X-Linked Alport Syndrome: Natural History and Genotype-Phenotype Correlations in Girls and Women Belonging to 195 Families: A “European Community Alport Syndrome Concerted Action” Study

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Abstract. Alport syndrome (AS) is a type IV collagen hereditary disease characterized by progressive hematuric nephritis, hearing loss, and ocular changes. Mutations in the COL4A5 collagen gene are responsible for the more common X-linked dominant form of the disease characterized by much less severe disease in girls and women. A “European Community Alport Syndrome Concerted Action” (ECASCA) group was established to delineate the Alport syndrome phenotype in each gender and to determine genotype-phenotype correlations in a large number of families. Data concerning 329 families, 250 of them with an X-linked transmission, were collected. Characteristics of heterozygous girls and women belonging to the 195 families with proven COL4A5 mutation are compared with those of hemizygous boys and men. Hematuria was observed in 95% of carriers and consistently absent in the others. Proteinuria, hearing loss, and ocular defects developed in 75%, 28%, and 15%, respectively. The probability of developing end-stage renal disease or deafness before the age of 40 yr was 12% and 10%, respectively, in girls and women versus 90 and 80%, respectively, in boys and men. The risk of progression to end-stage renal disease appears to increase after the age of 60 yr in women. Because of the absence of genotype-phenotype correlation and the large intrafamilial phenotypic heterogeneity, early prognosis of the disease in X-linked Alport syndrome carriers remains moot. Risk factors for developing renal failure have been identified: the occurrence and progressive increase in proteinuria, and the development of a hearing defect.

Alport syndrome (AS) is characterized by progressive hematuric nephritis with ultrastructural and immunohistochemical changes of the glomerular basement membrane (GBM), frequently associated with sensorineural hearing loss (1–8). It is caused by defects in type IV collagen, a major structural component of basement membranes. Six type IV collagen genes have been cloned and characterized, localized in pairs on three chromosomes (9). The COL4A3 and COL4A4 genes, on chromosome 2, are involved in the rarer autosomal recessive forms of the disease (10–14), whereas mutations in the COL4A5 gene coding for the α5 chain of type IV collagen are responsible for the more frequent X-linked dominant form of AS (15,16). X-linked AS is clinically heterogeneous with a
wide variability in the rate of progression to end-stage renal disease (ESRD), the type of GBM changes, the presence or the absence of deafness, and other extrarenal manifestations, such as ocular changes or diffuse esophageal leiomyomatosis. However, whereas progression to renal failure is constant in boys and men, the course of renal involvement is much more variable in girls and women, remaining mild in the majority of them. Since the identification of the first COL4A5 mutations (17), more than 300 different mutations have been reported by different groups in Europe, Asia, and the United States (reviewed in [15,16,18–27]). Because of the high diversity of mutations, a large number of families whose members have AS have to be analyzed to evaluate genotype-phenotype correlations.

Thanks to the formation of the “European Community Alport Syndrome Concerted Action” (ECASCA) group and the recruitment of 195 families with a characterized COL4A5 mutation, we recently described the natural history of X-linked AS in boys and men and established genotype-phenotype correlations (28). Here, we analyze the clinical and pathologic expression of the disease in heterozygous girls and women belonging to these 195 families and attempt to correlate phenotype and genotype. To our knowledge, this is the first large-scale study of the natural history of X-linked AS in girls and women with proven COL4A5 mutation. It confirms the variable clinical course of the disease previously observed in smaller series of AS girls and women and documents the high rates of proteinuria and ESRD in heterozygous girls and women, especially in women over 40 yr.

Material and Methods

Inclusion Criteria

Inclusion criteria have been described previously (28). Briefly, inclusion of families was based on the classical criteria of AS: positive family history of hematuria with or without progression to ESRD; progressive sensorineural hearing loss; characteristic ocular changes (lenticonus and/or maculopathy); and characteristic ultrastructural changes of the GBM. Two additional signs were considered as new diagnostic criteria: diffuse esophageal leiomyomatosis involving the tracheobronchial tree and the female genitalia, and abnormal GBM distribution of the \( \alpha_3 \), \( \alpha_4 \), and \( \alpha_5 \) chains of type IV collagen (3,8). Data from a total of 329 AS-associated families were collected, and the 195 families with an identified COL4A5 mutation were selected for the study.

