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EDITORIAL COMMENTARY

Diagnosis of Alport syndrome without biopsy?

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Abstract Alport syndrome (AS) is genetically heterogeneous. The gene *COL4A5* is mutated in the more frequent X-linked dominant form of the disease whereas *COL4A3* or *COL4A4* are mutated in the autosomal recessive and dominant forms. Diagnosis of AS and determination of the mode of transmission are important because of the differences in prognosis and genetic counselling attached to these different forms. Recently, promising results have been obtained in *Col4a3*-null mice, an animal model for AS, with different therapeutic trials when administered early in the course of the disease, an additional reason for making early diagnosis of AS in children. Since the identification of the molecular basis of the disease, mutation screening is theoretically the best diagnostic approach, avoiding the use or renal or skin biopsy. However, for many reasons linked to the genetic heterogeneity of the disease, the large size of the three genes and the random distribution of the mutations all along these huge genes, this method is tedious, expensive and time consuming. Moreover, its sensitivity is reduced. For these reasons, evaluation of the expression of type IV collagen chains in the skin, and if necessary in the renal basement membrane, remains a useful tool for AS diagnosis. At this time, the indication for these different approaches, which are not mutually exclusive but complementary, depends on the patient clinical presentation and family history.

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Alport syndrome (AS) is characterized by the association of progressive hematuric nephritis with ultrastructural changes of the glomerular basement membrane (GBM), sensorineural deafness, and frequently, specific ocular changes. It is caused by defects in type IV collagen, the major component of basement membranes (BM). The disease is genetically heterogeneous [1]. Mutations in the genes *COL4A3, COL4A4* or *COL4A5* encoding the α3, α4 and α5 chains of type IV collagen, respectively, are responsible for the different genetic diseases [2]. X-linked inheritance, due to *COL4A5* mutations, is the most common mode of transmission whereas the autosomal recessive and dominant forms of the disease, linked to mutations in the *COL4A3* or *COL4A4* genes, are observed in 10–15% of families in European countries [3]. The distinction is important because of the differences in prognosis and genetic counselling attached to these different forms.

Diagnosis of AS was initially based on clinical and morphological criteria. According to Flinter et al. [4], it could be made if at least three of the following criteria were present: positive family history of hematuria with or without progression to end-stage renal disease (ESRD), progressive sensorineural hearing loss, characteristic ocular changes (anterior lenticonus and/or maculopathy), and ultrastructural changes (thickening and splitting) of the GBM. Then, diffuse esophageal leiomyomatosis observed in 2–5% of X-linked AS families, and abnormal GBM and/or epidermal basement membrane (EBM) distribution of type IV collagen chains have been included as additional AS criteria [3, 5]. But finally, whatever the number and value of clinical and morphologic criteria, identification of the mutation is decisive for the diagnosis of the disease. This diagnostic gold standard being theoretically available, Slajpah et al., in

this journal, recommend to do genetic analysis in the initial diagnosis of AS in young patients, an approach preventing the use of "invasive skin and renal biopsies" [6]. However, this assertion, based on the analysis of children belonging to X-linked families, is debatable. Indeed, from a practical point of view, the diagnostic approach of AS depends on the patient clinical presentation and family history, the laboratory availability, and also the limits of molecular diagnosis which, for many reasons including cost, time-consuming, and reduced sensitivity, has proven to be unable, until now, to solve all diagnostic problems.

The propositus may belong to a large informative family in which the diagnosis of AS has been previously established on the presence of the classical criteria, and the X-dominant mode of transmission ascertained by the vertical transmission, the severity of the disease in affected males and the absence of father to son transmission (Figure 1: Family 1). This presentation was that of the young hematuric patients studied by Slajpah et al. [6]. In individuals, such as the female patient III-6 presenting with hematuria, the highly likely diagnosis of AS may be easily confirmed by the finding of ocular or hearing defect, or, if mutation screening cannot be done, by linkage analysis showing the co-segregation of the disease with markers within or flanking the *COL4A5* gene. Determination of the status of at-risk asymptomatic individuals (for example the asymptomatic female patient III-4), for genetic counseling or in the objective of kidney donation, and prenatal diagnosis of affected offspring, based on linkage analysis, are also possible in this family, without any skin or renal biopsy. The situation is nearly similar if the propositus belongs to a recognized autosomal recessive AS family characterized by parental consanguinity and similar progression to renal failure in male and female siblings (Figure 1: Family 2). Linkage studies to the *COL4A3-COL4A4* locus allow determination of the heterozygous or homozygous status of at-risk hematuric individuals belonging to the affected sibship (Pts II-5 and II-7), identification

