Familial haematuria: when to consider genetic testing

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ABSTRACT

Haematuria is a common finding in children. It is important to identify the underlying cause whenever possible so that appropriate follow-up is organised, particularly if the child is at risk of developing renal impairment or renal failure in later life. Until recently nephrologists relied on renal biopsy with examination under the electron microscope to make a diagnosis, but genetic testing can often provide an answer, together with additional information about the pattern of inheritance, which is also useful for other family members.

FAMILY HISTORY

The reported incidence of microscopic haematuria is up to 1%.[1] Thirty to 50% of children with isolated, persistent haematuria have a familial condition, most frequently Alport's Syndrome.[2 3] It may not be possible to take a detailed three generation family history in every paediatric out-patient clinic, but it is always worth asking if there is a family history of kidney problems, especially if these have occurred in relatively young people. Clinicians should also enquire specifically whether the affected individuals are deaf, as this strongly suggests the possibility of an underlying diagnosis of Alport's syndrome (AS). Affected relatives in previous generations may not have had a formal diagnosis, but a history of early onset renal failure (late teens or early twenties) coupled with deafness, especially in males, is highly suspicious even if, at the time, it was diagnosed as Bright's disease, focal segmental glomerulosclerosis (FSGS), or some other non-specific nephritis. Urinanalysis is indicated for the parents and siblings of any child found to have haematuria.

ALPORT'S SYNDROME

AS is the commonest form of hereditary nephritis, with a gene frequency of between 1 in 5000 and 1 in 10 000. It accounts for 1–2% of end-stage renal failure.[4] AS is caused by defects in type IV collagen; the α 5 chain combines with α 3 and α 4 chains to form a large trimer which forms part of the complex protein network found in basement membranes in the glomerulus, the cochlear, and the lens and retinal pigment epithelium in the eye. The distribution of type IV collagen accounts for the clinical manifestations of AS: renal failure, deafness and eye signs.

Clinical diagnostic criteria for AS were suggested in 1988.[5] Any patient presenting with unexplained haematuria in whom three of more of the following also apply (in either the proband or a close affected relative) can be diagnosed clinically as having classic AS:

- positive family history of macro/microscopic haematuria, chronic renal failure, or both
- electron microscopic evidence of AS on renal biopsy
- characteristic ophthalmic signs (anterior lenticonus/macular flecks)
- high-tone sensorineural deafness.

The typical presentation of AS is a male with persistent microscopic haematuria from birth or early infancy, which becomes intermittently macroscopic during intercurrent infections. Males with X-linked AS usually have proteinuria by the age of 10 years, and develop progressive renal impairment, leading inevitably to renal failure, typically in the early twenties, although early treatment with ACE inhibitors can slow the decline in renal function. AS progressing to renal failure in early adult life is less common in females (because the majority of cases are X-linked); as a consequence, it may take longer for affected females to be diagnosed.

DEAFNESS

Progressive hearing loss affects 80% of patients with AS, often starting in the second decade, but it usually plateaus in adult life, so some hearing is retained. It is worth considering an audiogram in any child with haematuria if they are more than 10 years old, to screen for high-tone sensorineural deafness. The hearing loss is progressive for frequencies over 3000 Hz, and aiding is beneficial, but it can be difficult to persuade teenagers to wear their aids consistently in order to gain maximum benefit from using them. As deafness may precede symptoms of renal disease, all children with deafness of unknown cause (excluding congenital deafness) should have their urine tested for haematuria. Children in whom the diagnosis of AS has been made should have audiological assessments from 5 years.

EYE SIGNS

The characteristic ocular changes seen in some affected individuals include anterior lenticonus and retinal flecks. Some authors regard these features as pathognomic, and if they are present it may not be necessary to embark on additional tests in order to make a clinical diagnosis; however genetic testing additionally determines the pattern of inheritance and enables cascade testing of relatives who may be at risk. These eye changes develop in late childhood or early adult life, and can be visualised using a slit lamp ophthalmoscope. They rarely cause visual loss, but refractory errors are common. They are seen in 44–72% of males and 15–38% of females with X-Linked AS,[5–7] and in 87% of patients with autosomal recessive AS (ARAS).[8] Anterior lenticonus, where the lens bulges forwards through the thinned lens capsule, causes refractory errors, which may fluctuate, while the retinopathy,

