Syndrome of the month

Alport's syndrome

Frances Flinter

Alport's syndrome (AS) is a progressive glomerulonephritis which is associated with high tone sensorineural deafness and characteristic eye signs. It accounts for 0.6% of all patients who start renal replacement therapy in Europe, and is most commonly inherited as an X linked disorder with a gene frequency of 1 in 5000. During the last six years several type IV collagen genes have been implicated in the aetiology of AS, and mutation detection studies are enabling genotype/phenotype correlations to be made, as well as facilitating carrier detection and prenatal diagnosis.

(J Med Genet 1997;34:326-330)

Keywords: Alport's syndrome; glomerulonephritis.

History

It has been recognised for more than a century that renal disease can be inherited¹ and the family which Alport described in 1927² had already been reported several times during the previous 25 years.³⁻⁶ By 1927 three generations of this particular pedigree were affected, and Alport was the first author to comment that the occurrence of "nerve" deafness in most of the patients with haematuria probably represented a specific clinical syndrome, rather than being purely coincidental. He also noted that macroscopic haematuria was the commonest presenting symptom and that males were affected more severely than females.² Subsequently many more families were described and the eponym was adopted in 1961.⁷ The term Alport's syndrome has been used extensively for patients with a variety of clinically heterogeneous hereditary nephritides, including some without deafness,⁸ and even benign familial haematuria.⁹ Only a few authors used strict diagnostic criteria to define a clinically homogeneous subgroup of families with "classical" AS.^{10,11}

Classical (X linked) Alport's syndrome

In 1988, a set of four clinical diagnostic criteria was described which enable the identification of families which are affected with the same hereditary nephritis as Alport's original family.¹² For a diagnosis of AS to be made clinically, any patient (or other affected relatives) with unexplained haematuria must fulfil at least three of the four criteria listed below (different features may occur in different

subjects within the family): (1) positive family history of macro/microscopic haematuria, chronic renal failure (CRF), or both; (2) electron microscopic evidence of Alport's syndrome on renal biopsy; (3) characteristic ophthalmic signs, that is, anterior lenticonus or white macular flecks or both; (4) high tone sensorineural deafness.

Thus an affected male with a negative family history may be diagnosed as having AS with confidence only if he has all the typical clinical signs. The clinical criteria are considered in more detail below.

(1) POSITIVE FAMILY HISTORY

The importance of obtaining detailed medical information about relatives cannot be overemphasised. In one German study only 20% of patients with some form of familial glomerulonephritis were aware of renal disease in their relatives.¹¹ The same study found that AS accounted for 50% of all familial glomerulonephritides. Suspicions may be raised by deaths in early adult life of males in previous generations, as well as by deaths during pregnancy or delivery, and deaths ascribed to "Bright's disease". Formal urine analysis, ophthalmological examination, and audiograms in first degree relatives are often useful. Adult males who are potentially affected are far more likely to yield useful clinical information than females or children.

(2) RENAL BIOPSY

Light microscopy contributes little towards a diagnosis of AS. The results are normal in children under the age of 10, and even in adult life the findings are non-specific. They may include segmental sclerosis and obsolescence, tubular atrophy, interstitial fibrosis, and infiltration by lymphocytes and plasma cells with clusters of foam cells. An experienced pathologist may recognise thickening of the glomerular capillary walls by light microscopy.¹³ Standard immunofluorescence studies are normally negative, but there may be a reduction or absence of binding to the glomerular basement membrane (GBM) of antibodies to the NC1 domain of the $\alpha 3$ chain of type IV collagen (obtained from patients with Goodpasture syndrome). It has been suggested that mutations within the X linked Alport gene (coding for the $\alpha 5$ chain of type IV collagen) cause the synthesis of an abnormal $\alpha 5(\text{IV})$ chain which

Figure 1 Electron micrograph of renal biopsy specimen from patient with Alport's syndrome. The black arrow indicates the glomerular basement membrane (GBM), which is thickened and split.

causes the failure of stable incorporation of $\alpha 3(\text{IV})$ chains, so that the antigenicity of $\alpha 3(\text{IV})$ is masked.¹⁴ Some pathologists have attempted to look at the distribution of the $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains of type IV collagen in the skin, in the hope that these would reflect the findings on renal biopsy. However, a Japanese patient has recently been found to have normal binding of antibodies to all three chains in the skin, but absent binding in the GBM (I Naito, personal communication). Overall, the diagnostic value of immunofluorescence studies in AS is probably rather limited.

