

The Molecular Basis of X-Linked Spondyloepiphyseal Dysplasia Tarda

A. K. Gedeon,^{1,2} G. E. Tiller,⁴ M. Le Merrer,⁵ S. Heuertz,⁵ L. Tranebjaerg,⁷ D. Chitayat,⁸ S. Robertson,⁹ I. A. Glass,^{11,12} R. Savarirayan,^{9,10} W. G. Cole,⁸ D. L. Rimoin,¹³ B. G. Kousseff,¹⁴ H. Ohashi,¹⁵ B. Zabel,¹⁶ A. Munnich,⁶ J. Gecz,^{1,2} and J. C. Mulley^{1,3}

¹Centre for Medical Genetics, Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, and University of Adelaide Departments of ²Paediatrics and ³Genetics, Adelaide; ⁴Vanderbilt University School of Medicine, Nashville; ⁵Institute Necker and ⁶INSERM U393, Hôpital Necker-Enfants Malades, Paris; ⁷Department of Medical Genetics, University Hospital of Tromsø, Tromsø, Norway; ⁸Hospital for Sick Children, Toronto; ⁹Victorian Clinical Genetics Service and ¹⁰University of Melbourne Department of Paediatrics, Melbourne; ¹¹Queensland Clinical Genetics Service and University of Queensland School of Medicine, Brisbane; ¹²Medical Genetics, University of Washington-CHRMC, Seattle; ¹³Cedars Sinai Medical Center, Los Angeles; ¹⁴University of South Florida, Tampa; ¹⁵Saitama Children's Medical Centre, Saitama, Japan; and ¹⁶University of Mainz, Mainz, Germany

The X-linked form of spondyloepiphyseal dysplasia tarda (SEDL), a radiologically distinct skeletal dysplasia affecting the vertebrae and epiphyses, is caused by mutations in the *SEDL* gene. To characterize the molecular basis for SEDL, we have identified the spectrum of *SEDL* mutations in 30 of 36 unrelated cases of X-linked SEDL ascertained from different ethnic populations. Twenty-one different disease-associated mutations now have been identified throughout the *SEDL* gene. These include nonsense mutations in exons 4 and 5, missense mutations in exons 4 and 6, small (2–7 bp) and large (>1 kb) deletions, insertions, and putative splicing errors, with one splicing error due to a complex deletion/insertion mutation. Eight different frameshift mutations lead to a premature termination of translation and account for >43% (13/30) of SEDL cases, with half of these (7/13) being due to dinucleotide deletions. Altogether, deletions account for 57% (17/30) of all known *SEDL* mutations. Four recurrent mutations (IVS3+5G→A, 157–158delAT, 191–192delTG, and 271–275delCAAGA) account for 43% (13/30) of confirmed SEDL cases. The results of haplotype analyses and the diverse ethnic origins of patients support recurrent mutations. Two patients with large deletions of *SEDL* exons were found, one with childhood onset of painful complications, the other relatively free of additional symptoms. However, we could not establish a clear genotype/phenotype correlation and therefore conclude that the complete unaltered *SEDL*-gene product is essential for normal bone growth. Molecular diagnosis can now be offered for presymptomatic testing of this disorder. Appropriate lifestyle decisions and, eventually, perhaps, specific SEDL therapies may ameliorate the prognosis of premature osteoarthritis and the need for hip arthroplasty.

Introduction

Spondyloepiphyseal dysplasia tarda (SEDT) is a genetically heterogeneous disorder of vertebral and epiphyseal growth, commonly culminating in degenerative osteoarthropathy of the hip joint (Byers et al. 1978; Kaibara et al. 1983; Beighton 1997). The main clinical features of this progressive skeletal disorder are disproportionately short-trunked short stature due to platyspondyly, and dysplasia of the large joints, frequently necessitating hip replacements in the third decade of life. It is the pattern of inheritance and the characteristic radiological appearance of the vertebrae that delineate the X-linked

form (SEDL [MIM 313400]) from those with autosomal modes of inheritance (MIM 271600 and MIM 184100). The mean adult height in affected males, for all forms of SEDT, is <150 cm (Wynne-Davies et al. 1985), although SEDL results in a more pronounced short-trunk condition than do the autosomal forms (Iceton and Horne 1986).

