Autosomal recessive Alport syndrome: linkage analysis and clinical features in two families

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Abstract
Background. Genetic heterogeneity is a well-known feature of Alport syndrome (AS). Most families with AS show an X-linked dominant pattern of inheritance but about 15% of families show an autosomal inheritance of the disease. Autosomal recessive AS may account for 10% of the total number of cases and is caused by mutations in the COL4A3 and COL4A4 genes. The clinical spectrum of this rare disorder has not been well clarified.

Methods. We present two families with AS. Two affected members of these families have entered end-stage renal disease (ESRD) in their 30s, and the other three are older than 15 years and have normal serum creatinine. Four of the five patients have deafness but none have ocular abnormalities. Two have been transplanted and have not suffered from anti-GBM antibody nephritis. Men and women are equally affected. We have performed linkage analysis for chromosome 2 with the following markers: D2S279, COL4A3/4 DNTR, COL4A4 RFLP Hae III.

Results. We demonstrate that both families, one of them consanguineous, are linked to the COL4A3/4 locus.

Conclusions. We can conclude that the only significant difference between the X-linked and the autosomal recessive forms of AS lies in the fact that in the latter females are as affected as males; thus the idea that autosomal recessive AS causes ESRD during childhood must be discarded. Other clinical features such as age of deafness or the presence of post-transplant anti-GBM antibody nephritis show no differences between the entities. Thus an accurate familial study is mandatory in patients with AS, as the identification of the different patterns of inheritance may cause a great difference in genetic counselling. Linkage analysis is the only effective molecular diagnosis that can be performed nowadays.

Key words: Alport syndrome; autosomal recessive; clinical features; COL4A3; COL4A4; genes; hereditary nephritis; linkage analysis; transplantation

Introduction
The association of hereditary nephritis and deafness was first recognized by Alport as a definitive entity in 1927 [1]. Extensive research over the following decades has extended the knowledge on the clinical, genetic, and structural features of this entity. The clinical and pathological abnormalities of Alport syndrome (AS) comprise: (i) persistent haematuria and progressive renal disease, both symptoms also present in the family history, (ii) hearing loss, (iii) ocular abnormalities, and (iv) splitting and thinning of the glomerular basement membrane. In rare cases, benign smooth-muscle tumours of the oesophagus and female genitalia (leiomyomatosis) or haematological abnormalities (megakaryocytic thrombocytopenia) have been found to be associated with AS.

This syndrome is now recognized as a specific disease of type IV collagen chains, with symptoms affecting basement membranes from various organs. Type IV collagen is the main component of the glomerular basement membrane (GBM) and is composed of six genetically distinct chains, α1 (IV) and α6 (IV), and encoded by pairs of genes located in three different chromosomes. Each chain is characterized by a long collagenous domain and a short non-collagenous domain (NC1). Three alpha chains are arranged to form a triple helical molecule and these triple helical type IV collagen molecules comprise the GBM network. The human α1 and α2 (IV) chains are encoded by the genes COL4A1 and COL4A2, on chromosome 13 [2]. The COL4A3 and COL4A4 genes encode the α3 and α4 chains of type IV collagen respectively, and have been mapped to chromosome 2 [3]. And α5 and α6 chains respectively are encoded by the COL4A5 and COL4A6 genes on the long arm of chromosome X [4,5].

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In most cases (80%), this disease follows an X-linked pattern of inheritance and the molecular defect lies in the COL4A5 gene. Most of the remaining pedigrees are usually uninformative and the pattern of inheritance cannot be deduced. A small number of families have been described for which autosomal recessive inheritance is suggested [6–8] and also some cases of male to male transmission (autosomal dominant inheritance) have been recorded. COL4A3 and COL4A4 are the genes accounting for these rare autosomal varieties of Alport syndrome [9–12]. Autosomal recessive AS may account for 10% of the totality of families with AS [6,10]. Although the molecular basis of the autosomal recessive variety of AS seems to be well established, the phenotypic information on patients with this type of AS is somewhat sparse.