Patient Database and Statistical Analyses

A database was developed with ORACLE 6.09 software as described previously (28). Statistical analyses were performed by SAS software, version 8.01, and S+ software, version 3.4. Curves of the cumulative probability of occurrence of events (age at ESRD, age at detection of hearing loss) were computed according to the Kaplan-Meier method. For the statistical comparisons between curves, we used robust tests based on the Cox proportional-hazard model that take into account the intrafamilial correlations (29). The relationship between two types of events was also assessed by considering one as a time-dependent covariant of the other (30).

Results

Analysis of the pedigrees of the 195 X-linked families with identified COL4A5 mutation showed that 506 girls and women were obligatory carriers for the disease. Information was obtained for 349 carriers but was not complete for all of them, probably because of the low rate of presentation as a result of the frequently benign course of their disease.

Expression of the Disease in Girls and Women

Age at diagnosis varied from 1 d to 57 yr. Importantly, hematuria was detected before or at the age of 2 yr in 13 patients. Microscopic hematuria was found in 309 (95.5%) of 323 patients. Among the 14 without documented hematuria, 8 (all of them more than 25 yr old) were completely asymptomatic carriers, 1 had isolated proteinuria, 2 had chronic renal failure, and 3 had diffuse leiomyomatosis with normal urinalysis. Proteinuria developed in 176 of the 234 patients tested.

Hearing loss was observed in 28% of the patients, exceptionally before the age of 20 yr. Ocular changes were detected in 15% of patients. It consisted of asymptomatic maculopathy in most of them. Anterior lenticonus was observed in only three patients.

Ultrastructure of the GBM was examined in 28 girls and women. Thick GBM was observed in 3 patients and irregular alternation of thick and thin segments in 17. Diffuse GBM thinning was seen in six patients who underwent biopsy at the age of 8 to 21 yr. The GBM was also thin in two related male subjects (14 and 26 yr old, respectively) from two different families. Alternating thick and thin segments were observed in three related women, 21 to 40 yr old, belonging to three different families and in one related 22-yr-old man. The GBM was normal in the two remaining patients, aged 39 and 52 yr at examination. The 39-yr-old woman had diffuse esophageal leiomyomatosis with no renal symptoms; the other belonged to a family in which an affected male subject had typical alterations of the GBM. The GBM distribution of the \( \alpha_3(IV) \) chain, studied in nine patients, was normal in two and discontinuous in seven.

Follow-up data were available in 288 female patients. At their last examination, 34 patients (12%) had chronic renal failure, and 51 patients (18%) had reached ESRD. It occurred early, between the ages of 19 and 30 yr in 14 patients (28%), between the ages of 31 and 40 yr in 16 patients (31%), and after 41 yr in 21 patients (41%). Renal transplantation has been performed in 13 patients, and posttransplantation anti-GBM glomerulonephritis occurred in none.

Comparison between Male and Female Patients

As shown on Figure 1, 70% of men at the age of 30 and nearly 90% of them at age 40 had reached ESRD. In contrast, nearly all female patients had functioning kidneys at age 30, and only 12% had reached ESRD at 40. Similarly, the risk of developing deafness before the age of 40 yr is about 90% in male patients versus only 10% in girls and women (Figure 2). However, after the age of 60, 30% to 40% of the cohort of

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female patients regularly followed have developed ESRD, and 20% have hearing loss.

In an attempt to correct the bias linked to the loss to follow-up of approximately half of the patients—probably those with a benign course of the disease—we performed a parallel analysis taking in account 61 additional girls and women for which partial information was available (Figure 1). The derived curve probably gives more precise information on the actual risk for girls and women with X-linked AS of developing severe complications. However, in this analysis, we could not include data of 157 obligatory carriers appearing in the genealogical trees, for whom birth date was not provided and for whom no information at all was available.

**COL4A5 and COL4A5/COL4A6 Mutation Distribution in Families**

COL4A5 mutations consisted of 38 large deletions of the COL4A5 gene (also involving the COL4A6 gene in 12) and 157 small mutations. Among the small deletions, 31 were deletions, 9 insertions, and 12 nonsense mutations; except for three in-frame deletions (analyzed with missense mutations), they were expected to result in the synthesis of a truncated α5 chain. Seventy-five were missense mutations, 59 of them leading to glycine substitution, and 29 were splice-site mutations. These mutations have been described in detail previously (28).