The distinctive radiological signs in males affected with the X-linked form are the hump-shaped central and posterior portions of characteristically flattened thoracic and lumbar vertebral bodies (Bannerman et al. 1971; Harper et al. 1973). Although the initial presentation usually occurs in late childhood (age 5–10 years), with vague back pain and a decline in growth velocity, the skeleton is radiologically normal up to the age of 5 years (Heuertz et al. 1993; MacKenzie et al. 1996), and the diagnosis may not be made until early adulthood. Expression of the disorder can vary from relative freedom from musculoskeletal complaints to limited spine mobility and premature osteoarthritis of the major proximal weight-bearing joints (Iceton and Horne

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Address for correspondence and reprints: Dr. A. K. Gedeon, Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, 72 King William Road, North Adelaide, SA 5006, Australia. E-mail: agi.gedeon@adelaide.edu.au

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1986; Whyte et al. 1999). Limb lengths (usually expressed as arm spans) do not appear to be involved. The upper torso of an affected male, however, is commonly described as “barrel chested,” as a result of the shortened spine and consequently elevated ribs (MacKenzie et al. 1996; Whyte et al. 1999).

Over a 30-year period in Britain, >9% of skeletal dysplasia index cases in patients requiring orthopedic care were classified as SEDT, with 22% of these recorded as being the X-linked recessive form (Wynne-Davies and Gormley 1985). The estimated prevalence figure of 1.7/1,000,000 (Wynne-Davies and Gormley 1985) is, however, likely to be an underestimate, since it represents only those symptomatic patients who presented for treatment and in whom X-linked family history was documented or inferred. In the absence of clear radiographic evidence, it may also be that, in the past, atypical or isolated *SEDL* cases were classified as being autosomal SEDT. The true prevalence of X-linked *SEDL* has yet to be determined.

We recently identified the *SEDL* gene in Xp22 (MIM 300202), consisting of six exons and encoding a protein of 140 amino acids with a putative role in vesicular transport (Gedeon et al. 1999). We have subsequently shown that recombinant human and mouse *SEDL* proteins localize to perinuclear structures consistent with the functional location of the yeast ortholog (Gecz et al. 2000). Initial screening of the *SEDL* gene in three Australian families with *SEDL* identified three different dinucleotide deletions that effected frameshifts of the open reading frame (ORF) (Gedeon et al. 1999). The purpose of the present study was to determine the spectrum of novel mutations in the *SEDL* gene in 36 unrelated patients with clinically diagnosed *SEDL*. By characterizing additional *SEDL* mutations, we aimed to determine essential functional domains necessary for normal bone growth and skeletal development and to examine phenotype/genotype correlation. We report 21 pathogenic *SEDL* mutations accounting for the *SEDL* phenotype in 30 patients.

Subjects, Material, and Methods

Patients with SEDL

A total of 36 unrelated patients were analyzed for this study. Genomic DNA from 33 new index patients with *SEDL* from various populations were screened for mutations in the *SEDL* gene. The contributing clinician in each case established the diagnosis of *SEDL*, by clinical and radiological means, and obtained informed consent. All males in this study had disproportionately short-trunked short stature. In most cases, the diagnosis included radiography of the lumbar vertebrae and epiphyses, to visualize the characteristic humping of the centra

of the vertebral bodies. The source and ethnic origin of the 30 patients confirmed to have X-linked *SEDL* are presented in table 1, including those from three Australian families described elsewhere (Gedeon et al. 1999). Of the 27 new patients with *SEDL* in this study, 17 came from families in which X-linked inheritance was clear or strongly suspected on the basis of anamnestic evidence, with no male-to-male transmission of the phenotype. Several of these families have been characterized previously, and the disorder either was shown to segregate with or could not be excluded from the linked DXS16–DXS987 *SEDL* genetic interval (Heuertz et al. 1995; Bernard et al. 1996; Gedeon et al. 1999; Tiller et al. 2001 [in this issue]).

Clinical summaries were not available for all patients in the present report. In all subjects for whom anthropometric measurements were available, the upper-to-lower body-segment ratio (U:L) was <1.0, and arm spans exceeded adult heights (table 2). Clinical data on four new Australian patients—patients 2, 26, 28, and 30—as well as on patient 6 (proband III-1 in family 1 of Bernard et al. [1996]) are summarized in table 2, along with the three previously described Australian patients (Gedeon et al. 1999), referred to here as patients 3, 10, and 13.

