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Benign Familial Hematuria due to Mutation of the Type IV Collagen α4 Gene

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Abstract

Benign familial hematuria (BFH) is characterized by autosomal dominant inheritance, thinning of the glomerular basement membrane (GBM) and normal renal function. It is frequent in patients with persistent microscopic hematuria, but cannot be clinically differentiated from the initial stages of Alport syndrome, a severe GBM disorder which progresses to renal failure. We present here linkage of benign familial hematuria with the COL4A3 and COL4A4 genes at 2q35-37 (Zmax = 3.58 at Θ = 0.0). Subsequently, a glycine to glutamic acid substitution was identified in the collagenous region of the COL4A4 gene. We conclude that type IV collagen defects cause both benign hematuria and Alport syndrome. Furthermore, our data suggest that BFH patients can be carriers of autosomal recessive Alport syndrome. (J. Clin. Invest. 1996; 98:1114–1118.) Key words: hematuria • Alport syndrome • basement membrane • collagen • mutation analysis

Introduction

Hematuria is the presenting symptom in a large number of renal disorders. If the hematuria occurs in childhood, is isolated and is of glomerular origin, the diagnosis is difficult to reach. Benign hematuria, Alport syndrome and IgA-nephropathy all present with persistent hematuria. Particularly important is that this 75-yr-old man had a normal serum creatinine concentration. In the family of the mother (II:8) he had microscopic hematuria in both parents and his younger brother (III:11), but no other symptoms suggestive for Alport syndrome. Renal function was normal in the parents (II:7 and II:8; normal serum creatinine concentration). Microscopic hematuria was present in three sisters of the father (II:7) and in half of their offspring, as well as in the grandfather (I:1; Fig. 1). Particularly important is that this 75-yr-old man had a normal serum creatinine concentration. In the family of the mother (II:8) hematuria was also widely present. Her 76-yr-old father had hematuria and a normal creatinine level, her mother was healthy. It was con-

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1. Abbreviations used in this paper: BFH, benign familial hematuria; GBM, glomerular basement membrane.

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cluded that the family history on both sides strongly supported the diagnosis BFH. The index patient might have inherited the disorder form both parents, which could explain the aberrant morphology of the biopsy specimen. He is presently 16 years old and symptoms are hematuria and recently developed proteinuria (0.5 grams/liter). Hematuria was defined as a condition with 10 or more erythrocytes per mm³ urine, established by the quantitative sediment method of Gadeholt (12). Individuals with borderline values were repeatedly analyzed. All family members could unambiguously be scored as either affected or unaffected.

**Linkage analysis.** Blood samples were collected from family members and genomic DNA was isolated by a salting out procedure (13). The *COL4A3/COL4A4* CA repeat was PCR amplified using 125 ng of primers CA11F (5'-ATC TCT CAG GGT GCC TGC-3') and CA11R (5’-CTC ATT GAT ACA CAC AAA TGC A-3') and 50-100 ng DNA template in a standard Cetus buffer with 2 mM MgCl₂. After 3 min at 94°C, four initial cycles were performed of 1 min at 94°C, 1 min at 58°C and 1 min at 72°C followed by 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. One of the primers was fluorescently labeled and the PCR product was analyzed on an ABI 373A Automated Sequencer using the Genescan software package (Applied Biosystems, Foster City, CA). The Gly897Glu mutation was identified by SSCP and subsequent sequence analysis and is caused by a neutral mutation at the 1198glycine residue of the *COL4A4* gene. PCR was performed on 100 ng genomic DNA using 100 ng of intronic oligonucleotides A4IF10 (5'-AGGGACAAGA-3') and 714 (5'-CTTCTATTGACCTTTGGGAG-3') (9). After 5 min of denaturation at 96°C, eight initial cycles were performed of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C and a second round of 35 cycles using inner primers F100 (5'-CTCTTCTGGAAGCTC-3') and R101 (5'-GGTCCTATGGCTC-3'). The Gly897Glu mutation created an AluI restriction site and was screened at the genomic DNA level by PCR amplification using oligonucleotides F100 (5'-AGGGCTTCCCGACTCCA-3') at position 2750 and R101 (5'-GGTGGAGGTCCTATGGTCTC-3') at position 3017 (17); denaturation for 1 min at 92°C, 1 min 30 s of annealing at 55°C and extension for 2 min at 72°C. The Gly897Glu mutation created an AluI restriction site and was screened at the genomic DNA level by PCR amplification using oligonucleotides F100 (5'-AGGGCTTCCCGACTCCA-3') and 1106 (5'-ACCTGGAGGACAGTTGAG-3').

**Results**

**Linkage analysis.** Linkage analysis was performed with two novel polymorphic markers from the *COL4A3/COL4A4* locus. The most informative marker was a CA repeat, isolated from a YAC clone with an insert of 540 kb, containing both *COL4A3* and *COL4A4* genes (18). The other marker was a
HaeIII RFLP in one of the exons of the \textit{COL4A4} gene. Because the genomic structure of \textit{COL4A4} is not clarified yet, the exact numbering of exons is unknown, but most likely exon 8 is involved (counting from the 3' end). The RFLP is caused by a polymorphism, a neutral G to A nucleotide substitution at Glycine1198 (GGG into GGA) (17). The allele frequencies of this polymorphism were 0.5/0.5 (70 unrelated individuals tested).

Figure 2. Gly897Glu substitution in the type IV collagen α4 chain in BFH. Sequence analysis of type IV collagen α4 cDNA of the index patient and a normal control. The mutation, which changes the glycine (G) codon 897 GGG into the glutamic acid (E) codon GAG is indicated by an arrow. Type IV collagen chains consist of collagenous and non-collagenous (NC) domains. Three type IV collagen chains form a triple helix, which is interrupted by the substitution of a conserved glycine residue.

