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The Value of Clinical Criteria in Identifying Patients with X-Linked Alport Syndrome

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Background and objectives: Alport syndrome (AS) is a predominantly X-linked hereditary nephritis associated with high-tone, sensorineural deafness and characteristic eye signs. Clinical diagnostic criteria were defined in 1988. Most cases result from mutations in the X-linked collagen gene *COL4A5*, with mutations in the autosomal genes *COL4A3* and *COL4A4* on chromosome 2 accounting for the rest. Mutation analysis of *COL4A5* with a combination of sequencing and multiplex ligation-dependent probe amplification has been available for several years. The objective of this study was to determine the utility of clinical diagnostic criteria in identifying patients likely to have a *COL4A5* mutation.

Design, setting, participants, & measurements: Clinical information was available on 206 patients whose DNA was received for testing between 1994 and June 2008; predictive tests for a known familial mutation, samples from duplicate family members, and incompletely screened samples were excluded. One hundred and twenty-eight patients (62.1%) had a pathogenic *COL4A5* mutation.

Results: The mutation detection rate in families fulfilling zero, one, two, three, or four diagnostic criteria was 0%, 18%, 64%, 89%, and 81%, respectively. Sixty-seven percent of patients with *COL4A5* mutations meeting only two diagnostic criteria had not had a complete clinical assessment. In two thirds of families meeting four diagnostic criteria without an identified *COL4A5* mutation, autosomal inheritance was confirmed or suspected.

Conclusions: The authors recommend *COL4A5* analysis in any patient meeting at least two clinical diagnostic criteria. *COL4A3* and *COL4A4* analysis should be considered if a *COL4A5* mutation is not detected and primarily if autosomal inheritance is suspected.

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Alport syndrome (AS) is a predominantly X-linked hereditary nephritis associated with hematuria, progressive renal failure, high-tone sensorineural deafness, and characteristic eye signs.

The family reported by Alport in 1927 had previously been described by others; however Alport commented that the presence of 'nerve' deafness in most patients with hematuria probably represented a specific clinical syndrome. He also noted that males were more severely affected than females, which is typical of X-linked inheritance (1). Subsequently pathognomonic eye findings (anterior lenticonus/macular flecks) were reported (2) and characteristic changes within the renal glomerular basement membrane (GBM) were observed under the electron microscope (3).

AS is caused by defects in type IV collagen, a major structural component of the GBM. Most AS (85%) is due to mutations in *COL4A5* located on the X-chromosome; the remaining 15% is accounted for by mutations in the autosomal collagen genes *COL4A3* and *COL4A4* on chromosome 2, which can be inherited in a recessive or dominant pattern depending on the specific mutation.

AS is the most common hereditary nephropathy, with a gene frequency of 1 in 5000 to 1 in 10,000. Studies suggest AS causes 0.6% of cases of chronic renal failure in Europe (4), but individual renal failure registries suggest a figure of up to 5%.

In 1988 a set of four clinical criteria was described (5), facilitating the diagnosis of AS in clinical practice if the proband and other family members between them meet at least three of the following:

1. Positive family history (FH) of macro/microscopic hematuria or chronic renal failure
2. Electron microscopic evidence of AS on renal biopsy
3. Characteristic ophthalmic signs (anterior lenticonus and macular flecks)
4. High-tone sensorineural deafness

Because of the genetic heterogeneity of AS, a comprehensive three-generation FH should be taken. For full clinical evaluation of a family, the proband and other relevant family members should have urinalysis, a formal ophthalmological examination, and an audiogram. Examination of the eyes with a slit lamp ophthalmoscope is required to detect the pathognomonic changes of AS, but these may be hard to detect in younger

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patients, especially females. Hearing loss is a nonspecific feature of several hereditary nephropathies. Sensorineural hearing loss is also frequently reported in patients with chronic renal failure, although the exact etiology of this hearing impairment remains unclear (6). Renal biopsies should be examined by light and electron microscopy. Light microscopy is generally uninformative in children under 10 years of age and is nonspecific in adults; however, electron microscopy reveals areas of thickening and splitting of the GBM, which is characteristic of AS. In early childhood and in females, the only evidence of AS may be thinning of the GBM, which can be misdiagnosed as thin basement membrane disease (7).