Mutation Detection

In each patient, each of the four exons comprising the *SEDL* ORF, as well as their exon/intron boundaries, were analyzed by direct sequencing of PCR products, through use of flanking intronic primers (Gedeon et al. 1999). PCR conditions were 35 cycles of 30 s each of 94°C, 58°C, and 72°C. The PCR-amplification product was purified by QIAquick centrifugation according to the manufacturer's (QIAGEN) instructions. A 100-ng aliquot of the purified sample was cycle sequenced with the ABI prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer Applied Biosystems), in the presence of equal-part halfBD™ (Genpak). The sequencing primers were the same as those used for template amplification (Gedeon et al. 1999). An X-specific reverse primer, 3XR (CAT ATT ATA CCA TAT CAT TAC AAT AGC), was used for amplification or sequencing in those male samples in which the exon 3 primers amplify the Y copy. The sequencing reactions were electrophoresed on ABI automated sequencers, and the resultant chromatograms were analyzed by Lasergene software (DNA Star) and compared against normal sequences (GenBank).

Mutations (fig.1) are numbered from 1, beginning at the first nucleotide of the translation start (den Dunnen and Antonarakis 2000). For each mutation identified in a subject, segregation with the affected phenotype was confirmed either in family members, when available, or by screening of ≥ 50 control chromosomes, to exclude

Table 1

Ethnic Origins and Mutations Identified in the *SEDL* Gene in 30 Patients with SEDL

Patient Number/ (DNA Number)	Ethnic Origin	Gene Region	Nucleotide Change	Predicted Amino Acid Change	Reference/Source (Clinician)
1 (SED2)	French	Intron 2	IVS2-2A→G	...	Family 2, Szpiro-Tapia et al. (1988); Heuertz et al. (1993, 1995) (M.L.M., S.H.)
2 (9275)	Australian	Exon 3del	Exon3del	Gross	Present study (I.A.G.)
3 (4660)	Australian	Exon 3	53-54delTT	F18X	IV-2 in family 1, Gedeon et al. (1999)
4 (SED1)	French	Intron 3	IVS3+5G→A	...	Family 1, Szpiro-Tapia et al. (1988); Heuertz et al. (1993, 1995) (M.L.M., S.H.)
5 (10008)	Norwegian	Intron 3	IVS3+5G→A	...	Family 9, Heuertz et al. (1993, 1995); present study (L.T.)
6 (10191)	English	Intron 3	IVS3+5G→A	...	III-1 in family 1, Bernard et al. (1996) (D.C.)
7 (861-99)	German	Intron 3	IVS3+5G→A	...	Present study (G.T.)
8 (SEDL-IL)	American	Intron 3	IVS3+5G→A	...	Present study (G.T.)
9 (10009)	Danish	Exon 4	G139T	D47Y	Present study (L.T.)
10 (6513)	Australian	Exon 4	157-158delAT	M53fsX87	II-3 in family 3, Gedeon et al. (1999)
11 (SED6)	French	Exon 4	157-158delAT	M53fsX87	Family 13, Heuertz et al. (1993, 1995) (M.L.M., S.H.)
12 (SEDL-MX)	Mexican	Exon 4	T182A	L61X	Present study (D.R.)
13 (4937)	Australian	Exon 4	191-192delTG	V64fsX87	III-6 in family 2, Gedeon et al. (1999)
14 (SED7)	French	Exon 4	191-192delTG	V64fsX87	Family 8, Heuertz et al. (1993, 1995) (M.L.M., S.H.)
15 (10043)	Norwegian	Exon 4	C218T	S73L	Present study (L.T.)
16 (SEDL-FL)	North American	Intron 4	IVS4-9-12del	...	Present study (B.K.)
17 (1018-99)	German	Intron 4	IVS4-4-11del	...	Present study (B.Z.)
18 (SED5)	French	Exon 5	241-242delAT	M81fsX87	Families 5 and 6, Heuertz et al. (1993) (later known as family 21, in Heuertz et al. [1995]) (M.L.M., S.H.)
19 (10639)	Canadian	Exon 5	262-266delGACAT	D88del;I89fsX100	Present study (D.C.)
20 (SED4)	French	Exon 5	C271T	Q91X	Family 4, Szpiro-Tapia et al. (1988); Heuertz et al. (1993, 1995) (M.L.M., S.H.)
21 (SED3)	French	Exon 5	271-275delCAAAGA	Q91del;E92fsX100	Family 3, Szpiro-Tapia et al. (1988); Heuertz et al. (1993, 1995) (M.L.M., S.H.)
22 (10010)	New Zealand	Exon 5	271-275delCAAAGA	Q91del;E92fsX100	Ice-ton and Horne (1986) (W.C.)
23 (SEDL-IN)	East Indian	Exon 5	271-275delCAAAGA	Q91del;E92fsX100	Present study (W.C.)
24 (R91-15)	Japanese American	Exon 5	271-275delCAAAGA	Q91del;E92fsX100	Present study (D.R.)
25 (SEDL-MI)	Finnish	Exon 5	272-273delAA	Q91fsX100	Present study (G.T.)
26 (10554)	Native Australian	Exon 5	320-321insT	I107fsX116	Present study (R.S.)
27 (10823)	French	Intron 5	IVS5-4-10 delTCTTTCGinsAA	...	Present study (A.M.)
28 (9574)	Australian	Intron 5	IVS5-2A→C	...	Present study (S.R.)
29 (SGL3845)	Japanese	Exon 6	T389A	V130D	Present study (H.O.)
30 (9983)	Australian	Exon 6	Exon6del	Gross	Present study (S.R.)

