Autosomal dominant Alport syndrome linked to the type IV collagen $\alpha_3$ and $\alpha_4$ genes (COL4A3 and COL4A4)


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Abstract

Background. Alport syndrome is a hereditary nephritis that may lead to end-stage renal disease (ESRD) in young adult life and is often associated with sensorineural deafness and/or ocular abnormalities. The majority of families are X-linked due to mutations in the COL4A5 gene at Xq22. Autosomal forms of the disease are also recognized with recessive disease, having been shown to be due to mutations in the COL4A3 and COL4A4 genes on chromosome 2. Familial benign haematuria has also been mapped to this region in some families.

Subjects and methods. We describe a large family with autosomal dominant Alport syndrome in which males and females are equally severely affected and one member with a mild sensorineural deafness reached ESRD aged 35 years. Renal biopsy in four affected patients demonstrated characteristic thickened and split glomerular basement membranes on electron-microscopy.

Results. Genetic linkage analysis using markers on chromosome 2q demonstrated co-segregation of the disease with the markers D2S351 and D2S401 with a maximum lod score of 3.4 at zero recombination. Linkage to the COL4A4 gene was confirmed using an intragenic COL4A4 polymorphism. Mutation analysis has revealed a missense Leu36Pro mutation in exon 5 of the adjacent COL4A3 gene in the unaffected mother, which may lead to a more severe phenotype in affected family members carrying this mutation.

Conclusion. Mutations in the COL4A3 and COL4A4 genes can cause a spectrum of glomerular basement membrane disease ranging from autosomal recessive Alport syndrome to autosomal dominant Alport syndrome and familial benign haematuria.

Key words: familial benign haematuria; hereditary nephritis; type IV collagen

Introduction

Alport syndrome is a progressive hereditary nephropathy associated with characteristic ultrastructural abnormalities of the glomerular basement membrane (GBM). The majority of families demonstrate X-linked transmission of the disease [1]. Classically males develop haematuria and deafness at a young age and progress to ESRD in their early 20s. Other associated features include eye signs [2] (anterior lenticonus, perimacular flecks), leiomymatosis [3], and possibly macrothrombocytopenia. Females tend to be less severely affected. Renal biopsy of patients with Alport syndrome often shows minimal abnormality on light-microscopy and standard immunofluorescence. Electron-microscopy, however, shows typical changes with thickening and splitting of the glomerular basement membrane [4].

In 1988 the Alport locus was mapped to Xq22 [5] and when the COL4A5 gene was cloned and mapped to the same region [6] it became a very strong candidate as the Alport gene. This was confirmed in 1990 by the finding of mutations in the COL4A5 gene in three kindreds with Alport syndrome [7]. Since then more than 200 mutations in the gene have been described [8,9]. Autosomal forms of Alport syndrome are also known to occur and in 1993 mutations in COL4A3 and COL4A4 were described in families with autosomal recessive Alport syndrome who had a very similar phenotype to the X-linked disease [10,11]. Autosomal dominant disease is also described but is much less common [12].

In this study we performed genetic linkage analysis on a large family with autosomal dominant Alport syndrome using intragenic markers and microsatellite markers on 2q flanking the COL4A3/COL4A4 loci.
of sarcoidosis diagnosed by node biopsy. Maximum proteinuria was 8.6 g/24 h. His renal function deteriorated and he reached end-stage renal failure aged 35 years. Audiometry revealed a mild high-tone sensorineural deafness. His father had a long history of microhaematuria, proteinuria (+ + on urinalysis) and hypertension, with subsequent documented renal failure (serum creatinine 200 μmol/l) prior to his death aged 68 years from myocardial infarction.

