Autosomal-dominant familial hematuria with retinal arteriolar tortuosity and contractures: A novel syndrome

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Background. Autosomal-dominant forms of hematuria have been mostly related to mutations in the COL4A3/COL4A4 genes. Patients with thin basement membrane (BM) disease do not have extrarenal manifestations, while those with Alport syndrome often present with hearing loss, anterior lenticonus, and dot-and-fleck retinopathy.

Methods. We performed a phenotypic study and a candidate gene approach in a four-generation family presenting with autosomal-dominant hematuria associated with extrarenal manifestations. Renal biopsy was analyzed for determination of BM thickness and expression of chains of type IV collagen. Linkage to 18 candidate genes/loci was investigated using polymorphic microsatellite markers.

Results. In all affected patients, hematuria without proteinuria was associated with muscular contractures and retinal arteriolar tortuosity responsible for retinal hemorrhages. Cardiac arrhythmia, Raynaud phenomena, and brain MRI abnormalities were also observed. Despite the presence of red cells in tubule sections, no glomerular abnormalities were found by electron microscopy. Expression of type IV collagen chains and glomerular BM thickness was normal. We searched for a molecular defect affecting either BM or angiogenesis. Linkage analyses of genes encoding BM components (COL4A3/COL4A4, COL6A1, COL6A2, COL6A3, FBLN1), and angiogenic factors or their receptors (VHL, ANPT1, ANPT2, TIE, TEK, NOTCH2, NOTCH3, NOTCH4, DLL4, JAG1, JAG2) and of the facio-sapulo-humeral dystrophy and 3q21 loci failed to show segregation of the disease with those gene loci.

Conclusion. We have identified a new inherited hematuria syndrome associated with retinal vessel tortuosity and muscular contractures. We recommend performing a fundus examination in patients with familial hematuria and episodes of visual impairment, as well as a urinary analysis in patients with retinal arterial tortuosity or congenital muscular contractures.

Key words: familial benign hematuria, retinal arteriolar tortuosity, muscular contractures, hypogammaglobulinemia, leukoencephalopathy.

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We first report an inherited autosomal-dominant hematuria syndrome in a four-generation family not linked to the COL4A3/COL4A4 locus and with no GBM ultrastructural abnormalities. This syndrome is remarkable for the association of hematuria with systemic non-renal signs that cosegregate with the renal disease, including muscular contractures, arteriolar retinal tortuosities responsible for repeated, benign hemorrhages, brain vascular abnormalities, and hypogammaglobulinemia. The presence of those unusual extrarenal manifestations led us to perform gene linkage studies focused on candidate genes encoding GBM components or involved in vasculogenesis or angiogenesis processes.

METHODS

Patients

The pedigree of our French Caucasian family is shown in Figure 1. Six affected members from the third and the fourth generations were investigated in our Department at Tenon Hospital. Partial medical records of the affected patients of the first and the second generations were also available.

Histologic study

A renal biopsy was performed in patient IV-1, and processed for light microscopy, immunofluorescence, and electron microscopic studies according to standard procedures used in our laboratory [13]. In addition, 3 μm frozen sections of the kidney biopsy specimen were incubated with monoclonal antibodies to the α1, α3, and α5 chains of type IV collagen (Alport syndrome diagnostic kit, Wieslab AB, Lund, Sweden), then with fluorescein isothiocyanate (FITC)-labeled secondary antimouse antibody (Dako, Carpinteria, CA, USA). The thickness of the GBM was measured on ×15,000 power micrographs. A total of 50 measurements in five loops was done.

Genetic linkage analyses

Venous blood samples from family members II-3, III-1, III-2, III-3, IV-1, IV-2, and IV-4 were obtained after informed consent, according to the French legislation. Genomic DNA was prepared according to standard methods. We tested our family for linkage to 18 gene loci (Table 1). Haplotype analysis was performed using polymorphic microsatellite markers spanning the genetic interval of each candidate gene and locus.

