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Possible Digenic Disease in a Caucasian Family with *COL4A3* and *COL4A5* Mutations

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Keywords

COL4A3 · *COL4A5* · Alport syndrome · Digenic inheritance · Familial hematuria · Genetic diseases · Thin basement membrane

Abstract

Microscopic hematuria is a common feature of patients with Alport syndrome, a familial nephropathy due to mutations in *COL4A3, COL4A4* or *COL4A5*. These genes encode for α3, α4, and α5 type IV collagen polypeptide chains (collagen IV α345), crucial for the structural component of the glomerular basement membrane. Even patients with mild phenotype, namely isolated microhematuria (X-linked females with thin basement membrane on electron microscopy or heterozygous carriers of *COL4A3* or *COL4A4* mutations), can potentially progress to proteinuria and to end-stage renal disease. Recent pedigree analyses provided evidence for digenic inheritance of Alport syndrome by concomitant mutations in *COL4A3*/*COL4A4* or *COL4A4*/*COL4A5*. We describe a Caucasian family with concomitant *COL4A3* and *COL4A5* mutations, consisting of a novel c.4484A>G *COL4A3* (p.Gln1495Arg) mutation and a previously reported c.1871G>A *COL4A5* (p.Gly624Asp) mutation. Our segregation analysis raises the possibility that Alport syndrome resembles also digenic inheritance by *COL4A3*/*COL4A5*.

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Introduction

Microscopic hematuria comprises a clinical entity with sporadic or inherited renal diseases due to heterogeneous causes. Amongst familial hematuria defects of the glomerular basement membrane (GBM) due to collagen IV alpha chains abnormalities are the most common. These defects of the GBM are characterized by a wide and continuous clinical and histopathological spectrum, from hematuria to end-stage renal disease, from thin basement membrane (TBM) to severe thickening, splitting, and complete remodeling of the GBM [1–3]. Moreover, hav-

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ing only a thin basement does not rule out a progressive disease [4], supporting the idea that a TBM is not always benign. In order to simplify diagnostic terminology and improve diagnosis and treatment, Kashtan et al. [5] proposed a new classification system of genetic collagen IV α345 molecule disorders, which implicates that the former term TBM nephropathy is not a nosological entity but a histopathological finding. Patients with TBM should be redefined as forms of autosomal Alport syndrome (e.g., heterozygous mutations in *COL4A3* or *COL4A4*) to avoid lack of close follow-up and to optimize appropriate treatment. Inheritance is predominantly X-linked (approximately 80%) and caused by mutations in the *COL4A5* gene, less cases are inherited in an autosomal recessive or autosomal-dominant manner [6–8].

Here, we describe the findings of a novel p.Gln1495Arg mutation in *COL4A3* (c.4484A>G) and a previously reported p.Gly624Asp mutation in *COL4A5* (c.1871G>A) in a Caucasian pedigree, causing a thin GBM with not so benign familial hematuria and comprising a complicated Alport syndrome and challenge for genetic counselling. Our analysis extends our understanding of Alport syndrome as a digenic disease [9, 10] caused by concomitant mutations of *COL4A* genes encoding type IV collagen polypeptide chains crucial for structural alterations of GBM.

Methods

Patients

All patients were entered into genetic testing after having given written informed consent.

DNA Isolation

Mutation analysis was carried out on genomic DNA isolated from 200 μL of EDTA-Blood using EZ1 DNA Blood 200 μL Kit, QIAGEN EZ1 DNA Blood card, as suggested by Qiagen's supplementary protocol with EZ1 Advanced XL instrument.

Sanger Sequencing

The coding region and intron-exons boundaries of the *COL4A3* (Ensembl Transcript ID ENST00000396578) and *COL4A5* (Ensembl Transcript ID ENST00000361603, ENST00000328300, ENST00000505728) were amplified by polymerase chain reaction (PCR) using flanking intronic primers. Primer details are available upon request. The promotor region of *COL4A3* was also amplified. PCR amplification was performed on GeneAmp PCR System 9700 (Applied Biosystems™). After PCR, the products were enzymatically purified. Direct sequencing of the purified PCR products was performed on capillary sequencing devices ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) using BigDye™ Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems™) according to manufacturer's instructions. *NPHS2* (Podocin) sequencing was performed as previously described [11].