A large study of 195 families with a proven *COL4A5* mutation found a FH of hematuria or ESRD in 88.5%, hearing loss in 82.5%, ocular changes in 44%, and ultrastructural GBM changes in 98% (8), consistent with the diagnostic criteria proposed in 1988.

In addition to the four clinical criteria, immunohistochemical (IHC) analysis of basement membrane type IV collagen expression with skin or renal biopsy has also been reported as a useful diagnostic tool. The lack of expression of α5(IV) collagen in the skin epidermal basement membrane (EBM) has been described in approximately 80% of male X-linked AS patients, with 20% of males having normal expression (9). Females demonstrate a mosaic α5(IV) collagen expression due to Lyonisation. Generally the pattern of expression of α5(IV) collagen in the EBM mirrors that of the GBM, however in autosomal recessive AS there is a lack of expression of α5(IV) collagen in the GBM with normal expression in the skin, Bowman's capsule, and tubular basement membrane. Although skin biopsy can provide a cheap and simple diagnostic test, discordance of expression between the EBM and GBM has been reported (10). In addition IHC GBM changes are found in fewer AS families compared with ultrastructural GBM changes (8). Therefore IHC is not always reliable and is not widely available in the United Kingdom.

The DNA laboratory, Guy's and St. Thomas' (GSTS) Pathology, at Guy's Hospital is the only diagnostic service in the United Kingdom to offer *COL4A5* analysis. Before 2005 *COL4A5* analysis was performed in a research setting; since 2005 the diagnostic laboratory has screened for mutations in patients with suspected X-linked AS. Multiplex ligation-dependent probe amplification (MLPA) was added to the diagnostic service testing in 2007. This technique facilitates analysis of genomic rearrangements of *COL4A5* and when combined with unidirectional direct sequencing of *COL4A5*, the sensitivity of testing is >95%. Families meeting clinical diagnostic criteria for AS, but without an identified pathogenic mutation in *COL4A5*, may have a rare deep intronic mutation that can only be detected efficiently with RNA studies; a mutation in an autosomal gene, *COL4A3*/*COL4A4*; or another clinical diagnosis.

The aim of this study was to determine if the clinical diagnostic criteria proposed in 1988 were helpful in determining the chance of finding a *COL4A5* mutation in an unselected series of individuals referred for diagnostic *COL4A5* analysis.

Materials and Methods

The diagnostic DNA laboratory (GSTS Pathology) received 250 requests for diagnostic *COL4A5* analysis between January 2005 and May 2008, including samples re-evaluated with MLPA if a negative result had previously been found by screening before 2005 and excluding multiple samples from the same family or predictive tests (a test in an unaffected individual for a known familial mutation). All samples received for mutation testing were accepted. Clinical assessment was performed by the referring clinician (clinical geneticist or nephrologist). To establish the clinical indications for testing, we retrospectively sent questionnaires to the clinicians of the 250 patients to elicit the clinical features of the proband and their family and the extent of clinical assessment performed. One hundred fifty questionnaires were returned completed (60%). In addition, 56 individuals with a *COL4A5* mutation identified before 2005 had a questionnaire completed from a notes review at Guy's Hospital. Information was collected on the proband and close relatives about the presence of hematuria, renal failure, characteristic ophthalmic signs, and/or high-tone sensorineural hearing loss, as well as the results of renal biopsies performed. Clinical information was analyzed to identify the number of diagnostic criteria met by the proband and family to establish the extent of the clinical assessment.

A total of 56 individuals had a mutation detected in the research laboratory. All individuals tested by the diagnostic service had *COL4A5* analysis performed by screening of 51 exons by heteroduplex analysis (conformation-sensitive capillary electrophoresis, $n = 5$; temperature-gradient capillary electrophoresis [TGCE], $n = 93$) or unidirectional direct sequencing ($n = 52$) plus MLPA for the detection of gross deletions and duplications if initial screening was negative. Only pathogenic mutations as determined by a protocol in accordance with current CMGS guidelines (11) were included in the analysis. Variants of unknown clinical significance were classified for the purpose of this study as a *COL4A5*-negative result.