**Correlations between Genotypes and Phenotypes**

In female patients, some differences in the probability of developing ESRD were observed according to the type of mutation, with the lowest rate of progression for missense mutations (Figure 3), but in contrast to what has been observed in boys and men, none of the differences were statistically significant. Moreover, a large intrafamilial variability for the occurrence and the age at ESRD was observed, whatever the type of mutation. In 12 families (two large rearrangements; three insertion, deletion, or nonsense; three splice sites; and four missense mutations), one woman had progressed rapidly to ESRD before the age of 31 yr. Analysis of these families showed that in four of them, severe renal disease was also observed in one relative female (ESRD at 30, 30, 33, and 36 yr, respectively), but renal function was normal in 15 other carriers (aged 13 to 83 yr). In family 4, ESRD occurred in two women at ages 54 and 59 yr, respectively, but renal function was normal in two others aged 21 and 61 yr. In the other families, no ESRD was observed in the six related carriers aged 40 to 82 yr.

No correlation could be established with the severity of the renal disease in boys and men (Figure 4). For example, in family 10, one woman with frameshift mutation developed ESRD at 28 yr, and the disease was also severe in related boys and men who developed ESRD between 13 and 25 yr of age. Conversely, in family 8, with missense mutation, 10 related men of a woman who experienced ESRD at 27 yr of age developed ESRD at various ages between 22 and 76 yr, and at more than 50 yr in five of them.

Twenty-eight percent of female patients developed hearing loss.
loss; no significant correlation was observed with the various types of mutation (Figure 5) or the occurrence of hearing loss in related boys and men. Ten patients with AS had diffuse esophageal leiomyomatosis. In all of them, a deletion removing the 5’ end of both the COL4A5 and the COL4A6 genes, with a breakpoint located in the second intron of COL4A6, was identified. None of these patients had developed ESRD at the age of 23 to 53 yr, and three had normal urinalysis findings. However, two of them were proteinuric at 23 and 26 yr, respectively. Five patients died of leiomyomatosis complications or superimposed cancers between the ages of 39 and 53 yr.

Abnormal thick or alternatively thick and thin GBM was observed in 19 patients with various types of mutation (Table 1). Two female patients (one with a large COL4A5-COL4A6 deletion and DEL, and the other with a missense mutation) had normal GBM. Thin GBM was predominantly seen in patients with missense mutation. But overall, no significant correlation could be established between the type of mutation and the GBM ultrastructural changes. GBM staining for α3 (IV) (and, when tested, for the α4 (IV) and α5 (IV) chains) was discontinuous in seven girls and women with different types of COL4A5 mutations. Conversely, it was normal in one female subject with glycine substitution and another with in-frame small deletion (Table 2).

Prognostic Features

The renal prognosis of AS in women is unpredictable at an early age. During the course of evolution, the occurrence of proteinuria has an ominous prognosis (Figure 6) (P = 0.001). The risk for developing ESRD is also higher in girls and women with, rather than without, hearing loss (Figure 7) (P = 0.02). Among the three patients with lenticularus, one developed ESRD at 24 yr, whereas the two others had normal renal function at 63 and 52 yr, respectively. Diffuse leiomyomatosis is not specifically associated with progression of the renal disease.

Discussion

We have previously reported the natural history of the disease in male patients belonging to 195 X-linked AS families with identified COL4A5 mutation (28). Here, we focus our report on the expression of the disease in related girls and women in an attempt to evaluate the actuarial risk of developing renal failure and deafness and to establish genotype-phenotype correlations. This is the first large-scale study of the natural history of X-linked AS in girls and women with proven COL4A5 mutation. It confirms the variable clinical course of the disease previously observed in smaller series of AS girls and women and documents the high rates of proteinuria and ESRD in heterozygous girls and women, especially in women over 40 yr.