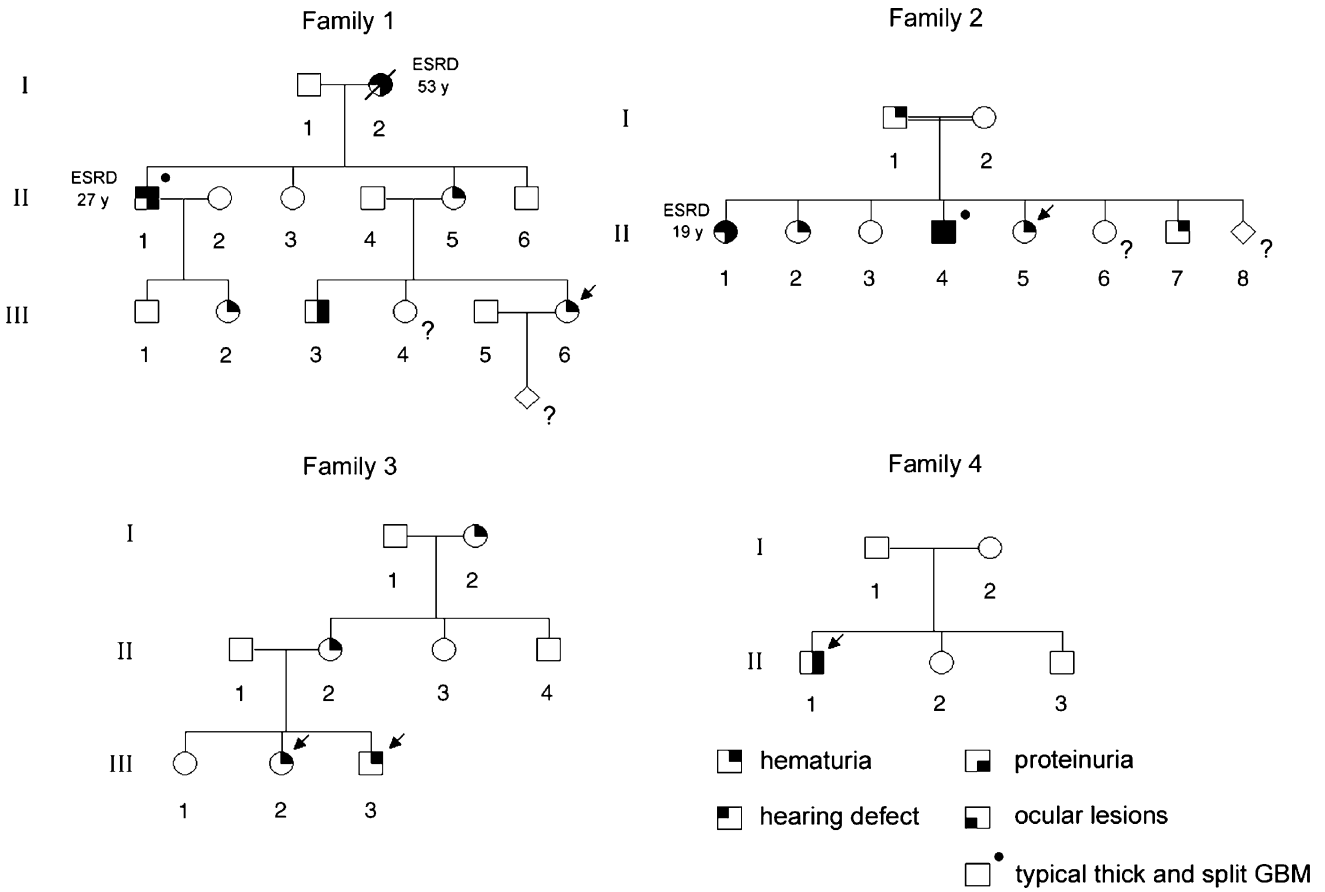

Fig. 1 The diagnostic strategy of AS in hematuric individuals has to take in account clinical and family data. In family 1 with known X-linked dominant AS, diagnosis of the disease in individual III6 may be easily confirmed by linkage analysis and/or mutation screening. The same is true for individual II5 in family 2 with established autosomal recessive AS. In both families, molecular genetic diagnosis of at-risk individuals, and prenatal diagnosis are possible. There is no need for biopsy. In family 3 with dominant transmission of isolated hematuria, the first diagnostic step should be skin biopsy for analysis of α5(IV) chain distribution in the epidermal basement membrane. In family 4, diagnosis of AS in patients II1 presenting with sporadic hematuria/proteinuria will be based on renal biopsy allowing immunohistologic and electron microscopic analysis of the GBM

of asymptomatic heterozygotes for genetic counselling, and prenatal diagnosis.