Results

Clinical information was available on 206 patients: 132 male and 74 female. One hundred twenty-eight individuals (62.1%), 92 male and 36 female, had a *COL4A5* mutation; in the remaining 78 individuals (37.4%), 40 male and 38 female, no *COL4A5* mutation was identified.

The 128 pathogenic mutations identified in *COL4A5* included 63 missense (60 affecting glycine residues), 12 nonsense, and 15 frameshift mutations; 15 gross duplications or deletions; 3 small deletions; 19 mutations resulting in aberrant splicing; and 1 complex rearrangement. A total of 101 mutations were novel (34 have since been reported by the research laboratory) and 27 mutations have been reported elsewhere. All mutations were classified as pathogenic using CMGS guidelines (11).

Results for individuals with positive and negative mutation results and the number of patients in whom a full clinical assessment had been completed is shown in Table 1. Only 54 individuals (42.2%) with a positive result and 47 (60.2%) of those with a negative result had had a complete assessment. In most incomplete assessments ophthalmological information was not available; however, when the proband was a child with hematuria, renal biopsy had not always been performed on them or any relative (Table 2). The referring clinicians rarely provided data on IHC studies.

The *COL4A5* mutation detection rate in patients fulfilling

Table 1. Diagnostic criteria reached in 128 individuals with a *COL4A5* mutation and 78 individuals without an identified *COL4A5* mutation

Diagnostic Criteria Met	Individuals with Identified *COL4A5* Mutation				Individuals with No Identified *COL4A5* Mutation			
	Male	Female	Total	Number with Complete Clinical Assessment	Male	Female	Total	Number with Complete Clinical Assessment
0	0	0	0	—	5	3	8	3 (38%)
1	3	4	7	2 (29%)	15	17	32	19 (59%)
2	33	16	49	16 (33%)	13	15	28	17 (61%)
3	45	14	59	23 (39%)	6	1	7	5 (71%)
4	11	2	13	13 (100%)	1	2	3	3 (100%)
Total	92	36	128	54 (42%)	40	38	78	47 (60%)

Table 2. Details of clinical assessment performed

Clinical Criteria	*COL4A5*-Positive Individuals (n = 128)			*COL4A5*-Negative Individuals (n = 78)		
	Present	Absent	Unknown	Present	Absent	Unknown
Positive family history	120	8	0	52	26	0
Positive renal biopsy	107	2	19	40	28	10
Ophthalmological signs	20	45	63	8	47	23
Audiometry (SNHL)	87	33	8	21	47	10

SNHL, sensorineural hearing loss.

Table 3. Mutation detection frequency according to number of diagnostic criteria reached in all 206 individuals

Number of Diagnostic Criteria Reached	Individuals with Identified *COL4A5* Mutation	Individuals with No Identified *COL4A5* Mutation	Percent Mutation Detection for Diagnostic Criteria
0	0	8	0
1	7	32	17.9
2	49	28	63.6
3	59	7	89.3
4	13	3	81.3

Table 4. Mutation detection frequency according to number of diagnostic criteria reached in 101 individuals with complete clinical assessment

Number of Diagnostic Criteria Reached	Individuals with Identified *COL4A5* Mutation	Individuals with No Identified *COL4A5* Mutation	Percent Mutation Detection for Diagnostic Criteria
0	—	3	0
1	2	19	9.5
2	16	17	48.5
3	23	5	82.1
4	13	3	81.3

zero, one, two, three, or four diagnostic criteria was 0%, 17.9%, 63.6%, 89.3%, and 81.3%, respectively (Table 3).