Screening of other siblings revealed four sisters and one brother with microhaematuria and proteinuria. Renal function was only mildly impaired (Table 1). Audiograms were normal in these patients. The proband’s mother (II3) had no evidence of microhaematuria on three occasions, and had normal renal function. No family member showed evidence of eye signs, platelet abnormalities, or leiomysomatosis. There were no nail or patellar abnormalities. All family members were caucasian and born in Northern Ireland. There was no evidence of consanguinity. Renal biopsy was carried out on four patients. Light-microscopy showed normal cellularity without foam cells. Immunohistochemistry using monoclonal antibodies directed against individual type IV alpha chains was not performed. Electron-microscopy revealed normal basement membranes which were irregular in density and outline with areas of fibrillation characteristic of Alport syndrome.

Criteria for diagnosis

A member of the family was considered affected if microscopic haematuria was found on a minimum of three occasions by dipstick urinalysis. Two young children in the fourth generation (aged 1 and 3 years) were tested. Each showed a trace of protein only on one of three occasions and in view of their ages were considered as possibly affected and were excluded from the linkage analysis.

DNA analysis

DNA was extracted from venous blood of family members and analysed for inheritance of informative microsatellite polymorphisms.

Polymorphic markers

All flanking microsatellite oligo-primers were obtained from Research Genetics, Inc. (Huntsville, AL, USA). The polymerase chain reaction (PCR) was used to amplify the polymorphic region in a reaction volume of 10 μl containing 25 ng DNA, 200 μmol dNTP, 0.25 μM each primer (one of which was end-labelled with [32P]ATP), 1.5 mM MgCl2, 1 × PCR buffer, and 0.2U Taq polymerase. Samples were denatured for 3 min at 94°C followed by 35 cycles of amplification at 94°C for 1 min, 55°C for 1 min, and finally extended at 72°C for a further 5 min. PCR products were separated by electrophoresis on a 6% denaturing polyacrylamide gel and visualized by autoradiography.

Intragenic markers for COL4A3, COL4A4 and COL4A5 were analysed. The 2B6 marker was used for COL4A5 [13]. An intragenic COL4A3 microsatellite polymorphism had previously been isolated by Toshio Mochizuki and primer sequences and PCR conditions were sent by him (personal communication). A conformational polymorphism in exon 7 of COL4A4 was used as an intragenic marker for this gene. PCR was performed on 100 ng DNA in 25 μl reaction containing 50 ng primers one of which was end labelled with 32P. Thirty cycles of 94°C 1 min, 50°C 1 min and 72°C 1.5 min were performed. (Primer sequences (711/712) available on request from B. Smeets, Nijmegen).

Single-strand conformational polymorphism (SSCP) [14]

Five microlitres of PCR product was mixed with 5 μl formamide loading buffer and denatured for 5 min at 94°C. The samples were run on a 0.5 × MDE gel (AT Biochem, Malvern, PA) with 10% glycerol in 0.6 × TBE for 16 h at 6W at room temperature.

Linkage analysis

Lod scores were calculated using the MLINK program from the computer package LINKAGE (v 5.1) [15]. A gene frequency of 0.0001 was assumed for the disease.

Mutation analysis

Exons 1–5 of COL4A3 were amplified by PCR (primers and conditions as published) [10] and analysed by SSCP. Any polymorphic regions were sequenced using an Applied Biosystems 373A automated DNA sequencer.

Results

Table 1. Clinical details of affected family members

<table>
<thead>
<tr>
<th>Family member</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Creatinine clearance (ml/min)</th>
<th>24 h urinary protein (g)</th>
<th>Deafness</th>
<th>Eye signs</th>
<th>Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>II2</td>
<td>Died</td>
<td>M</td>
<td>–</td>
<td>4+</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>III2</td>
<td>39</td>
<td>M</td>
<td>ESRF</td>
<td>8.6</td>
<td>Mild</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>III4</td>
<td>37</td>
<td>F</td>
<td>108</td>
<td>0.3</td>
<td>No</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>III6</td>
<td>36</td>
<td>F</td>
<td>69</td>
<td>3.4</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>III8</td>
<td>35</td>
<td>F</td>
<td>93</td>
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<td>No</td>
<td>No</td>
</tr>
<tr>
<td>III12</td>
<td>32</td>
<td>F</td>
<td>70</td>
<td>2.7</td>
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</tr>
<tr>
<td>III15</td>
<td>28</td>
<td>M</td>
<td>71</td>
<td>2.5</td>
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</table>
Autosomal dominant Alport syndrome linked to the type IV collagen α3 and α4 genes