Hematuria, usually microscopic, is the cardinal feature of the disease (2,31) and was the presenting symptom in nearly all girls and women. During the course of the disease, it was present in 96% of girls and women. This result is in agreement with the study by Flinter et al. (4) and Dagher et al. (32) who found hematuria in 100% of female carriers when repeated urinalysis was performed. Proteinuria developed in 75% of girls and women. Hearing loss, usually occurring after 30 to 40 yr of age, was observed in 28% of patients, and ocular changes, mostly macular flecks, were detected in 15%. Ten patients had diffuse esophageal leiomyomatosis; as previously shown, there is no correlation between the presence of leiomyomatosis and the severity of the renal disease (33).

Ultrastructural changes of the GBM were found in 26 of 28 patients and consisted of typical thick and split or alternatively thick and thin GBM in 19 patients. Interestingly, in six patients, the GBM was diffusely thin, a lesion not specific of benign familial hematuria. GBM immunohistochemical staining for the α3-α5(IV) chains, performed in nine patients, was discontinuous in seven. This specific anomaly was observed in patients with missense or frameshift mutation, but normal expression of the chains was also seen in two patients with deletion or missense mutation.

The prognosis of X-linked AS is usually regarded as favorable in girls and women (2,31,33). Actually, 51 girls and
women developed ESRD, and 55% of them reached this stage before the age of 40 yr, the youngest at the age of 19 yr. The risk of developing ESRD or deafness before the age of 40 yr is 12% and 10%, respectively. It is clearly different in related boys and men, in whom the risk of developing ESRD and deafness before the age of 40 yr is about 90% (28). Similar results have been found by Flinter et al. (4), who analyzed renal function in 113 female patients with X-linked AS: 15% of them had manifested chronic renal failure at an average age of 40 yr. However, we were surprised by the severity of the long-term outcome of the disease in our group of female patients: in them, the probability of having ESRD by the age of 60 is about 30%. This rate could be overestimated because approximately one-third of girls and women, likely to be

### Table 1. X-linked Alport syndrome

<table>
<thead>
<tr>
<th>GBM</th>
<th>Large Rearrangement</th>
<th>Missense</th>
<th>Splice Site</th>
<th>Nonsense Deletion</th>
<th>Insertion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Thin</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Thick, or thick and thin</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>20</td>
</tr>
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</table>

*Correlation between the type of COL4A5 mutation and the type of GBM ultrastructural changes in 28 female patients. GBM indicates glomerular basement membrane.

### Table 2. Immunohistochemical data, clinical status, and COL4A5 mutation state in nine patients with examination of α-chain expression

<table>
<thead>
<tr>
<th>GBM α3/α5 (IV)</th>
<th>Phenotype/Genotype</th>
<th>Hematuria (yr)</th>
<th>Proteinuria (yr)</th>
<th>HT (yr)</th>
<th>Renal Function</th>
<th>Hearing Loss</th>
<th>Ocular Changes</th>
<th>GBM</th>
<th>Last FU (yr)</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td>11</td>
<td>19</td>
<td>—</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>Thick and thin</td>
<td>26</td>
<td>Del 3BP, ex 33</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>19</td>
<td>19</td>
<td>21</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>27</td>
<td>Miss ex 27b</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>3</td>
<td>1</td>
<td>35</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>Thin</td>
<td>21</td>
<td>Miss ex 36b</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>1</td>
<td>9</td>
<td>—</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>36</td>
<td>Miss ex 35b</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>17</td>
<td>17</td>
<td>—</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>Thick and thin</td>
<td>47</td>
<td>Miss ex 26</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>7</td>
<td>9</td>
<td>—</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>Thin</td>
<td>19</td>
<td>Miss ex 26</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>26</td>
<td>26</td>
<td>—</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>Thick and thin</td>
<td>28</td>
<td>Miss ex 41b</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>14</td>
<td>14</td>
<td>ESRD 24</td>
<td>×</td>
<td>AL</td>
<td>×</td>
<td>Thick and thin</td>
<td>24</td>
<td>Del 1BP, ex 41</td>
</tr>
<tr>
<td>+/−</td>
<td></td>
<td>4</td>
<td></td>
<td>—</td>
<td>N</td>
<td>—</td>
<td>—</td>
<td>Thick and thin</td>
<td>15</td>
<td>Splice site intron 15</td>
</tr>
</tbody>
</table>

* + indicates normal distribution; +/−, discontinuous distribution; GBM, glomerular basement membrane; FU, follow-up; ND, not determined; HT, hypertension; AL, anterior lenticonus; Del, deletion; Miss, missense; and ex, exon.

b Four of the seven missense mutations resulted in glycine substitution.