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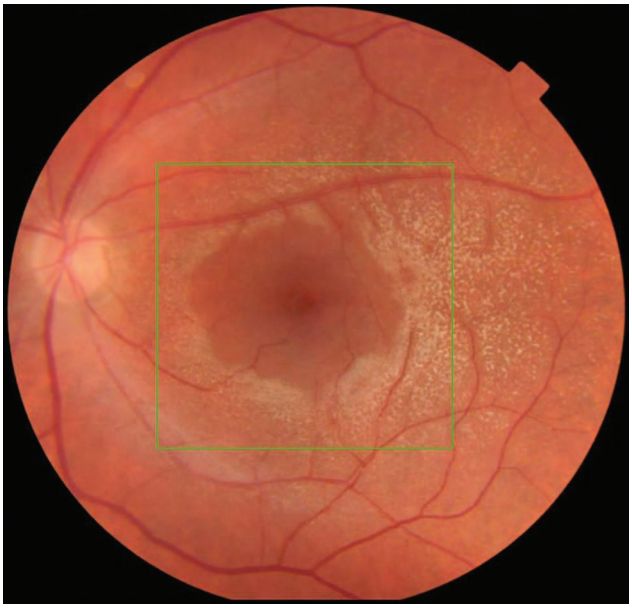

Figure 1 Retinal photograph from a patient with Alport's syndrome showing an abnormal left macula, with an abnormal foveal reflex ('lozenge sign') and surrounding 'dots and flecks'. Image kindly provided by Mr Moin Mohamed.

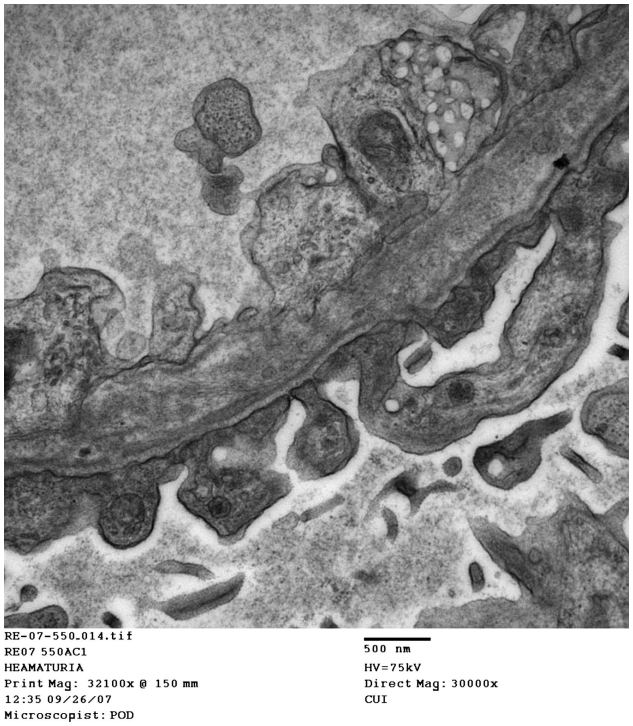

Figure 2 Electron micrograph of a glomerulus from a kidney in a patient with Alport's syndrome. Note the extensive thickening and splitting of the glomerular basement membrane. Image kindly provided by Dr Catherine Horsfield.

consisting of white or yellow macular flecks, is not associated with visual abnormalities (figure 1). An ophthalmology review, especially performed by someone with experience of AS, may be helpful in young people of 15 years or more, although the eye signs may not appear until adult life. If a boy's mother has haematuria, it is worth asking the ophthalmologist to have a look at her eyes at the same time, as she may have ophthalmological features of AS if she is a carrier of the X-linked (XL) disease, providing an important diagnostic clue.

RENAL BIOPSY

Until recently, renal biopsy, with electron microscopy, was a routine part of the investigation of a child with unexplained haematuria in whom AS or Thin Basement Membrane Nephropathy (TBMN) was suspected, but this has now largely been replaced by genetic testing looking for mutations in the type IV collagen genes.

The characteristic features seen in renal biopsies from patients with AS viewed under the electron microscope include variable thickening and splitting of the glomerular basement membrane, with lamellation giving a 'basket weave' appearance, and interposition of electron lucent areas containing dense particles (figure 2).

