The Bench to Bedside Transition

**COL4A5-associated X-linked Alport syndrome in a female patient with early inner ear deafness due to a mutation in MYH9**

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Abstract

Alport syndrome (ATS) is a type-IV collagen inherited disorder, caused by mutations in *COL4A3* and *COL4A4* (autosomal recessive) or *COL4A5* (X-linked). Clinical symptoms include progressive renal disease, eye abnormalities and high-tone sensorineural deafness. A renal histology very similar to ATS is observed in a subset of patients affected by mutations in *MYH9*, encoding non-muscle-myosin Type IIa – a cytoskeletal contractile protein. *MYH9*-associated disorders (May–Hegglin anomaly, Epstein and Fechtner syndrome, and others) are inherited in an autosomal dominant manner and characterized by defects in different organs (including eyes, ears, kidneys and thrombocytes). We describe here a 6-year-old girl with haematuria, proteinuria, and early sensorineural hearing loss. The father of the patient is affected by ATS, the mother by isolated inner ear deafness. Genetic testing revealed a pathogenic mutation in *COL4A5* (c.2605G>A) in the girl and her father and a heterozygous mutation in *MYH9* (c.4952T>G) in the girl and her mother. The paternal *COL4A5* mutation seems to account for the complete phenotype of ATS in the father and the maternal mutation in *MYH9* for the inner ear deafness in the mother. It has been discussed that the interaction of both mutations could be responsible for both the unexpected severity of ATS symptoms and the very early onset of inner ear deafness in the girl.

**Keywords**: *COL4A5*; early sensorineural hearing loss; *MYH9*

Introduction

Alport syndrome (ATS) is a progressive hereditary nephropathy characterized by ultrastructural anomalies of the glomerular basement membrane (GBM). The nephropathy is associated with haematuria, proteinuria and progressive renal insufficiency frequently leading to end-stage renal disease (ESRD). Extrarenal manifestations include high-tone sensorineural hearing loss and ocular abnormalities (anterior lenticonus, retinal abnormalities and corneal lesion) [1, 2]. ATS is a genetically heterogeneous disorder caused by mutations in type IV collagen, the major component of basement membranes in the kidneys, ears and eyes [3]. It comprises six homologous α-chains (α1–α6) encoded by the *COL4A1–COL4A6* genes. Mutations in any of the *COL4A3*, *COL4A4* and *COL4A5* genes may cause ATS [3]. Mainly, there are the following patterns of inheritance: X-linked inheritance (85%), due to mutations in *COL4A5* (OMIM #303630), as well as autosomal recessive (14%) and autosomal dominant (1%) forms, linked to mutations in *COL4A3* (OMIM #120070) or *COL4A4* (OMIM #120131) on chromosome 2q36–37 [1, 2]. There is a significant genotype–phenotype correlation in affected males. In females, these correlations are not observed. Clinical symptoms and kidney biopsies from female heterozygous carriers of *COL4A5* mutations have a widely variable appearance. The pathophysiological mechanisms are uncertain, but likely multifactorial. Even within the same family, the phenotypic variability between heterozygous females is high [4–6].

*MYH9* is localized on chromosome 22q11–13 [7, 8] and expressed in the kidney [9], cochlea [7] and platelets [10]. Furthermore, it is up-regulated during granulocytic differentiation [9]. *MYH9* encodes non-muscle-myosin IIA (MYHIIA) – a cytoskeletal contractile protein - which is part of a hexameric enzyme complex that plays a role in several important cellular functions, including cytokinesis, cell motility and maintenance of cell shape [9]. There are several rare disorders caused by mutations in *MYH9* that share overlapping features. Epstein syndrome (OMIM #153650) is an autosomal dominant disorder characterized by thrombocytopenia, giant platelets, nephritis and deafness [11]. May–Hegglin anomaly (OMIM #155100) is characterized by thrombocytopenia, giant platelets and Dohle body-like inclusions in peripheral blood leucocytes. Fechtner syndrome (OMIM #153640) has the platelet defect accompanied by nephritis, hearing...
loss and eye abnormalities. The findings of nephritis, hearing loss and occasional cataracts in Fechtner and Epstein syndromes are reminiscent of ATS. Sebastian syndrome (OMIM #605249) is similar to May–Hegglin anomaly, but has a different ultrastructural appearance of the leucocyte inclusions. Seri et al. [7] suggested that these four disorders are not distinct entities, but rather represent a single disorder with a continuous clinical spectrum, for which they proposed the term ‘MYH9-related disease’. Non-syndromic deafness without any irregularities in blood cells (DFNA17; OMIM #603622) is also caused by a mutation in MYH9 and inherited in an autosomal-dominant manner [10]. MYH9-related disorders are very rare diseases. In all reported individuals – with the exception of patients affected by non-syndromic deafness – macrothrombocytopenia and leucocyte inclusions are present at birth [12]. The onset of sensorineural hearing loss is distributed from the first to the sixth decade, 33% manifest before the age of 20 years, 31% between the ages of 20–40 years and 36% after the age of 40 years [12]. Nephropathy and cataracts can develop at any time between infancy and adulthood [12].