Analysis

For sequence data quality control was used Sequencing Analysis Software version 6.0 (Applied Biosystems™). Mutation analysis was performed by SeqPilot Software with module SeqPatient (JSI Medical Systems Software, version 4.4.0). Sequences were mapped to genome hg19, GRCh37). Detected changes of nucleotides and amino acids were recorded. The putative pathogenicity of the detected variants was evaluated by Polyphen-2 and MutationT@ster.

Case Presentation

The index patient (Fig. 1, IId, marked with an arrow) was a 55-year-old German man, who regularly came for follow-up visits to our renal transplant care center. When he was 10-year-old, microscopic hematuria and proteinuria were detected. A renal biopsy was performed, but was without any histopathological findings. Electron microscopy was not performed at that time. He reached end-stage renal disease at the age of 47 years and received a living organ donation from his wife. Three years after renal transplantation, he developed microscopic hematuria and proteinuria up to 4.8 g/g creatinine while his renal function remained stable (creatinine of 1.1 mg/dL). A biopsy from his renal transplant showed no pathologic findings when using light microscopy, whereas electron microscopy showed global foot process effacement leading to the diagnosis of minimal-change glomerulonephritis. His family history revealed that several family members had hematuria in a dominant trait (depicted in Fig. 1). His parents (Ia, Ib) had died and also one of his brothers (IIe) had died of lung cancer when he was 33 years old. The index patient's eldest sister had hematuria with proteinuria up to 1.5 g/g creatinine due to biopsy proven diabetic nephropathy. She suffered from diabetes mellitus type 1 similarly to one of her daughters. The second oldest sister (IIb) had hematuria with a biopsy showing a thin GBM. The index patient's daughters were both affected with microhematuria. In addition, the younger one had proteinuria up to 1.7 g/g creatinine, which developed during her pregnancy. She also underwent renal biopsy showing focal segmental glomerulosclerosis (FSGS), which was resistant to treatment with steroids and ciclosporin A. Remarkably, except the index patient, all family members had normal renal function and no hearing or ocular abnormalities. The findings prompted us to perform a genetic screening of all family members willed to participate. Mutational analysis revealed a hemizygote p.G624D, c.1871 G>A mutation in *COL4A5* (highlighted with dark gray marks in Fig. 1), a milder missense mutation previously reported to be as-

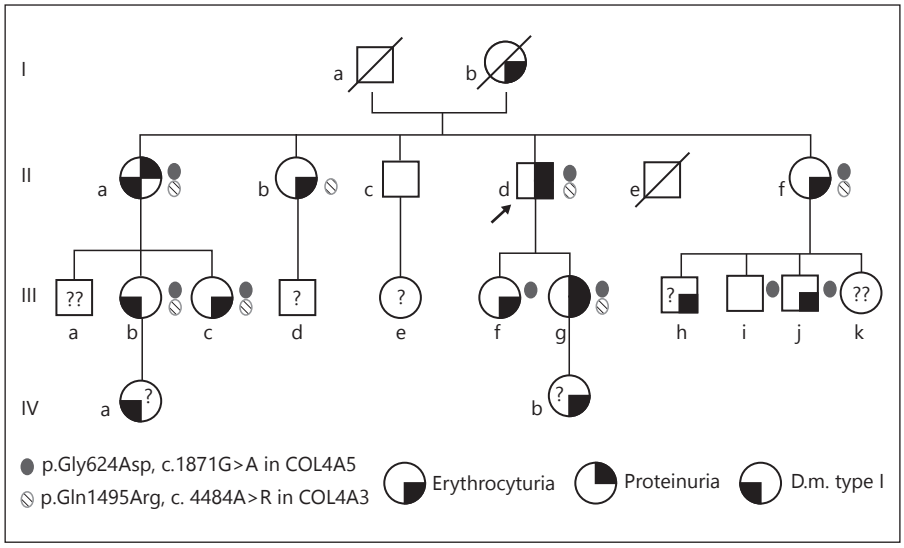

Fig. 1. Family members with identified hetero-/hemizygous *COL4A3* and *COL4A5* mutations and their clinical renal phenotype. Mutations are indicated with gray or striped marks on the right side. ? Individuals not genetically tested, ?? Phenotype unknown.