We describe the clinical features of five Alport patients belonging to two families, and demonstrate that they suffer from an autosomal recessive variety of AS, based on the family pedigree and on the analysis of microsatellites from the COL4A3/COL4A4 region.

**Subjects and methods**

**Clinical data**

**Family 1.** III-2. This patient is a 38-year-old woman who was diagnosed with AS at 30 years of age. At that time the patient underwent a blood analysis and an elevated level of creatinine was discovered. The urine analysis disclosed haematuria and proteinuria and the renal biopsy was conclusive of AS. Haemodialysis was initiated at 32 years of age and she received a kidney graft 6 months later. The graft is currently working properly and there are no signs of anti-GBM antibody nephritis. She has neither deafness nor any ocular abnormality. III-1. This is a 40-year-old male who was diagnosed with AS at 32 years of age by means of a renal biopsy. He had never had any symptoms related to the disease but when his sister was diagnosed he underwent some clinical tests that disclosed renal failure, proteinuria and haematuria. He started haemodialysis 2 years later. At 37 years of age he received a kidney graft and now has normal renal function with no signs of anti-GBM antibody nephritis. He has severe deafness that requires a hearing aid, but has no ocular abnormality. The parents are healthy. Neither the parents nor other members of the family have haematuria.

**Family 2.** II-1. This is a 15-year-old boy who presented with haematuria and proteinuria at 3 years of age. At the age of 2 years a severe sensorineural deafness made the use of a hearing aid necessary. He was diagnosed with AS at the age of 8 years by renal biopsy. Now he has a normal serum creatinine and a normal GFR, with persistence of haematuria and proteinuria. No ocular lesions are evident. II-2. This is a 16-year-old girl who was diagnosed with AS at 11 years of age in a family screening after the diagnosis of her brother. By that time she had haematuria and proteinuria but the audiogram was normal. Three years later her serum creatinine was normal but she developed deafness. Now the creatinine is still normal but her osmolarity test shows a decreased concentrating ability. No ocular lesions are evident. II-3. This is an 18-year-old patient who was diagnosed with AS at the age of 13 years. At that time she presented with gross haematuria, proteinuria, and initial deafness. She underwent a renal biopsy that confirmed the suspicion of AS. She has needed a hearing aid since then. Now she is doing well and her creatinine is 0.84 mg/dl (GFR 106 ml/min/1.73 m²). The osmolarity test shows a decreased concentration ability. No platelet alterations are evident. The parents have normal renal function and urine analyses. They are first cousins.

**Haplotype analysis**

Twenty millilitres of EDTA-anticoagulated peripheral blood was removed from each family member. DNA was extracted according to the salting out method [13]. All the available members of the family were typed for the following microsatellites from the COL4A3/4 region: D2S279, COL4A3/4 DNTR, COL4A4 RFLP Hae III [10,14]. PCR amplification was performed as described in previous reports [14]. The silver staining technique was used for developing the microsatellite analysis [15].

**Results**

The results of the microsatellite analysis are shown in Figure 1. In both families all affected siblings share the same haplotypes for this region. The children from family 2 are homozygous for all markers, which is clearly a sign of consanguinity. No recombinants have been detected. In family II only one of the three markers used was informative.

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Fig. 1. Pedigree of the kindreds. Open symbols, unaffected; solid symbols, affected; half-open-half-solid symbols, carriers; squares, men; circles, females. Haplotypes from the COL4A3/COL4A4 region are shown below each symbol; microsatellite alleles: D2S279, COL4A3/4 DNTR, COL4A4 RFLP Hae III. The chromosomes carrying the mutated gene are represented in bold.
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Discussion

Autosomal recessive AS is an infrequent form of AS. The fact that the disease is inherited, usually in a dominant sex-linked manner means that clinicians are usually only familiar with this mode of inheritance, which probably leads to an underestimation of the number of autosomal recessive AS families. Only nine mutations have been described up to now in the COL4A3/4 genes, accounting for nine different probands with AS [16]. The patients were either homozygous or compound heterozygous for the mutations. Six mutations that cause a premature stop codon and a splicing mutation have been described in the COL4A3 gene, a nonsense and a missense mutation have been described in the COL4A4, and finally a missense mutation (Gly1201Ser) in the COL4A4 gene has been described by Lennik et al. in a family with benign familial haematuria [9,14,16,17]. From these data it seems reasonable to postulate that mutations in the COL4A3/4 genes are responsible for a wide spectrum of renal disease ranging from benign familial haematuria to autosomal forms of AS.

