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

Henny H. Lemmink,* Willy N. Nillesen,† Toshibo Mochizuki,* Cornelis H. Schröder,* Han G. Brunner,‡ Bernard A. van Oost,*1 Leo A.H. Monnens,* and Hubert J.M. Smeets†1

Departments of *Pediatrics and †Human Genetics, University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands; ‡Department of Medicine, Division of Nephrology, Albert Einstein College of Medicine, Bronx, New York; †Department of Clinical Sciences of Companion Animals, University of Utrecht, Utrecht, The Netherlands; and ‡Division of Genetics, University of Limburg, Maastricht, The Netherlands

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, differentiation between Alport syndrome, which is a severe renal disorder, progressing to renal failure in most cases, and benign hematuria is mandatory for correct prediction of prognosis and genetic counseling. In benign hematuria electron microscopic analysis of renal biopsies shows thinning of the glomerular basement membrane (GBM)(1–3), while in Alport syndrome renal biopsies are characterized by irregular thickening and multilamination of the GBM (4). However, thinning of the GBM may be the only microscopic abnormality in younger Alport patients (5–7), which hampers the differentiation between the two disorders in childhood. Renal biopsies of 65 children with isolated hematuria persisting for at least one year revealed histological abnormalities consistent with Alport syndrome in eight cases and consistent with benign hematuria in 33 cases (3). A positive family history was detected in 23 of the 33 cases.

The major structural component of the GBM is formed by a type IV collagen network. As yet, six different type IV collagen chains have been identified and the respective genes were cloned. Mutations in the type IV collagen α1 (COL4A1) gene, which are present in patients with the autosomal recessive form of Alport syndrome (5–7), have been identified in some patients presenting with persistent microscopic hematuria. Since mutations in the type IV collagen α2 (COL4A2) gene are a normal finding in patients with mild Alport syndrome (8), it is likely that mutations in different type IV collagen genes may cause BFH. If the mutations were found in the β1- and β2-type IV collagen genes in Alport syndrome, The majority was present in the X-linked type IV collagen α5 (COL4A5) gene, but recently mutations in the type IV collagen α3 and α4 (COL4A3/ A4) genes have been reported in patients with the autosomal recessive form of this disease (8, 9). These latter two collagen genes are localized head to head on the tip of the long arm of chromosome 2 (10). Considering the similarities in GBM abnormalities, autosomal Alport syndrome and BFH could be the severe and mild forms of different molecular genetic defects in the same genes. Here we report linkage of the COL4A3/A4 locus to BFH in a large Dutch family (11) and the identification of a pathogenic mutation in the COL4A4 gene.

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 urinary sediment indicated a glomerular origin of the cells. Audiology 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 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-
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 GCG TGC C3') and CA11R (5'-CTC ATT GAT ACA AAC 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 in the PCR fragment, flanked by outer primers 945 (5'-GCCAAG-GGTGACATGTTGTATCA-3') starting at position 1871 and 946 (5'-CCCTCTGAGGAAGCTACCTCAG-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 56°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'-AAGGCCCT-TCCCCGGACTC3') at position 2750 and R101 (5'-CGGAG-GTCTATGAGTTCCTC-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'-AAGGCCCTTCCCCGGACTC3') and 1106 (5'-ACCTGGAGGACCAGTACG-3') and digestion with Alul restriction enzyme (BIBCO 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

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**Figure 1.** Segregation analysis of type IV collagen α3 and α4 markers in the BFH family. Haplotype markers were constructed for the type IV collagen α3/α4 CA repeat and the type IV collagen αα HaeIII RFLP. Type IV collagen α3/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).

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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 Glycine 198 (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 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 2898 of the fied [phocyte RNA followed by direct sequence analysis of ampli­

The maximum lod score ($Z_{\text{max}}$) of 3.01 was obtained in the paternal (11:7) side of the pedigree project no. C09.1022. We thank Marcy Speer (Duke University Medical Center, Durham, NC) for help in linkage analysis.

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**References**


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