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 G = 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 are all present with persistent hematuria. Particularly, differentiation between Alport syndrome, which is a severe renal disorder, progressing to renal failure in most cases, and benign 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 G = 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

Methods

Patients. The index patient (III:10; see Fig. 1) presented with hematuria at the age of 5 years. There were no concomitant abnormalities, i.e., glomerular filtration rate was normal and hypercalciuria was absent. There was also no hypertension. The morphology of the erythrocytes in the urine sediment indicated a glomerular origin of the cells. Audiography and fundoscopy revealed no abnormalities. Family history was negative for renal failure and deafness. One year later a renal biopsy was performed. Electron microscopy of the biopsy specimen showed regions with malformations of the glomerular basement membrane, typical for Alport syndrome, and regions which were thin. Further investigation of the family showed 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 off-spring, 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-
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 gram/liter). Hematuria was defined as a condition with 10 or more erythrocytes per mm² urine, established by the quantitative sediment method of Gade-holt (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 GCG TGC C-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 55°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 in the PCR fragment, flanked by outer primers 945 (5'-GGCAGAGGTGACATGTGTGATACA-3') starting at position 1871 and 946 (5'-CCCTCGAGAAGGTCAAACCTCCAG-3') starting at position 4074 (17) in a first round of 35 cycles; denaturation for 1 min at 92°C, 1 min 30 s of annealing at 60°C and extension for 3 min at 72°C. Two microliters out of the first PCR reaction was used as template for a second round of 35 cycles using inner primers F100 (5'-AAGGCCTTCCCCGAGCTCCA-3') at position 2750 and R101 (5'—CCGGAGGTCTTATGGCCT-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 Alul restriction site and was screened at the genomic DNA level by PCR amplification using oligonucleotides F100 (5'-AAGGCCTTCCCCGAGCTCCA-3') and 1106 (5'-ACCTGAGGACCCAGTGAC-3') and digestion with Alul restriction enzyme (GIBCO BRL, Gaithersburg, MD). If the mutation is present the 104-bp PCR fragment is cleaved in two fragments of 86 and 18 bp. The smallest fragment is not visible.

**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

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Segregation analysis of type IV collagen α3 and α4 markers in the BFH family. Haplotypes were constructed for the type IV collagen α3 or α4 CA repeat and the type IV collagen α4 HaeIII RFLP. Type IV collagen α3 or α4 CA repeat fragments are indicated as the length of the PCR fragment in basepairs (76, 78, and 80 bp). The HaeIII RFLP alleles are represented by 1 (HaeIII site absent) and 2 (HaeIII site present). The haplotype [78, 2], shown as black bars, cosegregates with BFH. Filled symbols indicate individuals with hematuria. Arrow indicates index patient (II-10).
HaeIII RFLP in one of the exons of the COL4A4 gene. Because the genomic structure of 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 Glycine198 (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 Alu I 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 ($\Theta$) of 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 897 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 Alul and the other family members and controls were screened for the presence of this site. If the Alul site is present, a 107 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 a4 chain is likely to be the pathogenetic mutation in the BFH family. First, the mutation changes a glycine in a conserved collagenous triple helical domain in the type IV collagen a4 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 a3, a4, and a5 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 a3 and a4 genes have been identified in patients with autosomal recessive Alport syndrome (8, 9). Two different mutations were reported in the type IV collagen a4 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 a4 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 compound heterozygote. The deep profit 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 a3 and a4 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 a3 and a4 genes (COL4A3 and COL4A4), manuscript submitted for publication). It is obvious that the type IV collagen a3 and a4 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 a3 and a4 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**


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