Figure 3. Segregation of Gly897Glu mutation in the BFH family. The presence of the mutation creates an additional AluI restriction site. Cleavage of the 104 bp PCR fragment in two shorter fragments of 86 and 18 basepairs is indicative for the presence of the mutation. Only the affected family members (filled symbols) show the shorter DNA fragment (86 bp), the smallest fragment of 18 bp is not visible. DNA fragment lengths are indicated in basepairs (bp).
Linkage analysis was performed in the paternal (II:7) side of the pedigree (Fig. 1). The mother (II:8) and her two sons, III:10 and III:11 (Fig. 1), were not included in the linkage studies, because BFH segregated also in the maternal line. The maternal relatives were not available for linkage analysis. A maximum lod score \( Z_{\text{max}} \) of 3.01 was obtained in the paternal (II:7, Fig. 1) line with the COL4A3/COL4A4 CA repeat at recombination fraction \( \Theta = 0.0 \) in our family. The \( Z_{\text{max}} \) obtained with the intragenic COL4A4 RFLP is 1.51 at \( \Theta = 0.0 \). All affected individuals share the haplotype [78, 2] for both markers (Fig. 1). This haplotype was linked to BFH with a \( Z_{\text{max}} \) of 3.58 at \( \Theta = 0.0 \).

**Mutation analysis.** RT-PCR was performed on total lymphocyte RNA followed by direct sequence analysis of amplified COL4A3 and COL4A4 cDNA fragments. A heterozygous G to A nucleotide substitution was identified at position 2898 of the COL4A4 gene in the patients III:10 and III:11 and in their father II:7, but not in their mother II:8. The glycine codon GGG at position 897 was changed into GAG, encoding a glutamic acid residue (Fig. 2). The mutation introduced a novel site for the restriction enzyme AluI and the other family members and controls were screened for the presence of this site. If the AluI site is present, a 104 bp PCR fragment will be cleaved in two smaller fragments of 86 and 18 bp (Fig. 2). All affected family members were heterozygous for the mutation (Fig. 3) and the mutation was not detected in the unaffected family members and 50 unrelated controls (data not shown).

**Discussion**

For several reasons, the Gly897Glu mutation in the type IV collagen \( \alpha_4 \) chain is likely to be the pathogenic mutation in the BFH family. First, the mutation changes a glycine in a conserved collagenous triple helical domain in the type IV collagen \( \alpha_4 \) chain (17). Every third amino acid is a glycine, because it is the only residue small enough to fit into the center of the triple helical molecule (19). If glycine residues are substituted by bulkier amino acids such as glutamic acid the triple helix structure will be distorted (Fig. 2). Unlike fibrillar collagens, type IV collagen triple helices contain 21–26 interruptions, which are assumed to provide flexibility to the molecule. However, the positions of these interruptions are highly conserved during evolution and the generation of an additional interruption may interfere with correct folding of the intricate type IV collagen trimers, consisting of type IV collagen \( \alpha_3, \alpha_4, \) and \( \alpha_5 \) chains. Substitutions for glycines in the collagenous triple helical domain are also a common cause of other collagen disorders, like osteogenesis imperfecta and Ehlers Danlos syndrome (20). Furthermore, the mutation cosegregates with BFH in our family and, finally, the mutation is absent from a control group of 50 individuals, which provides additional evidence for the pathogenicity of the defect.

In 1994, homozygous mutations in the type IV collagen \( \alpha_3 \) and \( \alpha_4 \) genes have been identified in patients with autosomal recessive Alport syndrome (8, 9). Two different mutations were reported in the type IV collagen \( \alpha_4 \) gene: a glycine to serine substitution and a serine to stop mutation (9). Similar to the Gly897Glu mutation in the BFH family presented here, the glycine is substituted in the triple helical domain of the type IV collagen \( \alpha_4 \) chain. Our data suggest that BFH patients can be manifesting carriers of autosomal recessive Alport syndrome. This is in line with the histological examination of the kidney biopsy and the presence of proteinuria in the index patient, who is a suspected compound BFH heterozygote. The definite proof awaits the detection of the second maternal BFH mutation and the progression of his renal disease.

In conclusion, this is the first report on a genetic defect explaining benign hematuria. The identification of type IV collagen defects, similar to those found in Alport syndrome, is important for genetic counseling and prognostic prediction. As yet, this prediction can not be based on the mutation only, but needs a complete investigation of family history, follow-up examination and renal biopsy. In a recent report linkage of the rare autosomal dominant form of Alport syndrome to the type IV collagen \( \alpha_3 \) and \( \alpha_4 \) locus was demonstrated in a large Northern-Irish family (Jefferson, J.A., A.P. Maxwell, A.E. Hughes, C.M. Hill, C.C. Doherty, and N.C. Nevin. Autosomal dominant Alport syndrome linked to the type IV collagen \( \alpha_3 \) and \( \alpha_4 \) genes (COL4A3 and COL4A4), manuscript submitted for publication). It is obvious that the type IV collagen \( \alpha_3 \) and \( \alpha_4 \) genes are strong candidates for the autosomal dominant form of Alport syndrome as well. This would complete the spectrum of phenotypes of type IV collagen \( \alpha_3 \) and \( \alpha_4 \) defects, ranging from neutral amino acid substitutions (8) and benign hematuria to mild autosomal dominant and severe recessive Alport syndrome. In this way, the type IV collagen mutations fit within the rule of extreme clinical and genetic variability of collagen disorders (21).

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**References**