Eight polymorphic markers across the region 2q35–37, where the COL4A3 and COL4A4 genes have been located, were then analysed. Positive lod scores were obtained with all markers (Table 2). The maximum two point lod score of 3.44 occurred between the disease gene and the locus D2S401 at zero recombination. Significant linkage was also found with the less informative COL4A4 single strand conformational polymorphism (SSCP) variant which was found to segregate with the disease throughout the family without recombination with a maximum lod score of 2.9.

The COL4A3 microsatellite was not informative for the disease in this family; however, analysis of recombination events allowed localization of the COL4A3 gene to a 12 cM region between the markers D2S130 and D2S396.

**Mutation analysis**

SSCP analysis of exons 1–5 (counted from 3' end) of COL4A3 was performed and a band shift in exon 5 was detected which segregated with one of the maternal haplotypes and not with the disease. Sequencing of this exon revealed a single T→C substitution changing the amino acid Leu36 into Pro (Figure 2). This muta-

![Fig. 2. ▼ marks the heterozygous T→C point mutation detected by automated sequencing. This converts CTT leucine residue at position 36 to CCT proline.](image)

<table>
<thead>
<tr>
<th>Marker</th>
<th>0</th>
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<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
</tr>
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<td>D2S128</td>
<td>-2.26</td>
<td>1.38</td>
<td>1.85</td>
<td>1.87</td>
<td>1.55</td>
<td>1.04</td>
<td>0.43</td>
</tr>
<tr>
<td>D2S130</td>
<td>-2.26</td>
<td>1.38</td>
<td>1.85</td>
<td>1.87</td>
<td>1.55</td>
<td>1.04</td>
<td>0.43</td>
</tr>
<tr>
<td>D2S136</td>
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<td>0.29</td>
<td>0.24</td>
<td>0.20</td>
<td>0.12</td>
<td>0.06</td>
<td>0.01</td>
</tr>
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<td>3.01</td>
<td>2.96</td>
<td>2.77</td>
<td>2.51</td>
<td>1.94</td>
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<td>0.18</td>
<td>0.16</td>
<td>0.13</td>
<td>0.07</td>
<td>0.04</td>
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<td>COL4A4</td>
<td>2.96</td>
<td>2.91</td>
<td>2.72</td>
<td>2.47</td>
<td>1.93</td>
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</tr>
<tr>
<td>D2S401</td>
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<td>3.38</td>
<td>3.12</td>
<td>2.82</td>
<td>2.15</td>
<td>1.41</td>
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</tr>
<tr>
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<td>1.36</td>
<td>1.87</td>
<td>1.55</td>
<td>1.04</td>
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<tr>
<td>D2S172</td>
<td>-∞</td>
<td>1.38</td>
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<td>1.87</td>
<td>1.55</td>
<td>1.04</td>
<td>0.43</td>
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<tr>
<td>D2S407</td>
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<td>1.04</td>
<td>1.55</td>
<td>1.6</td>
<td>1.37</td>
<td>0.95</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Table 2. Results of two-point linkage analysis between disease gene and chromosome 2 markers

Fig. 1. Renal biopsy electron-micrograph (×7000) from proband demonstrating a markedly irregular and thickened glomerular basement membrane with areas of fibrillation.
tion has previously been described as a non-pathogenic missense mutation [11]; however, in this family the unaffected members (II2 and III4) who do not carry the Leu36Pro mutation may have a less severe phenotype than those carrying the mutation (Table 1).