sociated with benign X-linked Alport syndrome [12–14], in all tested family members except sibling IIb with a biopsy proven thin GBM. Interestingly, in the latter one, we detected a heterozygous mutation p.Gln1495Arg c.4484 A>G mutation in *COL4A3*. This mutation was also found in several, but not all other family members (highlighted with striped marks in Fig. 1). In contrast, we did not detect any mutation in *COL4A4* gene. MutationT@ster and Polyphen-2 predicted that *COL4A3* mutation p.Gln1495Arg c. 4484 A>G is disease causing and possibly damaging (score 0.659), respectively. The 37-year-old individual IIIb showed microhematuria, whereas the 30-year-old individual IIIc showed proteinuria (Fig. 1). No mutations were found in *NPHS2* (Podocin) in individuals IIa, IIb, IId and IIIg. Mutations are depicted in Figure 2 comparing the unaffected sibling IIc without any pathological findings with sibling IIb with a thin GBM, with the index patient with minimal-change glomerulonephritis and with his daughter IIIg with FSGS.

Discussion

This case report describes a family with concomitant mutations in both *COL4A3* and *COL4A5* encoding α3 and α5 type IV collagen polypeptide chains crucial for the structural component of the GBM that comprise Alport syndrome. Our findings provide substantial evidence for possible digenic inheritance of Alport syndrome by concomitant mutations also in this combination of Alport genes, as to our knowledge only one single patient has been reported so far with concomitant *COL4A3*/*COL4A5* mutations, that is, a de novo *COL4A5* mutation combined with an inherited *COL4A3* mutation, which were associated with a notably severe phenotype [15].

Individual COL4A3 and COL4A5 DNA Mutations in Our Pedigree

Here, we report a novel *COL4A3* mutation p.Gln1495Arg c. 4484 A>G associated with the histopathological finding of a thin GBM in a family with inherited Alport syndrome. It is very likely that the *COL4A3* p.Gln1495Arg mutation is pathogenic since the *COL4A5* mutation p.Gly624Asp c. 1871 G>A mutation was not detected in the index patient's sibling with biopsy proven thin GBM. According to the new classification by Kashtan et al. [5] that patient finding depicts an autosomal dominant form of Alport syndrome. Interestingly, *COL4A3* p.Gln1495Arg has been previously suspected only as a polymorphism or a rare variant of unknown significance [7, 16]. In the database of the Exome Aggregation Consortium, the *COL4A3* p.Gln1495Arg DNA variant is listed in 6 cases in homozygous form, although the database does not contain information on the renal phenotype of the 6 cases and does not exclude that this variant may represent a hypomorphic mutation. Polymorphisms are indeed often detected in *COL4A3* and *COL4A4* which might cause non-progressive isolated microscopic hematuria that does not result in renal failure (previously called "benign familial hematuria") [17]. However, based on the minor allele frequency (MAF<0.01, e!GRCh37 Ensembl) and the clinical findings of our family, we suggest that the novel *COL4A3* p.Gln1495Arg c. 4484 A>G variant is a pathogenic mutation.