Under the electron microscope various definite ultrastructural lesions of the GBM are seen^{15 16} (fig 1). Initially the GBM may appear thin, particularly in children, owing to a reduction in the diameter of the lamina densa. Within the same specimen there may be areas where the lamina densa becomes thicker and the GBM appears split. Small electron lucent areas containing dense particles may occur within the lamina densa. GBM abnormalities may be patchy, alternating with segments of normal thickness, particularly in children and adult females,¹⁷ but serial renal biopsies show the progressive deterioration.^{15 18} Segmental areas of GBM splitting are not specific to AS, but the simultaneous occurrence of extensive thickening and splitting together with the inclusion of electron lucent areas containing dense granulations appears to be characteristic of AS, even though EM renal biopsy reports may only comment that the findings are "compatible with a diagnosis of AS".

(3) CHARACTERISTIC OPHTHALMIC SIGNS

The ophthalmic manifestations of AS were first reported in 1954,¹⁹ and the characteristic triad of signs is well recognised by ophthalmologists,²⁰ but only visible using a slit lamp ophthalmoscope. Patients with progressive renal disease and eye abnormalities in the absence of hearing problems are very rare,¹⁷⁻²¹ as the deafness usually precedes any eye signs.

Figure 2 Anterior lenticonus.

Figure 3 Macular flecks.

Figure 4 Peripheral coalescing flecks.

The three characteristic features are: (1) anterior lenticonus (fig 2), (2) macular flecks (fig 3), and (3) peripheral coalescing flecks (fig 4).

Patients may have one or more of these. Some authors believe that anterior lenticonus is diagnostic of AS,^{20 22} as all the patients with anterior lenticonus whom they studied had progressive renal disease. Anterior lenticonus causes a slowly progressive axial myopia, and rarely it may progress to anterior capsular cataract, for which surgical extraction is required. (Posterior lenticonus is not specific to AS.) Occasionally, lens opacities have been described in AS, but they are not specific.

White perifoveal flecks are also characteristic of AS. They do not affect the vision and fluorescein angiography of the macula is normal. Peripheral white flecks are less com-

mon. In one study,²³ 72% of affected males and 38% of affected females had anterior lenticonus, macular flecks, or both. The eye signs are rarely detected in childhood and usually become apparent at about the time the kidneys fail. The youngest case in whom the eye signs have been found was 13 years old, and it is not worth routinely screening children under the age of 12 years. The eye signs are much more likely to be detected in an affected adult relative.

(4) HIGH TONE SENSORINEURAL DEAFNESS

The detection of sensorineural deafness in a patient with haematuria should always suggest a diagnosis of AS, even in the absence of a positive family history or the diagnostic eye signs. It is important to perform an audiogram on any patient with unexplained haematuria even if the family history is negative, as the hearing loss may be subclinical at first, although it is usually bilateral.

The deafness is often progressive during childhood, particularly in males,^{23, 24} eventually necessitating the use of a hearing aid.²⁵ The hearing loss is usually static in adult life,¹⁷ and even the most severely affected patients retain some hearing capacity.²⁶ Occasionally hearing may improve after a renal transplant,²⁷ but this may represent a non-specific improvement in deafness attributable to the treatment of uraemia.²⁸ In males the deafness is present in 83% and presents clinically at an average age of 11 years,²¹ causing an average deficit of -66 dB. Fifty-seven percent of females are deaf, with an average deficit of -50 dB, but in females the deafness is often not symptomatic until middle age.

The underlying pathology of the hearing problems is not well delineated, but electron microscopic studies have shown a multilayered basement membrane in the *vas spirale*²⁹ consistent with the abnormalities found in the GBM and the lens capsule.