RESULTS

Clinical study

Pedigree. As depicted in the family tree (Fig. 1), the disease transmitted as an autosomal dominant trait. All affected patients presented with the association of renal involvement, retinal arterial tortuosities, and muscular contractures.

Renal involvement. Patients I-2 and II-2 presented with end-stage renal disease of unknown origin, which occurred after a septic shock and a postoperative shock, respectively. All affected patients in generations III and IV had isolated microscopic hematuria of more than 100,000 urinary red cells/mL. Gross episodes occurred in three patients. In the hematuric patients, proteinuria was <300 mg/day, and blood pressure and glomerular filtration rate were within the normal range. Renal CT scan demonstrated bilateral small cysts in three patients (II-2, III-1, III-3). A renal biopsy was performed in patient IV-1 (Fig. 2). Light microscopy showed no glomerular abnormalities, but disclosed red blood cells filling in some tubular lumens, thus confirming the glomerular origin of hematuria (Fig. 2A). No abnormal immunoglobulin or complement component deposits were detected by standard immunofluorescence methods. Indirect immunofluorescence study of the renal expression of the type IV collagen α1 (not shown), α3 (Fig. 2C), and α5 (Fig. 2D) chains was also normal. Electron microscopy examination of the kidney specimen did not show significant glomerular abnormality (Fig. 2B). The GBM appearance was globally normal, but with focal areas of dense folding. The average GBM thickness was 400 nm, within the normal range for gender and age [15]. No ultrastructural abnormality of podocytes and endothelial cells was observed.

Ocular involvement

All affected patients showed retinal arterial tortuosities in both eyes. They experienced episodes of superficial intraretinal hemorrhages responsible for transient visual impairment, but resolving spontaneously without visual sequelae. Funduspic examination and fluorescein angiography revealed the presence of bilateral tortuous retinal arteriolar vessels, especially in the macular
### Table 1. List, distribution, and function of candidate genes tested by microsatellite analysis

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Protein</th>
<th>Distribution/function</th>
<th>Human genetic disease</th>
<th>Microsatellite markers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BM components</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>COL4A3</td>
<td>a3 and a4 chains of type IV collagen</td>
<td>GBM</td>
<td>-AR forms of AS</td>
<td>D2S351, TOSH0, D2S159, D2S401[9]</td>
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<tr>
<td>COL4A4</td>
<td></td>
<td>GBM and mesangium</td>
<td>-AD forms of AS</td>
<td></td>
</tr>
<tr>
<td>COL6A1</td>
<td>a1, a2 of type VI collagen</td>
<td>BM and ECM including GBM</td>
<td>AD myopathy with contractures (Bethlem myopathy)</td>
<td>D21S1255, D21S266, D21S1260</td>
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<td>COL6A2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COL6A3</strong></td>
<td>a3 chains of type VI collagen</td>
<td>BM and ECM including GBM</td>
<td>AD myopathy with contractures (Bethlem myopathy)</td>
<td>D2S2348, D2S345, D2S338</td>
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<td>LAMA5</td>
<td>a5 laminin</td>
<td>BM</td>
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<td>FBLN1</td>
<td>Fibulin-1</td>
<td>BM</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Angiogenic proteins/receptors</strong></td>
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<td>ANGPT1</td>
<td>Angiopoietin-1</td>
<td>Mesenchyme surrounding developing vessels. Role in late stages of vasculogenesis</td>
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<td>D8S556, D8S1703, D8S1830</td>
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<td>Role in vascular remodelling</td>
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<tr>
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<td>Endothelial cells</td>
<td>Angiopoietin receptors</td>
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<td>Tie-2</td>
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<td>D9S259, D9S1121</td>
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<td>Arterial vascular smooth muscle cells</td>
<td>CADASIL</td>
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<td>Tumor suppressor protein Anti-angiogenic protein</td>
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<td>4q35ter</td>
<td>Unknown</td>
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<td>AD facio-scalpulo-humeral muscular dystrophy</td>
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<tr>
<td>3q21</td>
<td>Unknown</td>
<td></td>
<td>HERNS</td>
<td>D3S3685, D3S3564, D3S1289</td>
</tr>
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</table>