Table 2
Comparison of Phenotypes in Patients for Whom Anthropometric Measurements and Clinical Notes Were Made Available

SEDL Mutation and Patient or Family ID Number ^a	Adult Height (cm)	U:L	Arm Span (cm)	Severity	Family History of Affected Males (Reference)
Del exon 3: Patient 2	139.3 ^b	.7	160.4	Slowed growth at age ~6.5 years, scoliosis, hip pain referred to knees, back pain, arms extend to knees	Anamnestic evidence
IVS3+5G→A: Patient 6	156 ^c	.81	176	Arthralgia of hips, discomfort of limb joints and neck	One brother and maternal uncle (Bernard et al. 1996)
53-54delTT exon 3: Patient 3/IV-2 in family 1	137-160 ^d	<1.0	H+20	Barrel chests, kyphoscoliosis, osteoarthritis of femoral heads with hip replacements	Three generations (Gedeon et al. 1999)
157-158delAT exon 4: Patient 10/II-3 in family 3	140	.79	154.5	Large heads, upper thoracic kyphosis, normal limbs and joints	Three brothers (Gedeon et al. 1999)
II-4 in family 3 191-192delTG exon 4: III-5 in family 2	152.4 153	.82 ^e .72	167.4 161.5	Pain in lower back and hip, degenerative changes in hips and knees, limited movement in shoulders, bilateral hip replacement in one patient	Three maternal first cousins (Gedeon et al. 1999)
Patient 13/III-6 in family 2 III-7 in family 2	138 149	.78 .9	142.5 161.5		
IVS5-2A→C: Patient 28 320-321insT exon 5: Patient 26	152 146.7 ^f	.9 .67	168 164	Back pain on exertion, responsive to massage and analgesics, no kyphosis or scoliosis No kyphosis or scoliosis	Four brothers Maternal uncle and first cousin
Del exon 6: Patient 30	152.5	.83	170.5	Asymptomatic; playing sport to mid 30s, only two episodes of hip pain settled with anti-inflammatory medication	Three brothers

^a Patients are listed in order of the mutations, from 5' to 3', of the *SEDL* gene.

^b At age 16 years. SD -5.3

^c SD -4

^d Range among affected family members.

^e SD <-2.

^f At age 16 years.