The mutation detection in males was 70% (92 of 132) compared with 49% (36 of 74) in females, which was statistically significant when data for all numbers of diagnostic criteria met were analyzed together (P = 0.0043). However, when the mutation detection rate was analyzed separately for each number of diagnostic criteria reached, there was no statistically significant difference between males and females (one diagnostic criterion P = 1.0; two diagnostic criteria P = 0.0927; three diagnostic criteria P = 1.0; four diagnostic criteria P = 0.1357, using a two-tailed Fisher exact test).

A complete clinical assessment had been performed in 101 families (49.0%). The mutation detection in the patients with a complete assessment is shown in Table 4; mutation detection rates are similar to those for three and four diagnostic criteria and slightly lower for two diagnostic criteria. In these families, a characteristic renal biopsy appearance was the most predictive of a *COL4A5* mutation (odds ratio [OR] 46.6; 95% confidence interval [CI] 5.9 to 365.8) compared with a positive FH

(OR 4.3; 95% CI 1.3 to 14.4), sensorineural hearing loss (OR 4.4; 95% CI 1.9 to 10.3), or characteristic eye signs (OR 2.9; 95% CI 1.1 to 7.6).

Fifty-four families with a *COL4A5* mutation had undergone a complete clinical assessment: 53 (98.1%) had a characteristic renal biopsy appearance, 50 (92.6%) had a positive FH, 34 (63.0%) had evidence of sensorineural hearing loss, and 18 (33.3%) had characteristic eye signs.

Each diagnostic criterion was also reviewed separately. Results of renal biopsy were available on 177 probands/family members (86.0%) and this was the most predictive of a *COL4A5* mutation (OR 37.5; 95% CI 8.5 to 164.5). Family history was available on all probands and a positive FH gave an OR of 7.5 (95% CI 3.2 to 17.7). Audiometry had been performed in 188 probands/family members (91.3%) (OR 5.9; 95% CI 3.1 to 11.3). Eye examination had been performed in the fewest patients (120, 58.3%) and was the least predictive criterion (OR 2.6; 95% CI 1.0 to 6.5).

Discussion

Analysis of AS clinical diagnostic criteria in a large cohort of patients referred with suspected X-linked AS has confirmed that mutation detection frequency is highest when three or more diagnostic criteria are met. This is in agreement with the original proposal that at least three of four diagnostic criteria are required to make a clinical diagnosis of AS (5). The mutation detection frequency was 64% for patients meeting only two diagnostic criteria; however, 67% of these individuals had not had a complete clinical assessment performed and may also have other clinical features. The relatively high detection rate in individuals meeting only two diagnostic criteria suggests that in some cases it may be appropriate to proceed directly to mutation analysis before performing invasive tests or ophthalmological assessment, which can be challenging or uninformative in young children. The mutation detection rate for three and four diagnostic criteria is similar to the 82% reported by Martin (12) in 50 individuals with suspected AS. Interestingly, our results indicate that the mutation detection frequency is higher when three diagnostic criteria are met (89%) rather than four (81%). This may be a reflection of the incomplete clinical assessment in some individuals meeting only three criteria.

For the 78 individuals without an identified mutation, 58 had screening performed by TGCE and 20 had screening performed by direct sequencing; therefore, it is possible that a small proportion of mutations may have been missed using TGCE. Alternatively, a rare deep intronic mutation affecting splicing and only detectable by RNA analysis, another nephropathy, or a mutation in one of the autosomal genes may be responsible. Three individuals from three different families without an identified *COL4A5* mutation met four diagnostic criteria. Further mutation analysis in the autosomal collagen genes has now confirmed an autosomal recessive method of inheritance in one family, whereas retrospective review of another pedigree demonstrated autosomal dominant inheritance with male-to-male transmission. Two families meeting two diagnostic criteria but without an identified *COL4A5* mutation had evidence of consanguinity, which suggests that autosomal recessive rather than X-linked inheritance may be responsible. In addition, male-to-male transmission became apparent in at least two other families with two and three diagnostic criteria met. The true number of families with features of autosomal recessive or autosomal dominant inheritance may be higher because complete pedigrees were not always provided; these cases illustrate the importance of taking a good three-generation FH before referring for molecular analysis.

