability observed in our four patients, we suggest that the identified mutation has a profound impact on the protein, likely to be completely absent, and is sufficient to produce a uniform severe phenotype.

In this report, audiometric results showed pure-tone average hearing loss level of 52.5 and 54.75 for the left and right ears, respectively. All four patients had bilateral anterior subcapsular cataracts. Only one patient (VI-4) had bilateral anterior lenticonus. In 35 patients with autosomal-recessive AS reported in 2001, hearing loss was detected in 27 (77%); however, 8 patients who did not have hearing loss were young (8.3 years on average). Sixteen out of 26 (62%) patients tested for ocular symptoms were found to have positive findings; again, the average age of the 10 others who did not have eye findings was 10.0 years (Heidet *et al.*, 2001). In a series reported in 2012, 7 out of 12 patients had hearing loss, and only 1 out of 10 patients who underwent ocular examination had ocular lesions (Zhang *et al.*, 2012).

We conclude that although the relationship between various genotypes and phenotype in AS has not been fully elucidated, detailed family studies are helpful for genetic counseling, prediction of prognosis, the assessment of the risk for kidney transplantation, and for the follow-up and therapy of AS.

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Author Disclosure Statement

No competing financial interests exist.

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