Molecular genetics of Alport syndrome: the clinical consequences

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Progress in molecular genetics has been achieved through two different approaches: functional cloning (also called classical genetics) and positional cloning (previously called reverse genetics). The two approaches have been combined in recent years in Alport syndrome.

This statement deserves further demonstration.

The primary defect in Alport syndrome has been located at the glomerular basement membrane (GBM) [1]. Characteristic ultrastructural changes of the GBM have been documented. Immunohistochemical studies subsequently showed that GBM antigenicity, involving in some way the Goodpasture antigen, was abnormal in some (but not all) Alport patients: no fixation of anti-GBM antibodies was found in Alport kidneys, whereas these antibodies bind linearly along the GBM of normal and diseased kidneys.

Type IV collagen is the major component of the basement membranes. Each type IV collagen molecule is composed of three α chains, forming a central triple helical collagenous domain, bracketed at the amino-terminal end by the 7S domain and at the carboxyterminal end by the globular or non-collagenous NC1 domain. Adjacent type IV collagen molecules interact at both extremities to form the coherent network of the basement membrane. Five α chains have been identified: two major components, α1 and α2, and three minor components, α3, α4, and α5, more recently recognized [see Ref. in 2]. The Goodpasture epitope has been more precisely located in the NC1 domain of the α3 chain [3].

It is time to return to genetics. Genetic studies have established that X-linked dominant Alport syndrome is the most prevalent form (≥85%), whereas autosomal dominant or recessive inheritance is observed in the other kindreds [4]. By linkage analysis (positional mapping) the mutant gene for X-linked Alport syndrome has been mapped on the long arm of the chromosome, in the Xq-22 region [5]. Where are the mapped genes encoding for the five α chains? The genes encoding for α1 and α2 (COL4A1 and COL4A2) have been located on the distal end of the long arm of chromosome 13 [6]. S. Reeders and colleagues have assigned COL4A3 and COL4A4 to chromosome 2 [7]. K. Tryggvason’s group has mapped the gene encoding for α5 (COL4A5) by in situ hybridization to the Xq-22 region, where the Alport locus had been located [2]. Thus COL4A5 became an excellent candidate gene for X-linked Alport syndrome, and this was established by documenting mutations in COL4A5 in Alport families [8].

Progress in molecular genetics has clinical consequences in the management of Alport families. Firstly, genetic counselling is improved by the information furnished by molecular genetics. In some families the mode of inheritance can be ascertained by DNA testing [9]. This allowed us to exclude X-linked transmission in several families.

Correct identification of carrier females in X-linked families can often be achieved. The approach differs according to whether the COL4A5 defect has already been identified within the family (in about 20% of the cases), or not. In the latter case linkage analysis using intragenic polymorphic microsatellite markers, which are informative in about 75% of the families, is performed. This is clinically important since carrier females may have only slight or intermittent urinary abnormalities, and the diagnostic value of renal ultrastructural study has not been tested in them. Adequate identification of carrier females is crucial for genetic counselling since affected males always inherit the mutant gene from their mothers. Prenatal diagnosis can also be considered if requested by the parents. A preliminary survey (M. Lévy et al., unpublished) indicates that there is some demand on the part of the families, but that knowledge of the disease has to be improved in many kindreds to better delineate the place of prenatal diagnosis.
Secondly, DNA testing has shown that families with diffuse leiomyomatosis, sensorineural hearing loss, cataracts, and progressive nephritis belong to the spectrum of X-linked Alport syndrome [10]. Indeed, in three such cases, Antignac et al. have found deletions in the 5' end of COL4A5, expanding upstream, thus suggesting that leiomyomatosis might be due to the alteration of a second gene located upstream. A third continuous gene might also be involved whose defect would explain the high incidence of congenital cataracts [10].

Thirdly, there is molecular heterogeneity in X-linked Alport syndrome as in most human inherited disorders. More than 40 different mutations of COL4A5 have been identified. Major gene rearrangements (mainly deletions) are found in about 15% of the families, whereas the remaining DNA lesions are point mutations requiring more sophisticated, time-consuming methods of detection. No clear-cut genotype-phenotype correlation has yet been established. Recently, however, identical point mutations have been recognised in unrelated families (unpublished observations). Progress in molecular genetics will probably clarify our understanding of the pathogenesis. A defect in GBM antigenicity involving the Goodpasture antigen (\(z3\) NC1 domain) and the \(z4\) NC1 domain is found by immunofluorescence in most Alport families, whereas some other families have normal antigenicity [1]. What is the relationship between the biochemical defect involving the \(z5\) chain and that affecting \(z3\)? Why is there heterogeneity among families? The current hypothesis is that absence or abnormal conformation of the \(z5(IV)\) chain secondary to a mutated COL4A5 gene affects the integration of the \(z3\) and \(z4\) chains within the type IV collagen molecule. The lack of antigenicity seems to be restricted to juvenile, severe type X-linked Alport syndrome (with end-stage renal failure in affected males at 30 years of age or less) [1].

Fourthly, although the overall results of kidney transplantation in Alport patients are satisfactory [11], a small number of patients (1–5%) develop anti-GBM nephritis of the transplant. Elimination of critical epitopes of the GBM prevents the establishment of immunological tolerance and thereby predisposes to the development of post-transplant anti-GBM antibodies. It has been shown that these antibodies are directed against the NC1 domain of the \(z3\) chain, as in Goodpasture syndrome [12]. It is not yet determined whether anti-\(z5\) antibodies also develop. This complication cannot be predicted only from the severity of the COL4A5 lesions. Severe DNA lesions can be considered as those associated with a dramatic alteration of the gene product, i.e., large deletions or minor DNA lesions such as nonsense mutations introducing a stop codon or frameshift mutations leading to a truncated \(z5(IV)\) chain.

Among three patients with post-transplant anti-GBM nephritis that we have investigated, two had large deletions of COL4A5 and one no deletion by Southern analysis. However, six patients with large deletions in COL4A5 did not develop anti-GBM disease (Antignac et al., in preparation). In the family reported by Kashlan et al. two cousins developed anti-GBM nephritis after kidney transplantation whereas two other affected male patients had uneventful kidney transplantation [13]. Furthermore, in certain patients who had anti-GBM nephritis after a first transplantation, this complication did not recur at a second transplantation [14]. In addition to the gene defect, the immune response triggered by grafting may be involved. This possibility requires further study to identify Alport patients at risk of developing this complication.

Fifthly, autosomal dominant or recessive forms of Alport syndrome are found in about 15% of the families [4]. A defect in the X-located COL4A5 is therefore not involved in these cases. In recessive families, linkage to COL4A3 on chromosome 2 has just been demonstrated (B. Chan, C. Antignac, S.T. Reeder, 2nd Int. Workshop, New Haven, CT, 1993). Identification of mutations will confirm this finding in the near future.

References

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