The low number of families with autosomal recessive AS that have been described have provided very little data about the phenotype of this disease. From the cases reported, the disease seems very similar to X-linked AS, but has the peculiarity that females are just as affected as males. To date the cases reported in the literature only refer to juvenile AS. Two of the affected subjects that we present in this paper have entered end-stage renal disease (ESRD) in their thirties and the other three are more than 15 years of age and have normal serum creatinine levels. This fact may change the idea that the autosomal recessive form of AS is very aggressive with affected subjects always entering ESRD in childhood. On the other hand, as has already been reported, hearing loss is not a constant feature in this entity, which is also true for X-linked AS. It is not clear if this can be related to tissue-specific splicing [18] or if it may be a late and very slow progressive phenomenon. In the series reported herein, deafness seems to be more severe in males than females for all patients. Although none of the patients reported in this paper shows ocular abnormalities, it has been described that autosomal recessive AS may be associated with ocular lesion as for X-linked AS [19].

Post-transplant anti-GMB antibody nephritis has been described in both autosomal recessive [9,17] and X-linked AS [20–22]. Four of the five autosomal recessive AS cases reported in the literature have developed post-transplant anti-GMB nephritis. In the cases reported herein, two patients underwent renal transplantation and did not present anti-GBM nephritis. Thus this phenomenon seems to occur in an unknown percentage of autosomal recessive AS transplanted patients. All the patients with autosomal recessive AS and anti-GBM antibody nephritis described so far have mutations that result in the absence of the NC domain of the COL4A3 or COL4A5 protein. Although this explanation seems satisfactory, there have been descriptions of the deletion of the whole COL4A3 gene and absence of anti-GBM antibody nephritis [23]. Thus it seems obvious that factors other than the type IV collagen are implicated in this immune response.

The genomic structures of the COL4A3 and COL4A4 genes have not been entirely identified. Therefore mutation analysis of these genes using PCR-based methods have been restricted. As a result, genetic linkage analysis with highly polymorphic markers is still the method of choice in autosomal recessive AS. In this report, the efficacy of linkage analysis was confirmed in two families with autosomal recessive AS.

We can conclude that mutations in the z3 (IV) and z4 (IV) collagen chains lead to very similar AS phenotypes as hemizygous mutations in the X-linked form, where the gene for the z5 (IV) chain is affected. This fact makes the clinical diagnosis of this infrequent form of AS rather difficult. We suggest that clinicians must consider autosomal recessive AS when they face equally affected male and female patients within a family or when there is evidence of consanguinity in a given family. The relevance of considering this type of inheritance relies in genetic counselling and in the prognosis of the affected siblings. Patients affected with the recessive form of the disease must be advised that their offspring are not at risk of inheriting the disease, as they are only carriers for the disease. On the other hand the knowledge of this type of inheritance may help the clinician, when asked to provide a prognosis of the disease. From the cases reported herein one cannot assume that the disease will inevitably be very severe, with ESRD in childhood, but it seems reasonable to advise that females will be as affected as males.

Details of the arrangement of chain assembly in type IV collagen molecules of the GBM are still not fully understood but it seems reasonable to hypothesize that the z3, z4 and z5 chains have similar functions and are possibly present in the same heterotrimers, as mutations in any of them produce very similar phenotypes. The better understanding of the molecular genetic heterogeneity of these genes will allow us to improve our knowledge of the autosomal forms of AS and will probably facilitate the molecular diagnosis of these entities.

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References

3. Mariyama M, Zheng K, Yang-Feng TL, Reeders ST. Colocalization of the genes for the alpha 3 (IV) and alpha 4
(IV) chains of type IV collagen to chromosome 2 bands q35–q37. Genomics 1992; 13: 8009–8013


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