We report here a family with a female patient affected by haematuria, proteinuria and early deafness. Genetic testing in the index girl revealed both a mutation in COL4A5 and MYH9 as underlying cause of the clinical phenotype.

**Subject and methods**

**Index patient**

Figure 1a shows the pedigree of the family. Our index patient (IV-1), a 6 year old girl, was admitted to our hospital because of haematuria and proteinuria. Both had developed at the age of 2 years. Pregnancy and birth were uneventful. Post-partum, sensorineural high-tone hearing loss was detected. At the age of 6 years, physical and eye examinations were normal. Blood pressure was within the normal range (90/40 mmHg). However, urine analysis revealed a proteinuria of 670 mg/m² body surface area/24 h and a microhaematuria. At the same time, serum protein and albumin levels were normal (6.2 g/dL resp. 4.8 g/dL). The estimated creatinine clearance was 127 mL/min/1.73 m² (calculated by Cockroft and Gault). Renal ultrasound revealed a proteinuria of 670 mg/m² body surface area/24 h and a microhaematuria. At the same time, serum protein and albumin levels were normal (6.2 g/dL resp. 4.8 g/dL). The estimated creatinine clearance was 108 mL/min/1.73 m² (calculated by Cockroft and Gault). Renal ultrasound was normal. In the renal biopsy, characteristic histopathological lesions suggestive of ATS were identified. Electron microscopy revealed diffuse thinning and thickening of the glomerular capillary wall, and splitting of the lamina densa into multiple interlacing strands of electron-dense materials (Figure 2a). There were no detectable abnormalities of blood cell components, especially no giant platelets and no leucocyte inclusions. The platelet count was 289 000/μL.

**Father of index patient**

The father of our index patient (III-3) had suffered from haematuria since birth and developed ESRD at the age of 12 years. Haemodialysis was then initiated. At the age of 6 years, sensorineural high-tone hearing loss was detected and characteristic lesions of the cornea were identified by thorough ophthalmological examination. A renal biopsy, performed at the age of 9 years, revealed irregular structures of the glomerular capillary wall and a split lamina densa indicative of ATS (Figure 2b).

The sister of the father (III-2) was also affected by high-tone sensorineural hearing loss, haematuria and proteinuria. An uncle of the index patient’s father (II-1) had suffered from ESRD and deafness. Unfortunately, he died at the age of 56 years in the course of the disease.

**Mother of index patient**

The mother of the index patient (III-4) has suffered from high-tone sensorineural hearing loss since the age of 4 years. Urine analysis, blood pressure and renal ultrasound were normal. The estimated creatinine clearance was 127 mL/min/1.73 m² (calculated by Cockroft and Gault). No abnormalities of the eyes were identified. The blood cell components were normal, especially no macrothrombocytopenia and no leucocyte inclusions. The maternal grandparents of our index patient (II-4, II-5) were both affected by high-tone sensorineural hearing loss since childhood.

**Mutational analysis**

Blood samples were obtained from our index patient and her parents after informed consent. Genomic DNA was extracted from peripheral blood with the Qiagen® DNA Blood Kit according to the manufacturers’ protocol (Qiagen, Hilden, Germany). All coding exons and exon–intron boundaries of COL4A5 and MYH9 were amplified. For direct sequencing, the BigDye® dye terminator method was used (Applied Biosystems, Foster City, CA, USA). Capillary electrophoresis was performed using the ABI Prism 3130® technology (Applied Biosystems) and sequences were analysed with SeqView software.

**Results**

To confirm the suspected diagnosis of X-linked ATS, COL4A5 was examined by direct DNA sequencing in the index patient. In exon 31 of COL4A5, a heterozygous mutation (c.2605G>A, p.Gly869Arg) was identified (Figure 1b). Genetic testing revealed the identical mutation of COL4A5 in the hemizygous state in the father. In the index patient and her mother, a heterozygous mutation (c.4952T>C, p.Met1651Thr) in MYH9 was detected in exon 35 (Figure 1c).

