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Somatic mosaicism associated with a mild Alport syndrome phenotype

EDITOR—Alport syndrome (AS) is a hereditary nephritis characterised by haematuria, proteinuria, and chronic renal failure associated with progressive high tone sensorineural deafness and characteristic eye lesions (macular flecks and anterior lenticonus[1]). At the molecular level, X linked AS, which is the most common form, is caused by mutations in *COL4A5*, a type IV collagen gene expressed in the glomerular basement membrane of the kidney.[2] Mutations in *COL4A5* cause progressive kidney damage usually leading to renal failure in affected males in early adulthood (20-25 years, juvenile form). A small proportion of *COL4A5* mutations cause a later onset form of AS with ESRF in males at >31 years, although nephritis is apparent much earlier than this. Heterozygous females are generally mildly affected and often do not develop renal failure. We report here the identification of three apparently mosaic parents (two mothers and one father) of affected subjects. In the case of the mosaic father, an unusually mild AS phenotype was observed which may be a consequence of his mosaicism.

The *COL4A5* gene is composed of 51 exons spread over 250 kb of genomic DNA, which generate a 6.5 kb transcript encoding a 1685 amino acid protein[3] and has been the subject of several large mutation studies.[4–6] We have recently completed a screen of all 51 exons of *COL4A5* in 153 patients with suspected X linked AS using single strand conformation polymorphism (SSCP) analysis followed by direct sequencing of fragments showing mobility shifts, and have identified mutations in 77 of these families.[7] Where samples were available, other family members were analysed by SSCP both to provide accurate carrier diagnosis and to estimate the proportion of de novo mutations. In total, the mothers of 25 affected males and both parents of three affected females were screened, showing five de novo mutations and three instances of mosaicism.

In patient 15, AS is caused by a mutation in exon 26, 2208G→C, which changes glycine 669 to alanine and interrupts the Gly-X-Y repeat structure of the collagen triple helix.[7] The proband is an 11 year old male who presented with haematuria at 9 years, but has no reported deafness or eye lesions and has not yet developed renal failure. His mother, who also has haematuria but no other signs, was found by SSCP analysis to possess a reduced amount of the mutant allele and this was confirmed using DNA extracted from a second blood sample (fig 1A). As an additional control, an equimolar mixture of DNA from her son and an unrelated, unaffected male was analysed to show that the two alleles amplified equally (data not shown). DNA from both maternal grandparents has also been analysed and neither possesses the mutation (fig 1A). On the basis of these results we can be unequivocal that the mutation causing AS in this family arose somatically in the mother.

A splice site mutation (849-3c→a), which results in the in frame skipping of exon 12, causes AS in patient 47.[8] This man was diagnosed with haematuria and a renal biopsy showed irregular thickening and splitting of the glomerular basement membrane typical of AS before he developed end stage renal failure at the age of 17 years. He shows no loss of hearing or characteristic eye signs. His mother (who