Abbreviations are: BM, basement membrane; GBM, glomerular BM; ECM, extracellular matrix; AD, autosomal dominant; AR, autosomal recessive; BFH, benign familial hematuria; VMCM, venous malformations, multiple cutaneous and mucosal; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy; HERNS, hereditary endotheliopathy, retinopathy nephropathy and stroke.

area, without exudate or leakage at late frames of angiography (Fig. 3). No other ocular abnormalities were noted. In particular, there was neither anterior lenticonus and dot-and-fleck retinopathy, nor signs suggestive of Fabry disease.

### Muscular contractures

All affected patients experienced predominantly proximal muscular contractures from early childhood. Contractures were painful and paroxysmal. They could last a few seconds to a few minutes, and occasionally a few hours. Contractures were enhanced by exercise and alcohol ingestion. Electromyography was performed in four patients (III-1, III-3, IV-1, IV-4). Contractures were recorded in patient IV-4 and were electrically silent. Muscular testing disclosed only minimal proximal weakness in two patients (III-1, IV-1), without amyotrophia. No other clinical abnormalities were noted on neurologic examination. Persistently elevated levels of serum creatine kinase (from 2- to 7-fold the upper limit of normal) were measured in all patients. A muscular biopsy was performed in patient III-1. Histologic examination revealed mild nonspecific muscular changes without signs of metabolic disease, mitochondrial cytopathy, or microvascular change on ultrastructural examination.

### Central nervous system involvement

Patients III-1, IV-1, and IV-2 suffered from recurrent headaches, and patient IV-4 presented with a generalized seizure. Brain MRI disclosed predominantly posterior asymmetrical leukoencephalopathy on FLAIR...
Fig. 2. Renal biopsy specimen of the patient IV-4. (A) Normal glomerulus with numerous erythrocytes in proximal tubule lumen (Trichrome, magnification ×312). (B) Ultrastructural examination shows a uniform GBM with focal dense folding (→) (original magnification ×3000). Immunofluorescence examination with antibodies against α3(IV) collagen chain (C) and α5(IV) collagen chain (D) shows a normal distribution of α3(IV) and α5(IV) collagen chain (original magnification ×312).

Fig. 3. Fluorescein angiogram of the left eye of patient III-1 shows typical pattern of retinal tortuosities (→) affecting the second and third order arterioles, without leakage of the affected vessels. No capillary or vein abnormalities and micro-aneurysms are observed.

and T2-weighted imaging in patients III-3, IV-2, and IV-4 (Fig. 4). Brain MRI was normal in patient IV-1.

Other manifestations

In addition to renal, muscular, and ocular signs, some patients presented with other systemic manifestations, including Raynaud phenomena with nonspecific capillaroscopic changes (patients III-1, III-2, IV-1, IV-2, IV-4), and symptomatic cardiac arrhythmia (patients II-2, III-1 and IV-1). Mild hypogammaglobulinemia (4.4 to 7.1 g/L) was also detected in all affected patients from the two last generations without abnormalities of white blood count. There was neither macrothrombocytopenia nor leukocyte inclusions on blood smear examination.

Audiometry performed in patients III-2, IV-1, and IV-2 was normal, and no clinically detectable hearing loss was observed in other patients. Clinical examination of the skin was unremarkable in the affected patients. Skin biopsies were performed in patients III-3 and IV-3. Light microscopic and ultrastructural study of the biopsy samples was normal, without arteriolar abnormalities.