Frequently, however, the situation is not so simple. For example in family 3 (Figure 1), the young male proband (Pt III-3), his sister, mother and grandmother are hematuric, and careful examination failed to detect any extrarenal abnormality. Different diagnosis with opposite prognostic significance, especially for the propositus, may be discussed. Theoretically, the solution should be given by molecular analysis: linkage to and/or mutation in the *COL4A5* gene, if demonstrated, assert the diagnosis of X-linked AS. But linkage to or mutation in the *COL4A3* or *COL4A4* genes in the heterozygous state does not discriminate between familial benign hematuria and autosomal dominant or recessive AS (with hematuria in the heterozygous carriers), because of the large phenotypic heterogeneity associated with these gene mutations [7, 8]. If molecular analysis is unavailable or non informative, precise diagnosis may be achieved by skin biopsy if showing abnormal EBM distribution of the α5 chain of type IV collagen, a feature specific for X-linked AS, or by renal biopsy, allowing electron microscopic and immunohistologic examination of the renal BM. Regular follow-up examination of the young hematuric patients is another reasonable option, the occurrence of proteinuria or extrarenal symptoms leading to further diagnostic investigation.

In some patients, the glomerular disease is apparently sporadic, even after screening for hematuria of first-degree relatives. The early occurrence (before the age of 3 years) of persistent hematuria of glomerular origin, or the detection of hearing defect or eye abnormalities of the AS series, makes the diagnosis of AS highly probable. The confirmation could come from gene sequencing, the three AS genes being possible candidates in the absence of any family data. Alternatively, analysis of skin or renal basement membrane expression of type IV collagen chains may be done first and direct mutation screening. Moreover, in presence of sporadic persistent hematuria associated with proteinuria (Figure 1: Patient II-1 in family 4) without any symptom suggesting the diagnosis of AS, renal biopsy is the first investigation to be proposed because several diagnosis may be discussed, and no laboratory would accept to sequence the three AS genes in this situation. Examination of the renal tissue allows the exclusion of other hematuric glomerular diseases, the most frequent being IgA nephropathy, and the identification of AS lesions. In addition, immunohistochemical distribution of the α3, α4 and α5(IV) chains may orient toward the gene defect and the mode of transmission of the disease [9].

So what is the actual place of molecular analysis in the diagnosis of AS? Obviously at this time, with the exception of linkage analysis in informative families, mutation screening is not used on a large scale. The reasons are multiple. AS is genetically heterogeneous and the three AS genes, *COL4A3, COL4A4* and *COL4A5* are huge, containing 48 to 53 exons. Every exon has to be examined because there is no "hot spot" of mutation, most of them being private. The screening in most laboratories is based on single-strand conformation polymorphism (SSCP) analysis after PCR amplification of each exon followed by the sequencing of the abnormal one. This analysis is tedious, expensive and time-consuming. Very few laboratories in the world are able to take it in charge. Moreover, its sensitivity is rather low: according to the different laboratories using this technique, only 45 to 65% of mutations are identified. The detection rate may be higher when more sensitive methods are used: direct DNA sequencing, or RT-PCR and direct sequencing using *COL4A5* mRNA from culture skin fibroblasts [10, 11]. But these techniques are also long and expensive, not readily available for routine diagnosis. Recently, Torra described a new mutation screening strategy based on hair-root RT-PCR amplification of the complete *COL4A5* coding sequence followed by direct sequencing [12]. This simple, faster and more efficient method may change the diagnostic approach of X-linked AS by allowing rapid identification of *COL4A5* mutations. However, this method is not applicable to the screening of *COL4A3* or *COL4A4* gene which are not expressed in the skin. New molecular technologies have to be developed for allowing easier faster and less expensive screening of the AS genes.