Renal biopsy is an invasive, expensive procedure, however, associated with risks of bleeding and infection, and requiring general anaesthesia in younger children. As the histopathological features of AS evolve with time, renal biopsy in childhood may not be diagnostic, particularly in younger children. Carriers of AS have predominantly thin glomerular basement membranes. In some countries, skin biopsy with staining for α 5 type IV collagen is used diagnostically, but the false negative rate is significant and immunohistochemical studies on renal tissue and skin from the same patient may give inconsistent results.

INHERITANCE

AS is inherited as an X-linked condition in 85% of families. Female carriers nearly always have microscopic haematuria (95%), and if they have a kidney biopsy it may only show thin glomerular basement membranes unless renal impairment is present. Up to 30% of carriers of X-linked AS develop renal impairment over the age of 40 years, with about half of them reaching end-stage renal failure over 60 years of age, although these figures may be skewed by ascertainment bias. Twenty-eight per cent develop hearing loss, but later than in affected males.[7]

ARAS accounts for most of the remaining 15% of patients in whom a genetic diagnosis is confirmed molecularly. These patients have similar clinical findings to males with X-linked AS.

The risk of renal impairment in female carriers of X-linked AS and male/female carriers of ARAS is similar, and it is now recognised that all carriers of AS should be offered lifelong follow-up with monitoring of their blood pressure, and screening for proteinuria on an annual basis.

Dominantly inherited AS with typical extra renal features is very rare, accounting for <5% of cases of AS, and there is some debate as to whether or not it really exists. This is largely a question of semantics: we know that carriers of a single *COL4A3/4* mutation have thin glomerular basement membranes and an increased risk of hypertension and renal impairment, but most do not develop sensorineural deafness or the characteristic eye signs. They do not, therefore, fulfil the clinical diagnostic criteria for AS; but carrying a mutation in an autosomal type IV collagen gene undoubtedly acts as a predisposing factor for renal impairment. We do not know what other genetic variants and/or environmental factors may be required to precipitate more significant renal involvement, and labelling these patients as having autosomal-dominant AS may be confusing. Their offspring have a 50% chance of inheriting the familial mutation, but the risk of these children developing the clinical features of classic AS is small.

TBMN typically presents with asymptomatic microscopic haematuria, and the glomerular basement membrane is diffusely thinned. It has been considered the cause of so-called 'benign familial haematuria', and accounts for 15% of children biopsied for haematuria[3]; however, half these patients will be carriers of XLAS or ARAS, and the prognosis of their disease is not necessarily benign.

DIFFERENTIAL DIAGNOSIS

It is important to exclude common or serious conditions, such as urinary tract infection or renal tumours with urine culture and renal ultrasound examination. Other familial causes of haematuria are listed in table 1. Most of these conditions will have features suggestive of the diagnosis on history and examination.

Non-familial causes of haematuria also include other glomerulonephritides, such as postinfectious, ANCA-positive and Henoch-Schonlein nephritis, haemolytic uraemic syndrome, trauma, obstructive uropathies, vascular malformations, Nutcracker syndrome, infections such as malaria, schistosomiasis and tuberculosis, exercise and drugs.

GENETIC TESTING

Genetic testing for collagen IV defects should be considered in any child with unexplained haematuria. If proteinuria or hearing loss is present, together with a positive family history, then AS is the most likely diagnosis. As 10–15% of cases with X-Linked AS occur as the result of new mutations, the family history may be negative, and this should not deter clinicians from considering genetic testing. Identification of the genetic mutation(s) in a patient confirms the diagnosis, allows more informed advice to be given about prognosis, enables testing of other relatives, and facilitates genetic counselling and discussion about the possibility of prenatal diagnosis or preimplantation genetic diagnosis, if appropriate.

If genetic testing is negative or inconclusive, then renal biopsy is indicated unless the diagnosis can be confirmed on family history or by ocular examination in the patient or relatives with haematuria.