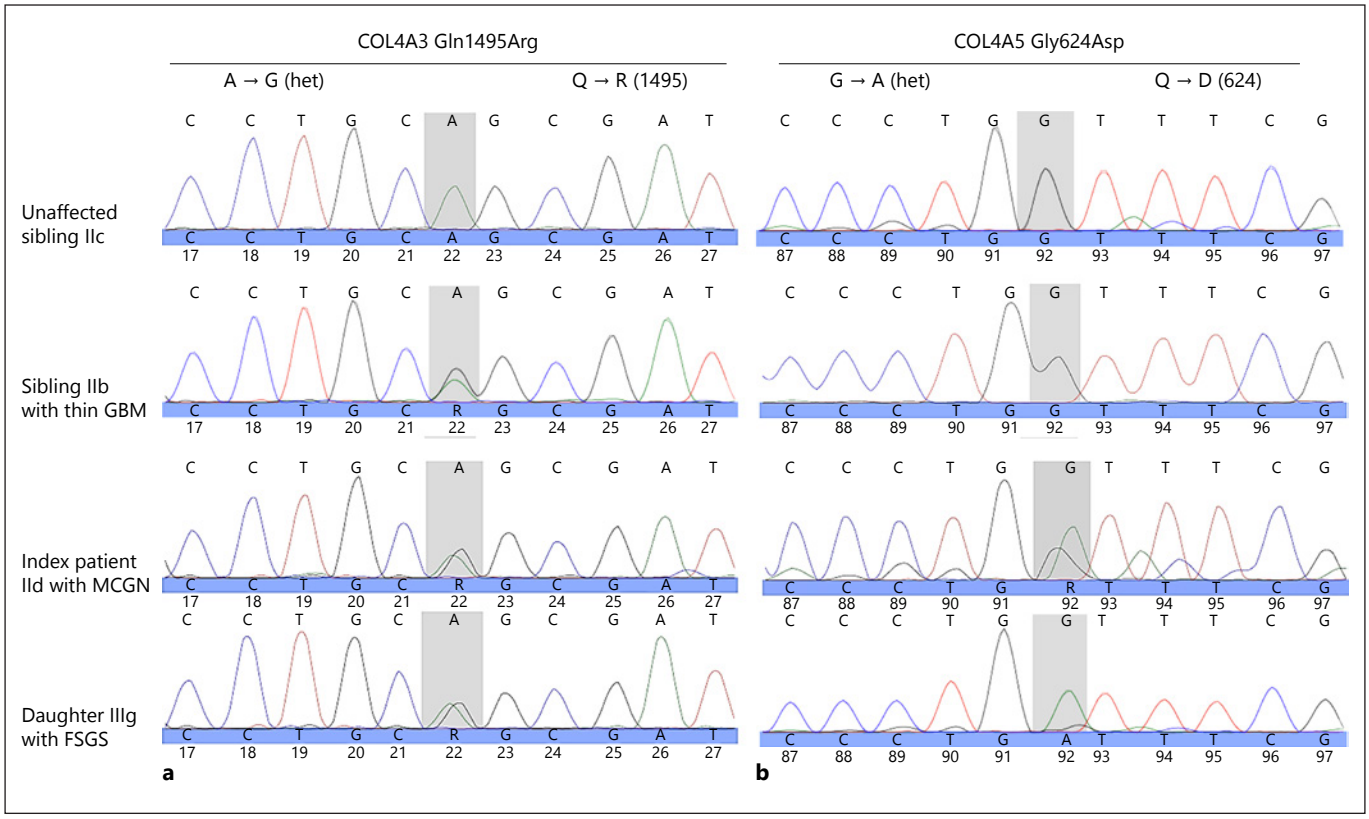

Fig. 2. Electropherograms showing the p.Gln1495Arg c.4484A>G mutation in *COL4A3* found in sibling IIb, index patient IId, and his daughter IIIg (**a**) and p.Gly624Asp c.1871G>A mutation in *COL4A5* detected in index patient IId and his daughter IIIg (**b**).

Our data are also in line with the idea that the *COL4A5* p.Gly624Asp mutation is a rather benign mutation causing X-linked Alport syndrome [12–14]. Interestingly, not all family members with the *COL4A5* p.Gly624Asp mutation had microscopic hematuria, but all family members with proteinuria above 1 g/g creatinine were detected to have both *COL4A3*/*COL4A5* mutations. Since the MAF of *COL4A5* p.Gly624Asp c.4484 A>G is suspicious high (>2% in the British population), we believe that this DNA variant is causing a very mild or even non-pathogenic phenotype. Of note, Pierides et al. [18] described this DNA variant as a hypomorphic Alport mutation, which can exhibit TBM with microhematuria and late-onset kidney failure in the Hellenic population. The prevalence of this variant in the German population is unknown. Therefore, it remains unclear which mutation caused proteinuria or whether both mutations together potentiate each other in phenotype, that is, cause a more severe phenotype in our family. Nevertheless, the relatively mild phenotype of individuals IIIb and IIIc could depend on their relatively young age and/or female gender, the latter with advantageous lyonization in order to express *COL4A5*.