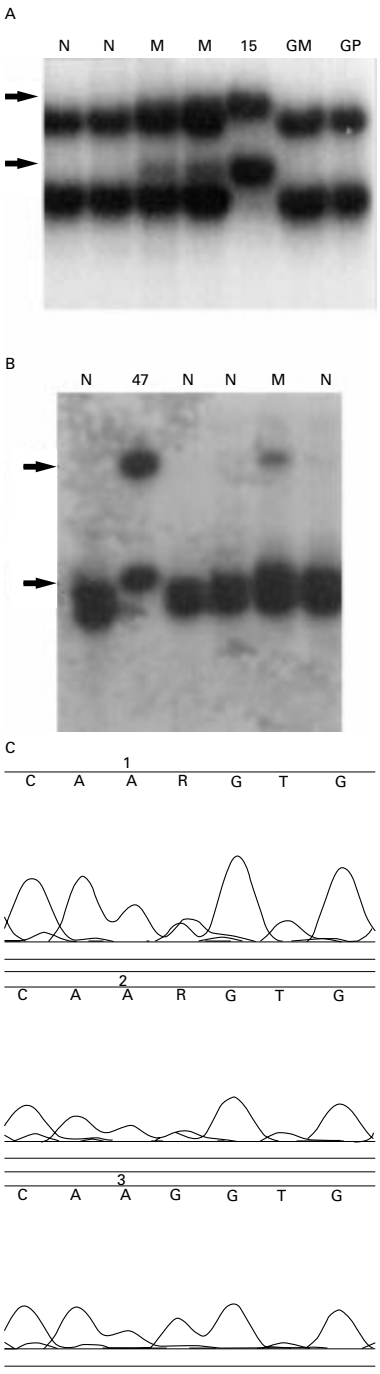

Figure 1 Somatic mosaicism in AS. Mosaicism is shown by SSCP analysis in (A) the mother of patient 15 using oligonucleotides flanking exon 26 (taccattgatttactcttgc and aataaattcctcacatac) and (B) the mother of patient 47 using oligonucleotides flanking exons 11 and 12 (ttgtcttctcttcttagg and gctctctttctttactcac). SSCP analysis has been described previously.[7] Briefly, genomic DNA was extracted from peripheral blood samples and internally labelled PCR products were generated and separated on an 8% polyacrylamide/5% glycerol gel. Proband samples are numbered, M indicates maternal samples, and N indicates normal controls. In the case of patient 15, the maternal grandmother (GM) and grandfather (GP) were also analysed. (C) In the father of patient 11, mosaicism was confirmed by direct sequencing of exon 25 using an ABI 377 automated sequencer. Top panel shows sequence from patient 11 (female heterozygote for 2114G→A, labelled R); middle panel shows father, presumed mosaic; bottom panel is a normal control.

does not have haematuria) possesses a very small amount of the mutated allele (fig 1B), while his maternal grandmother does not carry the mutation at all. It seems likely, therefore, that the mutation causing AS in patient 47 arose somatically in his mother, although DNA from the maternal grandfather (unavailable) would need to be examined to confirm this.

In patient 11, AS results from the mutation 2114G→A (G638S) in exon 25 of *COL4A5*.[7] In this case, the proband was a young female heterozygous for the mutation, who presented with haematuria at 4 years and in whom a diagnosis of AS was supported by a typical renal biopsy, although no other phenotypic features were observed. SSCP analysis of DNA taken from her father showed him to possess roughly equal amounts of the normal and mutant alleles (data not shown). This result has been confirmed with DNA extracted from a second blood sample by sequencing (fig 1C). It is unlikely that a Klinefelter karyotype (XXY) could explain these results, as such people are sterile; however, somatic mosaicism of XY/XXY cannot be ruled out. This result is interesting because while patient 11 was diagnosed at an early age, her father surprisingly showed no signs of AS until he went into renal failure at the relatively late age of 43. It may be that mosaicism in this father causes a mild form of AS, since some of the cells in his kidney may be expressing the normal α5 protein, resulting in a phenotype more akin to that of a heterozygous female than that of a hemizygous affected male. This is the first report of mosaicism apparently affecting the severity of AS.

Overall, this study has uncovered three probable somatic mosaics out of a total of 77 mutations found. This number is actually reduced to 3/28 when one includes only those cases where the parents were examined, giving a figure of 10.7% mosaic. This number falls into the range reported for a variety of diseases (0-30%) reviewed by Zlotogora.[9]

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KATE E PLANT*†
EILEEN BOYE*‡
PETER M GREEN*
DAVID VETRIE*§
FRANCES A FLINTER*

**Division of Medical and Molecular Genetics, UMDS, Guy's Hospital, London, UK*

†*Present address: Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK*

‡*Present address: Department of Cell Biology, Harvard Medical School, Boston, MA, USA*

§*Present address: The Sanger Centre, Cambridge, UK*

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