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A missense mutation in the *SEDL* gene results in delayed onset of X linked spondyloepiphyseal dysplasia in a large pedigree

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EDITOR—Spondyloepiphyseal dysplasia (SED) is a rare osteochondroplasia, characterised by disproportionate short stature with a short neck and trunk and barrel chest. The pelvis tends to be narrow and deep, the femoral neck short, and the femoral head flattened. Mild to moderate epiphyseal dysplasia of the large joints may also be seen. The latter may lead to premature secondary osteoarthritis with significant morbidity.[1] SED may occur sporadically; however, in many cases the family history indicates an inherited condition. In some of these pedigrees, the inheritance pattern seems autosomal dominant, while in others it is consistent with autosomal recessive or X linked recessive.[2]

Recently, mutations in the gene designated *SEDL*, located on Xp22, were identified as the cause of X linked spondyloephiphyseal dysplasia tarda in three families.[3] We have previously described a large kindred of British descent spanning four generations affected by SED.[1] Briefly, 14 males between the ages of 10 and 77 years were affected, with early adolescence development of progressive decline in growth rate accompanied by short stature, short trunk, and barrel chest. Although some of them had to limit their activities because of hip or back limitation of movement or pain, many continued with normal activity and were able to perform in the work place without impairment of function. There was no indication of other abnormalities previously reported in association with SED, such as mental retardation,[2] immune abnormalities and retinopathy,[4] cardiac dysfunction,[5] or hypogonadotrophic hypogonadism.[6] The female carriers in this pedigree had normal height. Although some of the females suffered from occasional mild back or hip pain, it did not affect their daily activity, nor was there objective radiological evidence of spinal or joint involvement compatible with

Table 1 Primer sets used for PCR of SEDL gene

Exon	*5'→3'*	*3'→5'*
3	CACTTAATTAGGAGCCATATATTGA (30 nucleotides upstream of the ATG)	GCAAGCCTCATTTAATTGTGGTGG (111 nucleotides downstream of the 5' of intron 3)
4	CAGAATGAAATGTCAGCATTCTCG (72 nucleotides upstream of the 3' of intron 3)	CTAAAAAAAGAAAAGTAGCCCCATAA (166 nucleotides downstream of the 5' of intron 4)
5	GAATACTGTATCAACGAACTGGTTG (70 nucleotides upstream of the 3' of intron 4)	CAATAGGCCAGTTTCCTGACAAAG (48 nucleotides downstream of the 5' of intron 5)
6	GATTTCCTTTCAGAAACTTAAGA (57 nucleotides upstream of the 3' of intron 5)	CTGAGTATACACCATTGTGGTGAC (49 nucleotides downstream of the TGA)

SED. In this large pedigree, linkage analysis performed using RFLP and microsatellite markers showed association between the Xp22 markers DXS1224, DXS16, DXS987, and DXS207 with SED.[1] We now have assessed this pedigree for the presence of mutations in the *SEDL* gene.

Material and methods

Genomic DNA was extracted from peripheral blood leucocytes of 13 family members and 50 normal blood donors as previously described.[1 5] Four X specific primer pairs, which flanked each of the four coding exons and a significant portion of the intron-exon junctions of *SEDL*, were used for PCR amplification (table 1). PCR reactions were performed with Elongase (Gibco-BRL) under the following conditions: 94°C for one minute followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 68°C for 60 seconds. The PCR products were sequenced using Thermosequenase kit (Amersham) as specified by the manufacturer.

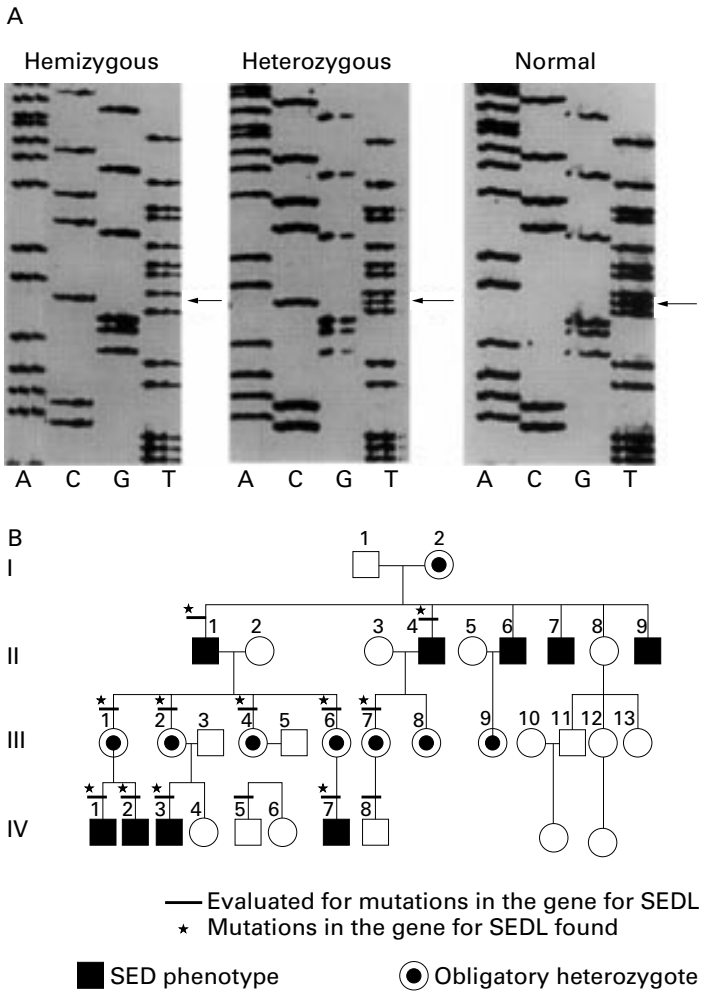

Figure 1 (A) SEDL sequencing and family pedigree. T→C substitution at nucleotide 248 in the SEDL gene cDNA in males with SED (hemizygous), while obligatory female carriers were found to be heterozygous for the mutation. (B) Pedigree of a four generation family with SED. Thirteen family members in generations II, III and IV were evaluated for mutations in the SEDL gene.