Genetic study

Our candidate gene approach was driven by pathophysiologic hypotheses (Table 1). We first tested genetic linkage to several genes encoding basement membrane components coexpressed in the kidney and in the muscle and vessel walls, including COL4A3/COL4A4, COL1A6, COL2A6, COL3A6, LAMA5, and FBLN1. We then extended our linkage analysis to candidate genes involved in vasculogenesis and angiogenesis processes, including Von Hippel Lindau gene and genes encoding angiopoietin-1, angiopoietin-2, Tie-1 and Tie-2 receptors, Notch2, Notch3, Notch4, and ligands of Notch proteins, including
Jagged1, Jagged2, Delta4. In addition, we tested linkage with the locus of the facio-scapulo-humeral dystrophy 4q35ter, and of hereditary endotheliopathy, retinopathy, nephropathy, and stroke (HERNS) 3q21. The linkage analyses failed to show segregation of the disease with any of the tested genes and loci.

DISCUSSION

We report a French Caucasian kindred affected with a novel syndrome defined by hematuria of glomerular origin, muscular contractures, and retinal arteriolar tortuosities. This syndrome was transmitted as an autosomal-dominant trait over four generations. All affected members presented with hematuria, muscular contractures, and retinal arteriolar tortuosities, while none of the family members had contractures or retinal arteriolar tortuosities in the absence of hematuria, which strongly suggested that a unique gene defect was responsible for those symptoms. However, we cannot definitely rule out the coexistence of several gene defects in this family. Some affected members also exhibited MRI brain abnormalities, Raynaud phenomena, cardiac arrhythmia, and hypogammaglobulinemia. Most of those manifestations, especially retinal arteriolar tortuosities and MRI abnormalities, point to a small vessel vascular disease for which 18 candidate genes have been investigated by genetic linkage analysis.

One of the most salient features of the syndrome is hematuria, which appeared during the second decade of life and remained “isolated” in affected patients of the third and fourth generations (i.e., without proteinuria, elevated blood pressure, or renal failure after more than twenty years follow-up). Partial medical records were available for patients I-2 and II-2, in whom renal failure could be related to additional conditions (i.e., acute sepsis and postoperative shock, respectively). Light microscopy examination of the kidney biopsy specimen from patient IV-1 did not show glomerular abnormalities, while red blood cells filled in some tubular lumens, confirming the glomerular origin of hematuria. Glomerular expression of α1, α3, and α5 chains of type IV collagen was normal, as were ultrastructural aspect and thickness of the GBM. Finally, haplotype analysis failed to show a linkage of the disease with the COL4A3/COL4A4 locus. These results indicated that the syndrome observed was distinct from usual forms of autosomal-dominant AS and FBH.

The absence of macrothrombocytopenia, and the characteristics of the renal disease, also ruled out Epstein and Fechtner syndromes, which are caused by mutations in the MYH9 gene (encoding the nonmuscle myosin heavy chain IIA) [16–18]. Because cases of AS were shown to be associated with retinal arteriolar tortuosities [19], the grouping of clinical signs led us to hypothesize that in our family, a common molecular defect affecting basement membranes was responsible for the multisystemic alterations. However, we failed to find a linkage with locus of genes encoding proteins coexpressed in the glomerular, muscular, and vascular basement membranes, including laminin-5, fibulin-1, and α1, α2, and α3 chains of type VI collagen.

Inherited retinal arteriolar tortuosity with superficial macular hemorrhages is a well-characterized autosomal-dominant disorder, although the underlying gene defect has not been identified so far. The disease has a benign course without long-term visual impairment, and arteriolar abnormalities are apparently limited to the retinal vascular bed [20, 21]. Retinal arteriolar tortuosity is occasionally observed in Fabry disease [22]. However, the diagnosis can be ruled out on the basis of the mode of transmission, and the absence of the characteristic inclusion bodies of glycolipid in podocytes by electron microscopy. Retinal arteriolar tortuosity has also been exceptionally reported in facio-scapulo-humeral dystrophy (FSHD), an autosomal-dominant muscular disorder characterized by proximal muscle weakness, amyotrophia, and contractures [23, 24]. Linkage with the FSHD locus (4q35ter) was excluded in our family.