**Discussion**

Basement membranes consist of a collagenuous triple helix network involving three collagen IV chains (α3(4)α5). Mutations in COL4A5 have been identified to cause X-linked ATS. Most cases have been reported with missense mutations in COL4A5 located primarily in the collagenuous
portion of the α5-collagen chains caused by unique glycine changes which are highly important for the steric arrangement of the collagen chain [13]. The genotype of mutations in COL4A5 seems to be a major determinant of the phenotype in affected males. Nonsense mutations and large deletions are more likely to lead to early ESRD than missense or splice site mutations. In females, these genotype–phenotype correlations are not observed [4, 5]. ATS is an important genetic cause of ESRD, accounting for 0.6% of ESRD in adult patients, 2.3% of paediatric kidney transplants and 1.9% of the paediatric dialysis population [14]. The most extensive natural history study of X-linked

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Fig. 1. (a) Pedigree of the family. (b) Extract of sequential analysis COL4A5 with the heterozygous mutation p.Gly869Arg (index patient). (c) Extract of sequential analysis MYH9 with the heterozygous mutation p.Met1651Thr (index patient).

Legend:
- carrier without any symptoms
- ? unknown
- ☐ deceased
- • affected by symptoms of ATS
- ○ affected by high-tone sensorineural hearing loss
AS was performed by the ‘European Community Alport Syndrome Concerted Action Study’ [5, 6]. Nearly 90% of males with X-linked ATS reach ESRD by the age of 40 years, while only 12% of heterozygous females experience kidney failure. After the age of 60 years, 30–40% of heterozygous females developed ESRD. Microscopic haematuria was detected in 100% of males and in 95% of heterozygous females. Proteinuria was present in 75% of heterozygous females [5, 6]. Sensorineural hearing loss typically becomes apparent by audiometry in late childhood or early adolescence [1]. By the age of 40 years, approximately 90% of affected males, but only 10% of heterozygous females, suffer from hearing loss [5, 6].

We report here a glycine-to-arginine mutation in the collagenous domain of the COL4A5 gene in our index patient and her father. This mutation has formerly been published as disease causing [15]. The paternal COL4A5 mutation accounts for the complete phenotype of ATS in the father with sensorineural inner deafness, ESRD and corneal lesions. It may also be responsible for the ATS symptoms in our female patient although the unexpected severity of the symptoms with very early deafness and the characteristic appearance of ATS in renal histology remained initially unexplained.

The finding of a pathogenic MYH9 mutation in the index patient and her mother explains the non-syndromic inner ear deafness in the mother. Provaznikova et al. [16] described the identical MYH9 mutation in two patients with bilateral sensorineural hearing loss, bleeding from minor cuts and one of them with suspected tubular reabsorption impairment.

It can be speculated that the coexistence of both mutations in COL4A5 and MYH9 in our index patient is responsible for the very early onset of inner ear deafness.

In part, interindividual variability in female heterozygous carriers of COL4A5 mutations might also be explained by mechanisms of X-inactivation. Several recent reviews focus on X-linked ATS and the mechanism of X-inactivation in females [4, 17, 18]. X-chromosome inactivation results in transcriptional silencing of one X-chromosome in females to attain gene dosage parity between XX female and XY male mammals [19]. Either the maternal or paternal X chromosome is randomly silenced through a complex cellular process, resulting in a female being a mosaic of cells with either an active maternal or paternal X chromosome [17]. As in ATS, X-linked inactivation has also been observed in females affected by X-dominant Rett syndrome. Here, carriers of MECP2 mutations may vary in neurological and haematological symptoms depending on the degree of X-inactivation [20]. In the present case of ATS, it should be discussed whether a possible skew in X inactivation in favour of the mutant COL4A5 could possibly contribute to the unexpected severity of ATS-specific histological abnormalities in the kidney biopsy specimen obtained from the index patient.

In summary, the interaction of both mutations (COL4A5 and MYH9) is likely to be responsible for the severity of ATS symptoms in the female patient and the very early onset of inner ear deafness.

With respect to the complexity of human genetics, we would like to point out that the restriction of the identification of a single genetic defect can be misleading. The presence of additional gene mutations should be considered, especially if the clinical course is exceptional.

Conflict of interest statement. None declared.

References


Fig. 2. (a) Electron microscopy (02/2010): glomerular basement membrane of the index patient. (b) Electron microscopy (11/1979): glomerular basement membrane of the father.

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