EFFECTIVENESS OF MUTATION SCREENING

A review in 2011 demonstrated the value of the clinical diagnostic features in identifying patients with X-linked AS: the *COL4A5* mutation detection rate in patients fulfilling zero, one, two, three or four diagnostic criteria was 0, 18, 64, 89 and 81%, respectively.[9] Subsequent analysis of the autosomal collagen genes has shown that most patients who did not have a *COL4A5* mutation despite having three or four diagnostic criteria, in fact have autosomal recessive AS.[10] X-linked AS is significantly more common than autosomal forms of the disease, so the *COL4A5* gene is usually screened first, unless parental consanguinity or early presentation in a female suggests that ARAS may be more likely.

Mutation screening in the collagen genes *COL4A3*, *COL4A4* and *COL4A5*, which code for the α 3, α 4 and α 5 chains of type IV collagen, respectively, is now routinely available to nephrologists and clinical geneticists. Haematuria is 100% sensitive for the presence of a type IV collagen mutation. Eighty-five per cent of patients in whom a type IV collagen mutation is found have X-linked AS with a mutation in *COL4A5*. Mutations in the *COL4A3* and *COL4A4* genes on chromosome 2 are found in the remaining 15% in whom a molecular diagnosis is possible, and these are usually recessively inherited. The cost of genetic tests is likely to fall rapidly with the introduction of next-generation sequencing technologies. Until recently, clinicians had to specify which genes they wanted sequenced first, as the tests were expensive and done consecutively; but the imminent move to next-generation sequencing will mean that all three genes can be sequenced simultaneously, together with *COL4A6*; (contiguous deletions involving a variable proportion of *COL4A5*, plus the first two exons of *COL4A6*, are associated with X-linked AS together with leiomyomatosis). Simultaneous screening of all relevant genes will speed up diagnosis and costs will fall. Some patients have coexisting mutations in the XL and the autosomal genes, and our relatively simplistic understanding of isolated single gene disorders is evolving as we identify more families in which mutations in several genes play a role in the underlying pathology. Mutations in *MYH9*, non-myosin heavy chain IIA on chromosome 22q, cause four clinical conditions with similar manifestations: May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome characterised by macrothrombocytopenia and variable renal involvement; these are extremely rare.[11] As collagen renal gene panels are developed for haematuria/AS, it is likely that other potentially relevant genes may also be included in addition to *MYH9*, for example, *NPHS2*, *NPHS1*, *UMOD*, *INF2* and *CFHR5*.

The most important diagnosis to make or exclude in a child presenting with familial haematuria is AS, and genetic testing, rather than a renal biopsy, is indicated in any child in whom this clinical diagnosis is a possibility. Even at a young age, and in the absence of a family history or any extrarenal features, genetic testing may still be indicated, particularly if it means that a renal biopsy can be avoided, as deafness or ophthalmological manifestations may only develop later.

Inevitably genetic testing cannot always give a definitive result. Most mutations or variations identified in the *COL4A3/4/5* genes are novel and may be unique to a particular family, so molecular biologists have to try and determine the likely pathogenicity of any variant that is identified. In some cases it is also necessary, on a research basis, to do further studies looking at the effect of a specific variant on the production of RNA extracted from hair root cells or a skin biopsy, before a call can be made as to the likely pathogenicity of a variant. DNA variant databases exist and are increasingly helpful resources for interrogation, but they rely on scientists being willing to submit their own data, together with phenotype information, in order to be really useful.[12]

Table 1 Other causes of familial haematuria

Cystic kidney disease	Autosomal-dominant polycystic kidney disease Autosomal-recessive polycystic kidney disease
Familial glomerulopathies	IgA nephropathy Familial C3 nephropathy (Complement Factor H-related 5) Familial lupus Familial FSGS Fibronectin 1 mutations (FN1)
Renal calculi	Familial hypercalciuria Primary hyperoxaluria Familial juvenile hyperuricaemic nephropathy Cystinuria
Haematological	Sickle cell disease or trait Haemophilias, von Willebrand's and other bleeding disorders
Neurocutaneous syndromes	Tuberous sclerosis
Interstitial diseases	Familial tubulo-interstitial nephropathy

FSGS, focal segmental glomerulosclerosis.

THE IMPORTANCE OF DIAGNOSING AS

The diagnosis of AS is important for many reasons. It is recognised that early therapy with ACE inhibitors improves the prognosis and may extend the life of the native kidneys by up to 13 years, as well as increasing life expectancy.[13] Proactive testing for the presence or absence of a familial mutation is indicated in every first-degree relative of a patient in whom a mutation is identified, so that affected individuals are diagnosed and referred to nephrologists early (preferably presymptomatically) and carriers identified.