Our findings are consistent with a large study of 195 families with proven *COL4A5* mutations (8). This study looked at six rather than four diagnostic criteria including a FH of hematuria, sensorineural hearing loss, characteristic eye signs, ultrastructural changes of GBM, diffuse esophageal leiomyomatosis, and abnormal GBM distribution of the α(IV) collagen chains by IHC. Leiomyomatosis, which is associated with a contiguous deletion of *COL4A5* and *COL4A6*, is rarely associated with AS and was found in only 5% of families in their study. IHC GBM changes were found in fewer families compared with ultrastructural GBM changes. These two criteria were not assessed in our study. Most families in their study (71%) met two or three diagnostic criteria, comparable with families with a proven *COL4A5* mutation in this study. In our families with an identified *COL4A5* mutation and a complete clinical assessment, 98% had a characteristic renal biopsy appearance, 93% had a positive FH, 63% had high-tone sensorineural hearing loss, and 33% had anterior lenticonus or macular flecks, consistent with their data for these four criteria.

It is important to remember that a small proportion of AS, approximately 12%, arises *de novo* (8). In this cohort, seven patients with only one diagnostic criterion were found to have a *COL4A5* mutation; one of these patients had a positive FH and five had characteristic biopsy findings; however only two of seven had been fully assessed clinically and more clinical criteria may be present. We have shown that a positive renal biopsy is most predictive of a *COL4A5* mutation; thus, suspicions may be raised clinically even with only one diagnostic criterion met.

Also of interest are the eight patients in which no diagnostic criteria were met; all were cases of isolated hematuria in young boys: five had undergone a renal biopsy without characteristic AS changes although in one case the original diagnosis of AS was later revised. This may suggest, although the numbers are small, that *COL4A5* mutations are unlikely in cases of isolated hematuria in young boys in the absence of other clinical features.

There are potential limitations to this study because the information entered into the questionnaires was provided by different clinicians. In some instances clinicians may have indicated that no ophthalmological signs or hearing loss were present without checking if a formal assessment had been performed. Furthermore, an ophthalmological assessment should only be performed by an experienced ophthalmologist proficient in recognizing clinical eye manifestations of AS. Anterior lenticonus is said to be pathognomonic of AS; however, eight individuals with a negative *COL4A5* result were reported to have characteristic eye signs (one case now confirmed as autosomal recessive inheritance). These individuals may have

autosomal AS, an undetected *COL4A5* mutation, or potentially an inaccurate diagnosis of eye changes. Inaccuracies in data collection may also have resulted from incorrect interpretation of renal biopsy reports. In most cases clinicians described renal biopsy reports in detail or forwarded the pathology report, but this did not happen in all cases. Data were rarely available on IHC studies and this may reflect that access to genetic studies is more readily available to clinicians in the United Kingdom.

In conclusion we recommend that clinicians take a detailed three-generation FH before initiating genetic testing for AS. Most cases are X-linked and are caused by mutations in the *COL4A5* gene, so it is reasonable to screen this gene first in most cases. However, in families with male-to-male transmission or consanguinity, autosomal dominant or recessive inheritance should be considered; in these families it is appropriate to proceed directly to *COL4A3* or *COL4A4* mutation screening, which has recently become available in the United Kingdom at Guy's Hospital (GSTS Pathology, DNA laboratory).

We recommend mutation analysis of *COL4A5* in families meeting two or more diagnostic criteria for AS, with a greater than 60% chance of detecting a mutation. Mutation screening can now be completed routinely in 8 weeks and if there is no urgency to make a diagnosis it would be reasonable to postpone renal biopsy until the results of mutation screening are available, particularly in children. Although *COL4A5* mutations were infrequently detected in families meeting only one diagnostic criterion, mutation analysis could be considered if a characteristic renal biopsy appearance was present because in our study this criterion was most predictive of detecting a mutation. If *COL4A5* analysis is negative, we recommend completing the clinical assessment if not already performed and reviewing the pedigree to consider autosomal inheritance, particularly in families meeting three or four diagnostic criteria.

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Disclosures

None.

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