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Mosaicism in Alport syndrome and genetic counselling

EDITOR—Alport syndrome is characterised by a progressive glomerulonephritis with typical ultrastructural changes in the glomerular basement membrane. The most frequent, semidominant, X linked type is the result of a variety of mutations (either point mutations or intragenic deletions) of the *COL4A5* gene encoding the α5 chain of type IV collagen.[1]

During SSCP scanning of the *COL4A5* gene, a shift in a segment including exon 44 and flanking intronic sequences was found in a 19 year old proband showing typical ultrastructural changes of the glomerular basement membrane (III.3 in fig 1). Sequence analysis showed a G→C transversion in the 5' splice site of intron 44 (position 4271+1). The mutation introduced an *Alu*I restriction site which divided a 66 bp fragment into two fragments of 39 + 27 bp. All 18 family members were tested using this restriction assay and the mutation was found in the proband's affected brother, his cousin, his mother, and two maternal aunts. Surprisingly, the proband's grandmother was a normal homozygote. The proband's grandfather was dead, but true paternity of all daughters could be (indirectly) ascertained by polymorphic markers.[4]

In this family the mutation is associated with juvenile Alport syndrome in males, suggesting that the splicing defect results in a low level or absence of the protein, in agreement with our previous findings on genotype-phenotype correlations.[1] Interestingly, we noted considerable clinical variability among heterozygous females (n=4), ranging from ESRD at 27 years to absence of microscopic haematuria at 37 years.

Our data strongly suggest mosaicism in the germ cells of either grandparent. Mosaicism in germ cells may be the result of either a mutation in a germ cell that thereafter undergoes mitotic divisions (giving rise to mosaicism confined to germ cells), or an early postzygotic mutation before separation of the somatic/germ cells (giving rise to mosaicism in both the tissues and germline). In the latter case, the phenotype may or may not be expressed in the mosaic subjects, depending on the proportion of mutated cells in the relevant tissues. In order to verify mosaicism in somatic tissues of the living grandmother (I.1 in fig 1), we used Amplification Refractory Mutation System (ARMS-PCR), a tool able to detect known mutations even when present in a low fraction of template molecules.[5] The primer sense for exon 44[1] was used in combination with the following specific antisense primers: normal (5'-GGTATAACTATCTTCAGGAATAAGTCTTAC-3') and mutant (5'- GGTATAACTATCTTCAGGAATAAGTCT TAG-3'). We performed ARMS-PCR on DNA extracted from grandmaternal peripheral blood using progressively lower stringency by lowering the temperature or increasing the PCR cycle number or both, with the aim of reaching a condition where even the very few mutated molecules present in the blood sample would be amplified. This condition was never reached, as the grandmother's DNA always gave the same results as normal homozygous female controls (data not shown).

On analysis of Xq22 DNA polymorphisms, the three carrier females in the second generation were homozygous for one of the maternal haplotypes, which therefore must have been present in the dead grandfather as well, while the single non-carrier female and the unaffected male carried the other maternal haplotype. These data might suggest that the mutation was present in the grandmaternal gonads on