Typical clinical course of an affected male with X linked AS

Sixty seven percent of males present with macroscopic haematuria during an intercurrent infection at an average age of 3½ years. The author has never known an at risk male with documented normal urine analysis at the age of 5 years to develop AS subsequently. All affected males subsequently develop proteinuria (never the other way round). Typically, deafness becomes clinically apparent at about 10 years, and the blood pressure begins to rise in the mid-teens. Renal function is usually deteriorating by 20 years, and 94% of males have abnormal renal function by 25 years. The average age of end stage CRF is 21 years, and it is unusual for males to retain normal renal function beyond the age of 30 years.³⁰

Typical clinical course in females with X linked AS

The clinical course in females is extremely variable. A few are as severely affected as males (presumably because of non-random X inactivation), but the majority are clinically asymptomatic throughout a normal lifespan.

Thirty six percent present with macroscopic haematuria at an average age of 9 years, and a further 40% are detected when they are found to have microscopic haematuria on routine urine analysis. Earlier reports have suggested a gene penetrance in adult females of 85%, implying that 15% do not even have microscopic haematuria,³¹ but other authors have found that all obligate carriers have microscopic haematuria (at least) by the age of 20 years.^{30, 32} One third become hypertensive (usually in middle age) and the risk of CRF may be as high as 15%,²³ although this figure may be artificially inflated by ascertainment bias.

Recent EDTA (European Dialysis and Transplant Association) data have suggested that for patients aged 15 to 29 years at the start of renal replacement therapy, males and females with Alport's syndrome have a superior survival to those with standard primary renal disease (S Rigden, personal communication).

Autosomal recessive AS

Autosomal recessive AS is much rarer than the X linked form, and heterozygotes often have microscopic haematuria. (It has been suggested that autosomal dominant benign familial haematuria is seen in manifesting heterozygote carriers of autosomal recessive Alport's syndrome (Lemmink *et al*, submitted for publication). Affected (homozygous) children develop chronic renal failure at a young age (often between 5 and 15 years of age), and usually have sensorineural deafness, but their eyes are often normal.³³⁻³⁵

Autosomal dominant AS

There are over 30 published pedigrees showing male to male transmission of a hereditary nephritis which has been called AS,⁸ but none would fulfil the criteria listed above.^{10, 12} Deafness is very unusual in these families, and none of the affected subjects has any of the characteristic eye signs, although a variety of non-specific eye abnormalities is described.^{36, 37} The histological evidence is also weak, with non-specific abnormalities of the GBM predominating in most cases.

Autosomal dominant hereditary nephritis is much rarer than classical X linked AS, and males and females are affected with equal severity. Macroscopic haematuria is rare and renal disease is usually diagnosed in adult life following the detection of microscopic haematuria, proteinuria, or hypertension. Chronic renal failure usually develops in middle age.³⁸⁻⁴¹

Extrarenal abnormalities

Apart from deafness and ocular signs, several other extrarenal abnormalities have been reported in association with AS. The most important is oesophageal, tracheobronchial, and genital leiomyomatosis.^{42, 43} Most case reports are French, and the phenotype is associated with a contiguous deletion involving COL4A5 (the Alport gene) and part of COL4A6, which is adjacent.⁴⁴

Macrothrombocytopenia (thrombocytopenia (TCP) and giant cell platelets) occurring in patients with AS has been reported,^{45 46} but in at least three of the reported families the TCP and renal disease segregate independently, and the association may be purely coincidental. Chance association probably also explains cases of AS with antithyroid antibodies⁴⁷ and AS with hyperprolinaemia/hyperaminoaciduria.²⁵

The type IV collagen genes

Type IV collagen, the main structural component of glomerular basement membranes, was isolated in 1971.⁴⁸ The two major polypeptide chains which associate to form a triple helical heterotrimeric molecule are $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$, and they are present in a 2:1 ratio. The genes for these two chains (COL4A1 and COL4A2) lie adjacent to each other on chromosome 13. The rarer $\alpha 3$ and $\alpha 4$ chains are coded for by a similar pair of genes on chromosome 2 and mutations here are associated with autosomal recessive AS.