Because of the present limits of mutation screening, immunohistochemistry of the skin (and when necessary immunohistochemical and ultrastructural analysis of renal BM) remains a precious diagnostic approach. Skin biopsy is less invasive and easier than renal biopsy. It gives faster results than molecular analysis, with the same sensitivity. Abnormal expression of the α5 chain of type IV collagen in the epidermal BM has been reported to be detected in 50-60% of X-linked AS patients, the most frequent genetic form of the disease, whereas the expression is always normal in autosomal AS [9, 13, 14]. In our experience, 18/24 children with identified *COL4A5* mutation had total or focal (in females) absence of the α5(IV) collagen chain in the skin BM. The anomaly was found in the 12 males with nonsense mutations or insertions/deletions changing the reading frame of the gene. Moreover, diagnosis of X-linked AS was unequivocally established in 20 additional patients, in 7 of which mutation screening was inconclusive. These data clearly show that genetic analysis and skin immunohistochemistry are not mutually exclusive but are complementary methods for attaining diagnosis and determining the mode of transmission of the disease. In our department, skin biopsy is often the first step in the diagnostic approach of AS in children. Once the diagnosis is asserted, pedigree and linkage analyses allow one to answer most of the important questions raised by this hereditary disease:

recognition of heterozygote carriers, genetic counseling and if there is a demand, prenatal diagnosis.

According to Pirson et al. [5], "recognition of the disease and identification of its mode of transmission have significant implications in terms of prognosis and genetic counseling of the patient and the extended family". Precise information on the disease, the gender-dependent rate of progression, and the mode of transmission should be given to the patient and/or family to assure appropriate use of prenatal testing [15]. However, in young children, is it ethical to make an early diagnosis of AS? The question is strongly debated because of the possible psychosocial consequences of the diagnosis, and the absence of effective treatment [16]. Actually, making the diagnosis of AS may result in psychological problems in the child and the family. But, contrarily to asymptomatic adults belonging to recognized AS families in which this diagnosis may be done with the purpose of kidney donation or genetic counselling, without any mental reservation, it is usually not proposed for asymptomatic children. It is discussed in children presenting with persistent microscopic hematuria frequently associated with episodes of gross hematuria, a source of permanent anxiety in patients and families leading to the demand of a precise diagnosis.

Until now, only a few therapeutic trials have been reported in AS patients, and none of them have unequivocally demonstrated their efficacy in term of prevention of the progressive course of the disease. In the study of Proesmans et al. [17] including 10 pediatric patients with proteinuria of at least 350 mg/24 h, and normal creatinine clearance at onset, angiotensin converting enzyme (ACE) inhibition transiently reduces urinary protein excretion but its reno-protection effect was not demonstrated. Beneficial long-term effects of cyclosporin administration has been reported by one group, based on the 8.4-year survey of eight AS patients having persistent proteinuria (46 to 207 mg/h) with reduced creatinine clearance (43.2 and 46 ml/min/1.73 m^2) in two of them, at onset of treatment: no deterioration in renal function and no aggravation of the histological lesions were observed at the end of the study [18]. Similarly, in Samoyed dogs with X-linked AS, high dosage of cyclosporin given from one month of age, slows the clinical and pathological progression of the disease, a beneficial effect observed despite significant increase in urinary protein excretion [19]. These positive results were not confirmed in two recent studies including 9 and 14 AS patients respectively, presenting with proteinuria, and normal renal function in most of them, at the initiation of cyclosporin treatment [20, 21]. Moreover rapid occurrence of nephrotoxicity was observed in seven of the nine patients reported by Charbit et al. [20], precluding the long-term use of the drug in AS patients. These results are disappointing, but, based on recent therapeutic methods tested on animal models, we can now expect that the diagnosis of AS will have therapeutic impact in the near future. In the *Col4a3*-null mice which develop proteinuria and renal lesions at eight weeks of age, and ESRD at 14 weeks, gene therapy by transgenesis of YAC carrying the human *COL4A3-COL4A4* locus, rescues the Alport phenotype [22]. This technique is not readily applicable to humans, but different pharmacologic approaches using metalloprotease inhibitors [23], vasopeptidase inhibitors (blocking the action of both ACE and neutral endopeptidase) [24], or antagonist of chemokine receptors [25] appear to be effective as judged by survival, proteinuria, renal function and histological changes, if they are administrated early in the course of the disease. Lastly, it has been shown that transplantation of wild-type bone marrow derived stem cells results in recruitment of wild-type podocytes and mesangial cells in damaged glomeruli, partial restorationof α3(IV) and consequently α4 and α5 (IV) expression in the GBM, and improved glomerular architecture and function [26, 27]. Globally these results are promising, and similar trials in humans may be developed in the near future. Because most of the beneficial effects obtained in AS mice are observed only if the treatment is administered before the development of proteinuria and severe GBM changes, it is now mandatory to recognize Alport syndrome early in the course of the disease. This is facilitated by an integrated approach to diagnosis.

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