Carriers are also at increased risk of hypertension and renal impairment, so they should also be offered regular, lifelong screening to check their blood pressure and test their urine for blood and protein. Carriers of XL and ARAS also benefit from the early use of ACE inhibition,[14] and should have at least an annual check up.[15] It is probably no longer appropriate to refer to carriers of ARAS, who have thin glomerular basement membranes, as having 'benign familial haematuria' now that we know that their prognosis is not always benign.

Some couples who know that they are at increased risk of having a child with AS may chose to have prenatal diagnosis or preimplantation genetic diagnosis, but this is usually only possible if the precise mutation(s) is known in their family.

HAEMATURIA IN POTENTIAL KIDNEY DONORS

Isolated haematuria is common in the general population and is sometimes identified in people who have volunteered to give a kidney, either to a relative or altruistically. Investigation is indicated to exclude urological disease and to identify glomerular pathology that would preclude donation. Glomerular pathology in potential live donors has been identified using thresholds as low as 1–3 red cells/μL.[16] Thin basement membrane disease is present in 10–50% of patients biopsied for persistent asymptomatic non-visible haematuria and although often considered to be benign, proteinuria has been described in 10–20% of patients and renal impairment in 5%. Genetic testing for *COL4A3/4* mutations is warranted, followed by *COL4A5* if no mutation is found in the autosomal genes. Potential donors who carry a type IV collagen mutation are not ideal candidates, but when there is no realistic alternative they may be very keen to proceed. It is essential that they are counselled very carefully and understand the possible risks to their own health in the future, but if they have normal blood pressure and no proteinuria, and are >45 years old nephrologists may consider that the risks are acceptable.[17] A report of six carriers of XL AS who donated kidneys to their affected children showed a decline in kidney function of 25–60% in four of the six donors over 2–14 years of follow-up, although in no case was creatinine clearance <40 mL/min.[18]

CLINICAL USEFULNESS OF GENETIC TESTING

There are many reasons why genetic testing may be useful in patients with familial haematuria (box 1). A clinical utility gene card for genetic testing in AS has been published and summarises the spectrum of mutations found in each of the relevant collagen genes.[19] The analytical sensitivity (proportion of positive tests if the genotype is present) is quoted as >95% for *COL4A5* sequencing of genomic DNA with multiplex ligation-dependent probe amplification (MLPA) and >95% for *COL4A3/4* as well; this figure may change as labs move from Sanger sequencing to next-generation sequencing. The analytical specificity (proportion of negative tests if the genotype is not present) is also >95% for all three genes. The clinical sensitivity (proportion of positive tests if the disease is present) is dependent on variable factors, such as age and family history, but is highest in families fulfilling three or more diagnostic criteria. Clinical specificity (proportion of negative tests if the disease is not present) is almost 100% in males by the age of 20 years and approaches 100% in males and females by the age of 20 years for autosomal recessive forms. The positive clinical predictive value (lifetime risk of developing the disease if the test is positive) is almost 100% with respect to end-stage renal disease in males for XL AS and 30–40% in females with XL disease, although this figure may be skewed by ascertainment bias, and does not reflect benefits from the recent recommendations in the use of ACE inhibition. It is also nearly 100% for autosomal recessive disease. Finally, the negative clinical predictive value (probability of not developing the disease if the test is negative in a family in which a mutation has been identified) is almost 100% for all forms of AS.

Box 1 Uses of genetic testing in familial haematuria

- To diagnose/exclude Alport's syndrome and prompt early intervention with ACE inhibitors.
- To diagnose/exclude carrier status of Alport's syndrome.
- To establish the mode of inheritance and hence the risk to relatives.
- To enable genetic testing of any unaffected relative who wishes to act as a kidney donor.
- To help predict the phenotype: for example, early or later onset chronic renal failure if the mutation has been reported previously.
- To facilitate prenatal diagnosis or preimplantation genetic diagnosis, if requested.
- To predict the risk of antiglomerular basement membrane disease post-transplantation if there is a large *COL4A5* deletion.

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