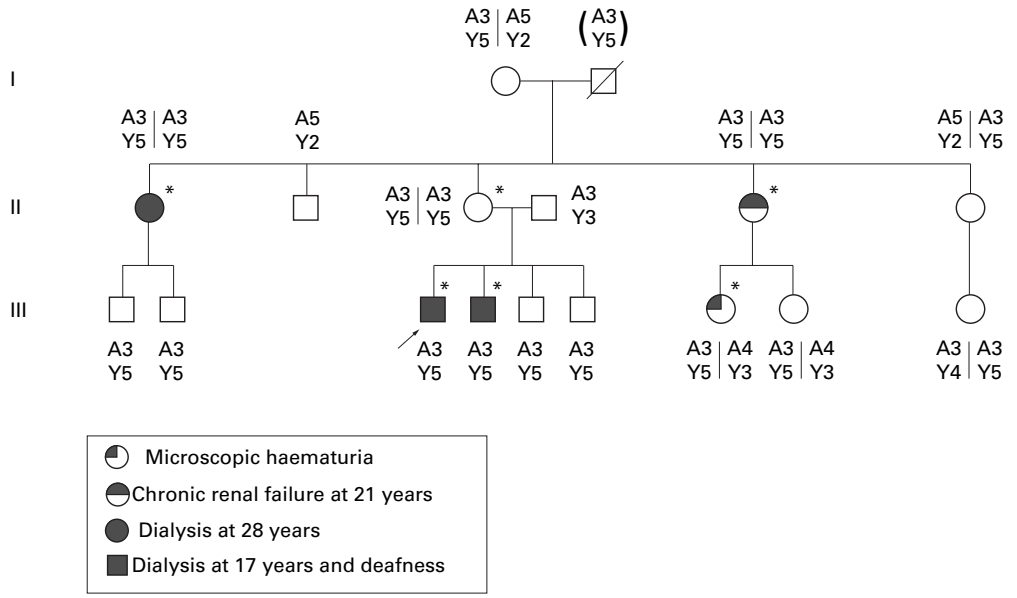

*Figure 1 Pedigree of the family and haplotype analysis at the COL4A5/COL4A6 locus. *Presence of mutation G→C at 4271+1 of intron 44. For haplotype analysis: A=2B6 polymorphism at the 3' end of the COL4A5 gene[2]; Y=A6YU2 polymorphism in intron 31 of COL4A6.[3]*

haplotype A3Y5, and that the mutated germ cells were indeed preponderant, since it was transmitted to all daughters sharing this haplotype. When mosaicism in the germline is diagnosed because of the birth of more than one affected child to apparently healthy parents, it has been reported that in 50% of the cases the mutation can be found in somatic cells.[6] The absence of the mutation in the blood of the grandmother suggests that she carried germline mosaicism only. However, since only blood was tested and some cases are reported in which a mutation not found in blood was shown in other tissues, such as muscle, buccal smear, or hair, somatic mosaicism cannot be completely excluded.[7 8]

An alternative explanation for the above results is mosaicism in the dead grandfather (I.2 in fig 1). Somatic mosaicism is not excluded even if he did not himself express Alport syndrome, and germline mosaicism would be compatible with transmission of a mutated *COL4A5* gene to three females and of a normal gene to one female; in fact, it is critical for the model that the latter subject, II.5, does not carry the mutation. In order unambiguously to assign the mutation to a paternal or a maternal haplotype, it would be necessary to discriminate which haplotype the three carrier females transmitted to the next generation, but unfortunately they were homozygous for all markers tested. Given these data, mosaicism in either the grandmother or the grandfather might be considered equally likely; both are asymptomatic and no difference in gender of mosaic subjects has been reported.[6]

Mosaicism is a well known phenomenon observed in several Mendelian diseases. Mosaicism of parental somatic/germ cells is reported at a rate of 6% in osteogenesis imperfecta type II, 5-10% in campomelic dysplasia, and at an even higher rate in facioscapulohumeral dystrophy, Duchenne muscular dystrophy, and haemophilia A and B (11-20%).[6] A recent review reports a rate of about 10% of somatic and germline mosaicism in retinoblastoma patients.[9] In contrast, in other diseases like achondroplasia and Apert syndrome, mosaicism was never detected in large series of cases, despite the high de novo mutation rate.

In agreement with a low fitness of male Alport syndrome patients, it is well known that a definite proportion is caused by new or recent mutations.[1 10] Among a sample of 16 (unpublished) mutations in which at least the parental DNA was tested, the present case was the only one with a large family available in which we could show that the mutation had originated in a previous generation. Of the remaining cases, one arose de novo in the grandfather who showed no evidence of mosaicism and 14 were inherited. From these proportions, it is hard to establish the frequency of mosaicism among parents of Alport syndrome patients. Additional cases, similar to the one we report here which originates from mosaic subjects in recent generations, may have gone undetected. In a recent communication by Plant *et al*,[11] somatic mosaicism was reported in 3/28 Alport syndrome families with a known *COL4A5* mutation (10.7%). In these three cases, the new mutation was detected as a mosaic in the blood of one parent who was oligosymptomatic (two cases) or asymptomatic (one case). In the same series, five de novo mutations were reported[12]; thus, the incidence of mosaicism among sporadic cases was 1/6 (16.6%). Since no cases of mosaicism had been reported in Alport syndrome until recently, it was current practice in genetic counselling to reassure parents when an apparently de novo mutation was found. Given our results and somatic mosaicism detected by others, more cautious counselling would be advisable in all cases with an apparently de novo mutation.