Linkage studies during the 1980s suggested a gene localisation for X linked AS on Xq22.⁴⁹⁻⁵¹ In 1990 two groups described a novel type IV collagen chain, $\alpha 5(\text{IV})$, and the gene, which mapped to Xq22.2, was cloned and called COL4A5.^{52 53} The COL4A5 gene contains 51 exons, covering 240-310 kb of genomic DNA which produces a 6.5 kb transcript, making it one of the longest collagen genes described to date. Several mutations were reported in unrelated pedigrees, which cosegregated with the disease,⁵⁴⁻⁵⁶ and since then several hundred mutations have been detected. Most are unique to the individual pedigree, and the few shared mutations may be explained by a common ancestry.⁵⁷

About 10-15% of patients have a large deletion which is detectable on Southern blotting. The largest deletion identified so far involves the loss of 450 kb of DNA, only about 10 kb of which lies within the gene, the rest of the deletion extending beyond the 3' end.^{55 58} Another patient is deleted for 50 out of 51 exons, having only exon 1 (E Boye, unpublished data). A further 25-30% of patients have smaller mutations, including nonsense, missense, and splice site alterations, and the mutations reported are scattered across the gene with no particular mutational hotspot.

In spite of enormous efforts, using a variety of techniques, to define the remaining 55% of mutations, these remain elusive.⁵⁹ It is possible that mutations in non-coding segments of COL4A5 account for a significant proportion of X linked cases. Linkage studies have not suggested a second locus for X linked families, and if another gene is involved it must lie nearby.

The COL4A6 gene lies upstream of COL4A5 in a head to head arrangement,^{60 61} but only a few patients have mutations here, and always in association with a deletion in COL4A5. These contiguous deletions extend from the 5' end of COL4A5 and include just the first two exons of COL4A6 in patients with AS and leiomyomatosis, and extend further

into COL4A6 in patients with AS and congenital cataracts without leiomyomatosis (C Antignac, E Boye, personal communication). No patient with AS has been found to have an isolated mutation just affecting COL4A6.

Mutations in the autosomal collagen genes COL4A3 and COL4A4 on chromosome 2 have been described in a few cases of autosomal recessive Alport's syndrome.⁶⁰

Once a mutation has been detected in a family, accurate carrier detection and prenatal diagnosis become possible. Molecular tests have shown that clinical screening, including urine analysis, ophthalmological examination, and audiograms, are very useful in at risk relatives, as females with no clinical signs are unlikely to carry the mutation (residual risk after negative clinical screen about 2%). Several prenatal tests have been performed and linkage studies using intragenic markers are available if no mutation has been detected.

Genotype/phenotype correlations

About 3-5% of AS patients who receive a cadaver renal transplant develop anti-GBM antibody nephritis, and 40-50% of these patients have a big deletion in COL4A5 (compared with 15% of AS patients overall). The grafts are lost rapidly, despite intervention, and recurrence of the problem in subsequent grafts is common. Two point mutations have also been associated with post-transplant anti-GBM nephritis, however, so other factors must also be relevant.⁶¹ Deletions within COL4A5 may also be associated with a more severe phenotype generally, including a younger age of CRF, early deafness, and the presence of eye signs. Missense mutations and splicing errors are less likely to be associated with eye lesions, juvenile renal failure, and anti-GBM antibody nephritis⁶¹ (EC Concerted Action, unpublished data).

Gene therapy

AS has a significant morbidity and mortality, and research into the possibility of gene therapy is under way in spite of the complexities involved with a disease caused by mutations in a big gene affecting an inaccessible target organ.

The whole COL4A5 cDNA will need to be transferred, together with appropriate regulatory gene elements, into renal glomeruli. This will require knowledge about gene regulation (promoters, tissue specific enhancers), and also appropriate transfer systems which can target the glomeruli. New animal models are needed urgently as the only mammal with an X linked nephritis is the Samoyed dog, and its nephritis is atypical, with proteinuria preceding haematuria. The affected dogs also have normal eyes and normal hearing. However, in vivo experiments in rabbits and pigs have resulted in no expression of a virus (carrying the Lac Z reporter gene) after direct injection into the renal artery initially, but very high expression in the glomeruli when the experiment was repeated after the use of vasodilators. The

durability of gene expression after transfer this way is not yet known.

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