In addition to retinal arteriolar tortuosity observed in all affected subjects, some of them displayed additional
clinical signs suggesting a systemic small vessel disease; indeed, three patients suffered from recurrent headaches, five patients had Raynaud phenomena, and white matter signal abnormalities were observed on brain MRI in three patients. Recently, Vahedi et al reported a kindred presenting with an autosomal-dominant syndrome that associated typical retinal arteriolar tortuosity with a central nervous system disease, causing infantile hemiparesis, migraine with aura, and leukoencephalopathy with dilation of perivascular spaces on brain MRI, but without hematuria or contractures [25]. This new syndrome adds to the spectrum of the hereditary cerebral small vessel diseases recently identified [26]. Among them, cerebral autosomal-dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL) is a systemic arteriopathy affecting mainly cerebral vessels, rarely retinal vessels [27]. Because CADASIL is due to mutations in the NOTCH3 gene [20], we, like Vahedi et al [25], excluded a linkage to the CADASIL/NOTCH3 locus. We also ruled out a linkage with genes encoding NOTCH2 and NOTCH4 proteins and the NOTCH protein ligands Jagged1, Jagged2, and Delta4. We have also tested segregation of the disease with the 3p21 locus, to which are linked three autosomal-dominant cerebroretinal hereditary syndromes, including the cerebroretinal vasculopathy, the hereditary vascular retinopathy, and HERNs, while in the latter conditions, retinal vascular abnormalities are clearly distinct from the ones observed in our family [28]. Interestingly, HERNs is associated with renal involvement including proteinuria, hematuria, and renal failure [29]. On ultrastructural examination of HERNs renal biopsy specimens, glomerular and peritubular capillary basement membranes presented a multilaminated aspect similar to the one observed in vascular basement membranes in the brain and other tissues [29], whereas basement membranes were ultrastructurally normal in patient IV-1 from our family. We failed to demonstrate a linkage with the 3p21 locus in our family.

Because we suspected a systemic small vessel disease, we reasoned that angiogenic proteins could be involved in podocytes and/or endothelial alterations, leading to abnormal permeability of the glomerular capillary wall to red blood cells. In our family, the increased permeability of glomerular and retinal arteriole basement membrane was remarkable for the absence of protein leakage, contrary to retinal neovascularization induced by VEGF-A in proliferative diabetic retinopathy and in animal models [30, 31]. On the other hand, angiopoietin-1 and -2 and Tie-1 and Tie-2 receptors are known to induce vasculogenesis and inhibit the increased permeability to proteins caused by VEGF-A [32]. However, linkage analyses with genes encoding angiopoietin-1 and angiopoietin-2, Tie-1, and Tie-2 were negative. There was no linkage either to the Von Hippel Lindau gene.

Muscular symptoms in our patients were characterized by a painful, transient activation of muscle contraction. Because no electrical activity was recorded during a muscle contraction on electromyography in one patient, we concluded that muscular symptoms were muscular contractures. The pathophysiology of contractures remains obscure. They were painful enough to lead the patients to consult at the emergency room in pediatric age. They were different from acroparesthesias observed in Fabry disease [33]. Because they were associated with vascular abnormalities, we hypothesize that they could be related to transient muscular ischemic episodes, given the fact that they were enhanced by exercise.

CONCLUSION

Our genetic study was limited to the testing of candidate genes. A genome-wide linkage analysis would be more powerful, but would require either a larger pedigree or identification of other families. We recommend systematically performing a fundus examination in patients with familial benign hematuria and episodes of visual impairment, as well as a urinary dipstick analysis in patients presenting with retinal arterial tortuosity or congenital muscular contractures. Gene identification of this novel hereditary disease might help understand the pathophysiology of some familial hematuria syndromes.

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