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Breast hypoplasia and disproportionate short stature in the ear, patella, short stature syndrome: expansion of the phenotype?

EDITOR—The ear, patella, short stature syndrome (EPS or Meier-Gorlin syndrome) is a rare disorder characterised by microtia, absent or hypoplastic patellae, and proportionate pre- and postnatal growth retardation. In 1994, published reports of the disorder were reviewed by Boles *et al*.[1] To date, over 17 patients have been described.[1–5] Inheritance is autosomal recessive as evidenced by an almost equal number of male and female patients, as well as affected sibs, occurrence of consanguineous matings, and the absence of clinical abnormalities in the parents. Here, we describe two unrelated patients with the EPS syndrome and breast hypoplasia. This is a hitherto unreported finding that may be a part of the syndrome in adult females. Furthermore, the disproportionate short stature which was present in our patients may be a skeletal manifestation of the EPS syndrome.

Patient 1 was the first child of non-consanguineous parents. Clitoral hypertrophy and hypoplastic labia minora were noted after birth. She was referred at the age of 14 years because of dysmorphic features and delayed breast development. Her menarche started at the age of 12 years and she had regular periods. Psychomotor development had been satisfactory. Hearing was normal. Physical examination showed disproportionate short stature (height 1.47 m (<3rd centile), arm span 1.33 m, arm span for height <<3rd centile). Head circumference was 53.3 cm (25th centile). There was microtia (ear length <3rd centile) and micrognathia (fig 1). She had a narrow thorax. Puberty was Tanner stage P5M1 (fig 2). A skeletal survey showed bilateral absent patellae. Endocrine studies were normal. She had been treated with ethinyloestradiol which resulted in minimal breast development.

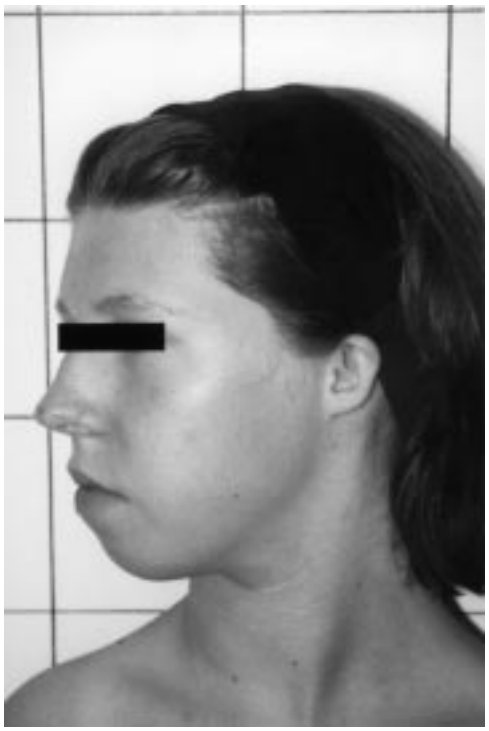

Figure 1 Case 1 aged 14 years. Note microtia and micrognathia.

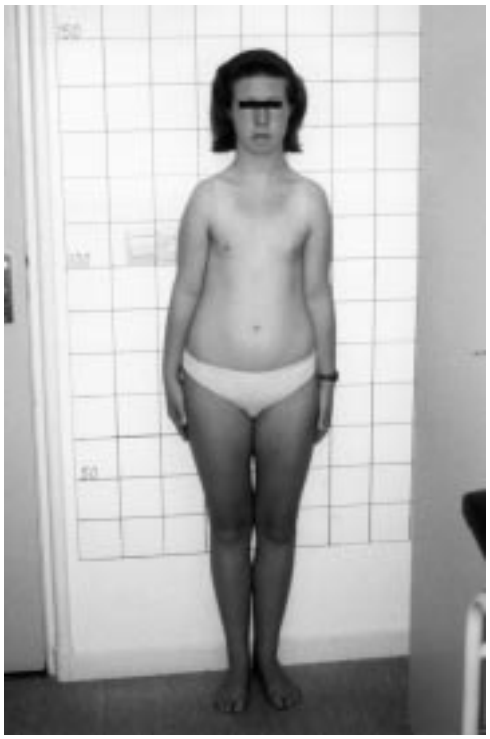

Figure 2 Case 1 aged 14 years. Note disproportionate short stature and breast hypoplasia.