Figure 6. Probability of end-stage renal disease according to the presence (n = 119) or absence (n = 43) of proteinuria.

Figure 7. Probability of end-stage renal disease according to the presence (n = 165) or absence (n = 55) of hearing defect.
less severely affected, were lost to follow-up, but it suggests that AS in girls and women is less benign than previously thought.

High levels of progression to ESRD have been reported in a few studies, but these were probably the result of the mode of selection of patients or a referral bias (34,35). In the analysis by Grünfeld et al. (34) designed to determine the predictive factors of renal prognosis in girls and women with AS, 40% of patients developed ESRD before (nine patients) or after (five patients) the age of 35 yr. But the 36 patients have been selected on the basis of persistent urinary abnormalities, and the mode of transmission of their disease is not determined. The 63% rate of progression to ESRD observed in the series of Chugh et al. (35) is clearly the result of a referral bias in favor of the most symptomatic patients because only 11 of 63 patients with AS assessed in their center were girls and women. On the other hand, it is interesting to note that in the Finnish experience, the median age at ESRD was 31.1 yr (range, 20.2 to 39.9 yr) in the 5 of 40 female patients from 25 families who had a progressive renal disease, not much later than in the 20 of 38 related boys and men who developed ESRD at a median age of 24.9 yr (range, 14.5 to 39 yr) (36).

We tried to determine whether the clinical course of the disease depends on the genetic defect. The lowest rate of progression to ESRD is associated with missense mutations, as in related boys and men; however, the association is not statistically significant. Similarly, the 11 girls and women with X-linked AS who developed ESRD in the recent study of Hertz et al. (27) bore different types of mutations from missense to frameshift, and in the series of Inoue et al. (22), the two girls with heavy proteinuria at, respectively, 13 and 15 yr of age had missense mutation. No genotype-phenotype correlation was detected regarding the occurrence of hearing loss.

A peculiar feature of X-linked AS is the large intrafamilial variability of the disease in girls and women. This heterogeneity was already seen in the family reported by Alport (1) and further completed by Crawford et al. (37): age at ESRD in the five girls and women with a progressive course varied from 12 to 83 yr. The same variability was observed in our series, and we did not find any correlation between the rapid progression to ESRD (before the age of 31 yr) in a given girl or woman and the severity of the disease in related male subjects or female subjects. This intrafamilial heterogeneity could be related to the random X-chromosome inactivation. Actually, 90% of the X chromosome with the normal COL4A5 allele was found to be inactivated in the kidney of one female patient with a severe Alport phenotype (38). In addition, Vetrie et al. (39) have shown that in peripheral leukocytes, the ratio of the relative activity of the mutant X chromosome to the X chromosome carrying the normal COL4A5 allele was not the same in all AS heterozygotes. However, this ratio was not correlated with renal function, a finding in agreement with the tissue specificity of X-chromosome inactivation patterns (40). Conversely, Nakanishi et al. (41), who also observed intrafamilial heterogeneity of female phenotype, found that the degree of α5(IV) chain expression in the epidermal basement membrane, used as a marker of X inactivation, was correlated with the severity of the renal disease. This finding was not confirmed by one of us (42).

At the clinical level, the absence of genotype-phenotype correlations and the intrafamilial heterogeneity of the phenotype make it impossible to predict the renal course in AS individual girls and women from family analysis or genetic studies. These patients have to be systematically followed on a longitudinal basis, and as previously shown by Grünfeld et al. (34) and as demonstrated in this study, the best ominous prognostic marker remains the occurrence and the progressive increase in proteinuria. The risk of developing ESRD is also higher in patients with, rather than without, hearing loss. These data have to be taken in account in the difficult discussion of kidney donation by related Alport heterozygotes. It cannot be accepted if the presumptive donor is young, or if proteinuria, hearing defect, hypertension, or renal insufficiency are present. Conversely, heterozygous women who have asymptomatic hematuria when older than 30 to 40 yr have a very small risk of developing late renal failure. If they are strongly motivated and well informed, they cannot be definitely refused as potential kidney donors.

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