Possibility of Digenic Inheritance

Using massively parallel sequencing, Mencarelli et al. [9] identified 11 patients with Alport syndrome in 6 European countries (except Germany) who had pathogenic mutations in 2 of the 3 collagen IV genes. Seven patients had a combination of mutations in *COL4A3* and *COL4A4*, whereas 4 patients had 1 or 2 mutations in *COL4A4* associated with mutation in *COL4A5* [9]. The authors found that individuals with the digenic disease exhibited an intermediate phenotype between the autosomal-dominant and the autosomal-recessive forms with a severity of renal involvement, estimated according to the mean age at onset of end-stage renal disease, that is, intermediate between those of the classic forms [9]. In the reported pedigrees, the "two-locus model" explained the variable expressivity of the disease within the same family better than simple Mendelian inheritance: the different genotypes at 2 loci, rough-

ly equal in importance, can explain the differences in age at onset of renal failure and in the severity of the symptoms. Similar findings were reported in a Chinese family with *COL4A3* and *COL4A4* digenic mutations mimicking an autosomal dominant inheritance [10]. Together, the results indicate that Alport syndrome is under digenic control by *COL4A3*/*COL4A4* and *COL4A4*/*COL4A5*.

There is only one report on a patient with Alport syndrome with an inherited *COL4A3* mutation combined with a de novo *COL4A5* mutation [15]. Our study reports the findings of at least 6 more cases on a pedigree with 2 concomitant *COL4A3*/*COL4A5* mutations. Since the patient in [15] and our index patient exhibited a more severe phenotype, the findings raise the possibility of digenic inheritance of Alport syndrome by mutations also in *COL4A3* and *COL4A5*. Since genetic testing is only performed in families with suspected GBM disease due to collagen IV alpha chains abnormalities, the prevalence of these mutations in subjects without a clinical phenotype is unknown. Nevertheless, on a conceptual level, our findings support the view that Alport syndrome is a genetic collagen IV α345 molecule disorder under digenic control by all 3 *COL4A3/4/5* gene combinations [5], that is, by *COL4A3*/*COL4A4* [9], *COL4A4*/*COL4A5* [9], and *COL4A3*/*COL4A5* (this study).

Alport Syndrome and Podocin Mutations

The association of FSGS with TBM has been reported, and it was speculated that either FSGS is secondary to TBM or that a coinherited glomerulopathy causes progressive renal disease [19–21]. Hypomorphic podocin variants may act as adverse genetic modifiers when coinherited with *COL4A3/A4* mutations, thus predisposing to FSGS and severe kidney failure [22, 23]. Our sequencing data argue against a possible role of *NPHS2* (Podocin) mutations in our family. Recently, Gast et al. [24] demonstrated that *COL4A3/COL4A4* and *COL4A5* mutations might cause FSGS in adulthood. In contrast, the hypothesis of a coexisting glomerulopathy was confirmed in a retrospective analysis of 658 renal biopsies, where TBM was usually associated with a benign phenotype and with a worse renal outcome only in the presence of another glomerulopathy [25].

In conclusion, our findings expand knowledge regarding causative *COL4A3* and *COL4A5* mutations of GBM disease due to collagen IV alpha chains abnormalities. The identification of novel mutations in patients with Alport syndrome, including histopathological findings of a thin GBM, is crucial to elucidate its pathogenic role and relevance in patients with familial hematuria.

Acknowledgments

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Ethics Statement

Patients provided written informed consent. This study did not require review/approval by the appropriate Ethics Committee. The studies were conducted in accordance with the principles contained within the Declaration of Helsinki.

Disclosure Statement

The authors declare that they have no relevant financial interests.

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