Results

Genomic DNA sequencing indicated that in all six affected males with SED a T to C substitution at nucleotide 248 in exon 5 was present (fig 1A). Sequencing in the forward and reverse direction of exon 5 PCR product, which was subcloned into pUC-19, confirmed the presence of the mutation. Five obligate female carriers were heterozygous for both the C and T at this position (fig 1B), which was not detected in the two unaffected male family members, nor was it found in 50 normal blood donors.

The mutation is predicted to cause a substitution of phenylalanine (F) by serine (S) at amino acid 83. The phenylalanine at this position is highly conserved, as it is identical in the SEDL protein homologue found in mouse, yeast, *C elegans*, and *D melanogaster*.[3] Several programs used to predict secondary protein structure, GOR IV,[7] HNNC,[8] and Predator,[9] indicated that the mutation would significantly alter the protein structure and introduce an additional alpha helix (fig 2).

The cosegregation over three generations of a mutation in the gene for *SEDL* and spondyloepiphyseal dysplasia further establishes the association of the genetic defect and SED. This change is found in all affected subjects examined and five obligate carriers are heterozygous. *SEDL* sequence was completely normal in two male family members who had no clinical evidence of SED, as well as in 50 unrelated normal donors.

Discussion

Contrary to previously reported mutations, which result in truncated message and probably in complete loss of SEDL function,[3] the impact of the T→C missense mutation described here may be different. The *SEDL* gene is relatively short and the substitution of the bulky phenylalanine side chain for a highly hydrophilic side chain of serine may cause a structural or functional change in the protein. The function of SEDL and its role in bone metabolism remains unknown; however, several methods to predict the structure and function of proteins have suggested a significant change. A phenotype/genotype correlation is difficult to determine; however, an amino acid change that does not alter the rest of the protein may not be as detrimental to the

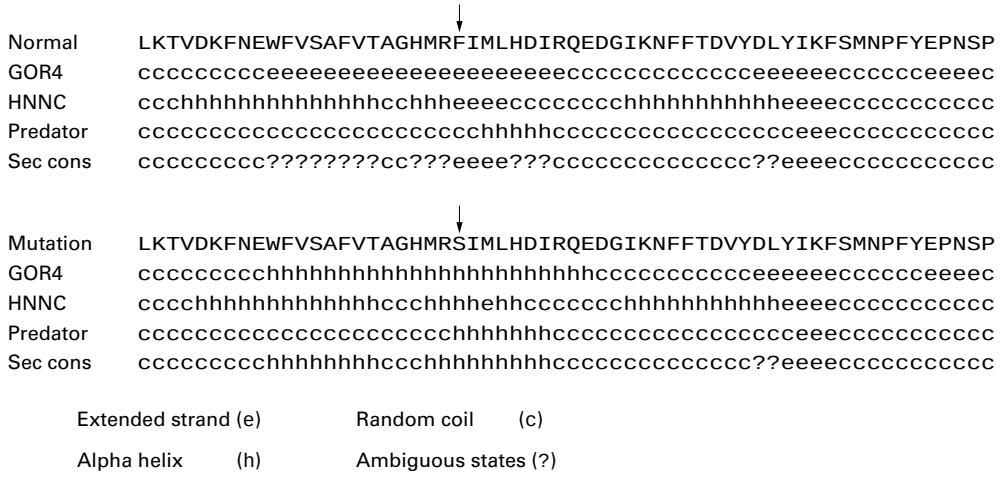

Figure 2 SEDL secondary structure. SEDL secondary structure was predicted to change from an extended strand (e) and random coil (c) in normal controls (normal) to an alpha helix (h) in SED patients (Mutation) with F→S mutation at position 83 (marked by arrow).

phenotype as a mutation that results in truncation of the protein. The affected patients in the family described here had very mild clinical symptoms. Contrary to other pedigrees with X linked SED, in which females heterozygous for the gene defect suffered from subtle skeletal abnormalities and arthritis by middle age,[10 11] the female carriers in the family described here had no objective evidence of SED. This may reflect the subtle impact of the C→T mutation on SEDL protein function.

In the family described here we were able to confirm that a male of uncertain status (IV.8) had no mutation in SED. Thus, the ability clearly to diagnose SED by molecular methods in children with joint or spine abnormalities is of extreme importance, as it may allow appropriate and early intervention.

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Molecular characterisation of a new case of microphthalmia with linear skin defects (MLS)

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EDITOR—Microphthalmia with linear skin defects (MLS) is a clinically complex and highly variable phenotype in XX subjects and has been considered to be at least partially determined by three features: the pattern of X chromosome inactivation; the extent of the Xp22.3 segmental monosomy; and the nature of the chromosomal anomaly (deletion or translocation).The recurring features of microphthalmia with linear skin defects, generally restricted to the face and neck in all the early reported cases, led to its designation as the MLS syndrome. The consistent association of these two manifestations with Xp22.3 segmental monosomy suggests that MLS is a contiguous gene syndrome.[1 2] However, the phenotype may be complicated by additional abnormalities which include sclerocornea, chorioretinal abnormalities, agenesis of the corpus callosum, hydrocephalus, infantile seizures, mental retardation, and congenital heart defects. To date, around two dozen cases of MLS syndrome