Discussion

Alport syndrome is a genetically and phenotypically heterogeneous disease. X-linked Alport syndrome is by far the commonest, but autosomal recessive and autosomal dominant forms have also been described and are said to be the mode of inheritance in 15% of families [12]. Flinter et al. [16] proposed that Alport syndrome can be diagnosed if at least three of the following four criteria are fulfilled: (1) positive family history of haematuria/chronic renal failure; (2) electron-microscopic evidence on renal biopsy of Alport syndrome; (3) high-tone sensorineural deafness, and (4) characteristic eye signs. The family described fulfills these diagnostic criteria but shows a milder phenotype than is usually found in classic X-linked Alport syndrome. The older age at end-stage renal failure, minimal deafness, and absence of eye signs is similar to the phenotype of previously described families with autosomal dominant hereditary nephritis [17].

Type IV collagen is the major structural protein of the GBM and forms the basic framework into which other constituents are bound. Mutations in the COL4A5 gene and the COL4A3/COL4A4 genes have been shown to cause X-linked and autosomal recessive Alport syndrome respectively. In this family an X-linked pattern of inheritance was unlikely from pedigree analysis, and involvement of the COL4A5 locus was excluded by linkage analysis. We then mapped the disease to the long arm of chromosome 2 and linkage was confirmed to the COL4A3/COL4A4 gene locus. The COL4A3 and COL4A4 genes are arranged close together and both are therefore very strong candidate genes for this disease. We can hypothesize that mutations in these genes produce abnormal \( \alpha^3(IV) \) and \( \alpha^4(IV) \) chains which fail to incorporate properly into the triple helix of type IV collagen, leading to a destabilization of the molecular superstructure. Heterozygous mutations may lead to a less severe phenotype than homozygous mutations as there are still normal \( \alpha^3(IV) \) and \( \alpha^4(IV) \) chains being produced.

Mutation analysis in this family has led to the detection of a missense mutation in exon 5 of COL4A3 on one of the maternal chromosomes which she has passed on to the majority of her children. This mutation changes an evolutionary conserved leucine residue to proline, but has previously been described in two families as non-pathogenic [11]. In our family the mutation is not pathogenic by itself, in that heterozygotes have no clinical features, but may lead to a more severe phenotype in affected patients who carry this

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**Fig. 3.** Family pedigree showing haplotypes for markers D2S130—D2S351—COL4A3 microsatellite—COL4A3 exon 5 Leu36Pro mutation (L/P)—COL4A4 SSCP—D2S401—D2S396. □ males, ○ female. Filled symbol is an affected individual. Roman numerals represent generations and numbers identify family members. The shaded haplotype segregates throughout the family with the disease. Recombination events in patient III8 allow localization of COL4A3 and COL4A4 between markers D2S130 and D2S396.
mutation. The proband’s father (II2), who did not reach ESRD, and patient III4 seem to have a less severe clinical phenotype and are the only two affected family members not to carry the Leu36Pro mutation. It is also possible that a second mutation may be present in the maternal COL4A3 or COL4A4, accounting for the more severe phenotype.

Familial benign haematuria (FBH) is a related glomerular basement membrane disorder characterized by isolated microhaematuria, normal renal function and typical ultrastructural changes on electron-microscopy. Recently linkage analysis has mapped this to the COL4A3 and COL4A4 loci [18]. We postulate that mutations in the type IV collagen genes result in a spectrum of disease ranging from thin basement membrane disease to autosomal recessive and autosomal dominant Alport syndrome. This variation in clinical expression due to different mutations in collagen genes is well recognized. Different mutations in the COL1A1 and COL1A2 genes have been shown to cause osteogenesis imperfecta, type VII Ehlers–Danlos syndrome, and osteoporosis, with the severity of the osteogenesis imperfecta ranging from a lethal neonatal form to a mild form with only minor fractures in childhood [19]. Recessive and dominant disease from mutations in the same gene has also been reported previously for the COL11A2 gene in osteochondrodysplasias [20]. We conclude that mutations in COL4A3 or COL4A4 can result in autosomal dominant Alport syndrome in addition to autosomal recessive Alport syndrome and familial benign haematuria and is entirely compatible with the known clinical variability of collagen mutations.

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