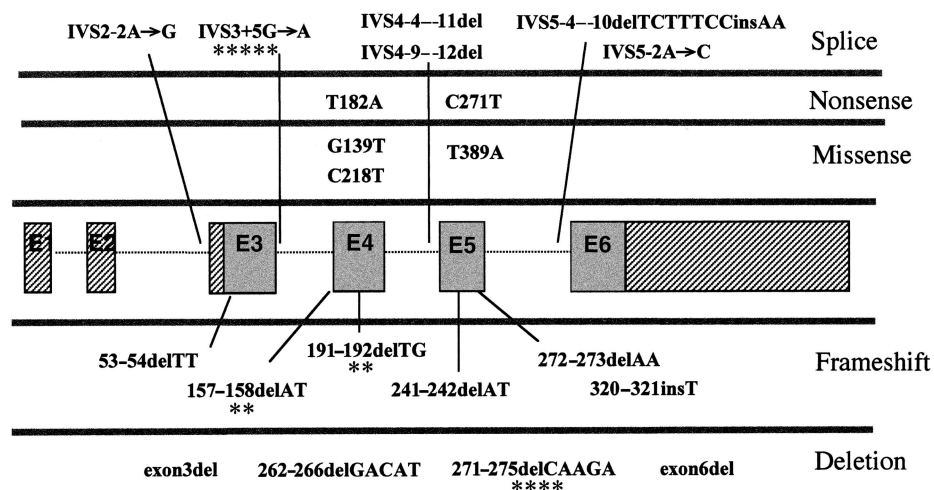


Figure 1 Schematic representation of the spectrum of mutations of the *SEDL* gene. Solid boxes represent the *SEDL* ORF, hatched boxes represent the UTR of the gene. Twenty-one different mutations of all types were found to have pathogenic consequences in 30 unrelated *SEDL* cases. Asterisks (*) indicate recurrent mutation events.

polymorphisms and rare variants. Additional family members were available for patients 1, 3–6, 8–11, 13, 14, 18–22, and 27 (table 1). Segregation of the mutation with the disorder was demonstrated as follows: by restriction-endonuclease digestion of the PCR product, followed by visualization on 1.5% agarose gels (when an enzyme site was created or destroyed); on 5% polyacrylamide gels (for small deletions); by single-stranded conformation analysis (SSCA) on either MDE™ (FMC Corporation) or 10% nondenaturing polyacrylamide gels; or by direct sequencing. To determine the extent of gross deletion of exon 3 in patient 2 and exon 6 in patient 30, extra primers were designed in exon 2 (SEDLE2F [CCC AAG TGT GAC AAC CAA AGA TG] and 1BR [CTT TAC CTC ACA GCA CAC AAT GG], amplicon 129 bp) and flanking exon 6 (6AF [GTG GTC TTT AGA CTT TGG AAT GTC] and 6BR [CAA CAG AGT GAG ACT CTG TAT CA], amplicon 504 bp).

RT-PCR

When available, RNA was extracted from peripheral blood lymphocytes, to examine the effect of *SEDL* mutations on *SEDL*-gene transcription and processing. RT-PCR experiments were performed on total RNA, as described elsewhere (Gecz et al. 2000).

To examine the consequence of the complex intron 5 deletion/insertion mutation in patient 27, RT-PCR was performed using either primers E1F2 (CTT CCG CGG AAA CTG ACA TTG C) and E4R1 (ATG CCC CGC AGT GAC AAA TGC C), from the 5' end of the *SEDL* gene (fig. 2a), or primers SEDLF1 (GGC CAC CAT GAT AAT CCA GT) and SEDL5R_x (GTA TAC ACC ATT GTG

GTG ACA TC), flanking the exon 5/exon 6 splice site (fig. 2b).

To examine the *SEDL* RNA of the exon 3 deletion in patient 2, primers from exon 1 (E1F2) and exon 6 (SEDL5R_x) were used. By these primers, which span the alternatively spliced exon 2 of *SEDL*, two RT-PCR products, of 679 bp and 537 bp, are amplified only from the X-linked *SEDL* gene; in the absence of exon 3 (as in patient 2), 567-bp and 425-bp products are generated instead (fig. 2c).

Haplotype Analysis

Disease-associated haplotypes were constructed by the genotyping of seven polymorphic loci flanking *SEDL* (DXS7109, DXS1224, DXS16, AFMa124wc1, DXS8022, AFM126ye11, and DXS987). The genetic distance covers 7 cM between DXS7109–2.6 cM–DXS1224–3.1 cM–DXS8022–1.3 cM–DXS987 (Nagaraja et al. 1997), with the *SEDL* gene mapped to the 170-kb interval between markers DXS16 and AFMa124wc1 (Gedeon et al. 1999). To determine whether recurrent mutations were due to identity by descent (IBD) from a founder mutation, the haplotypes of unrelated samples were compared. Haplotypes were also generated from two female CEPH controls representing four normal X chromosomes.

Results

Mutation Analysis in Patients with *SEDL*

In 27 new index patients, mutations were found that, together with three previously described dinucleotide deletions, account for the *SEDL* phenotype in 30 affected

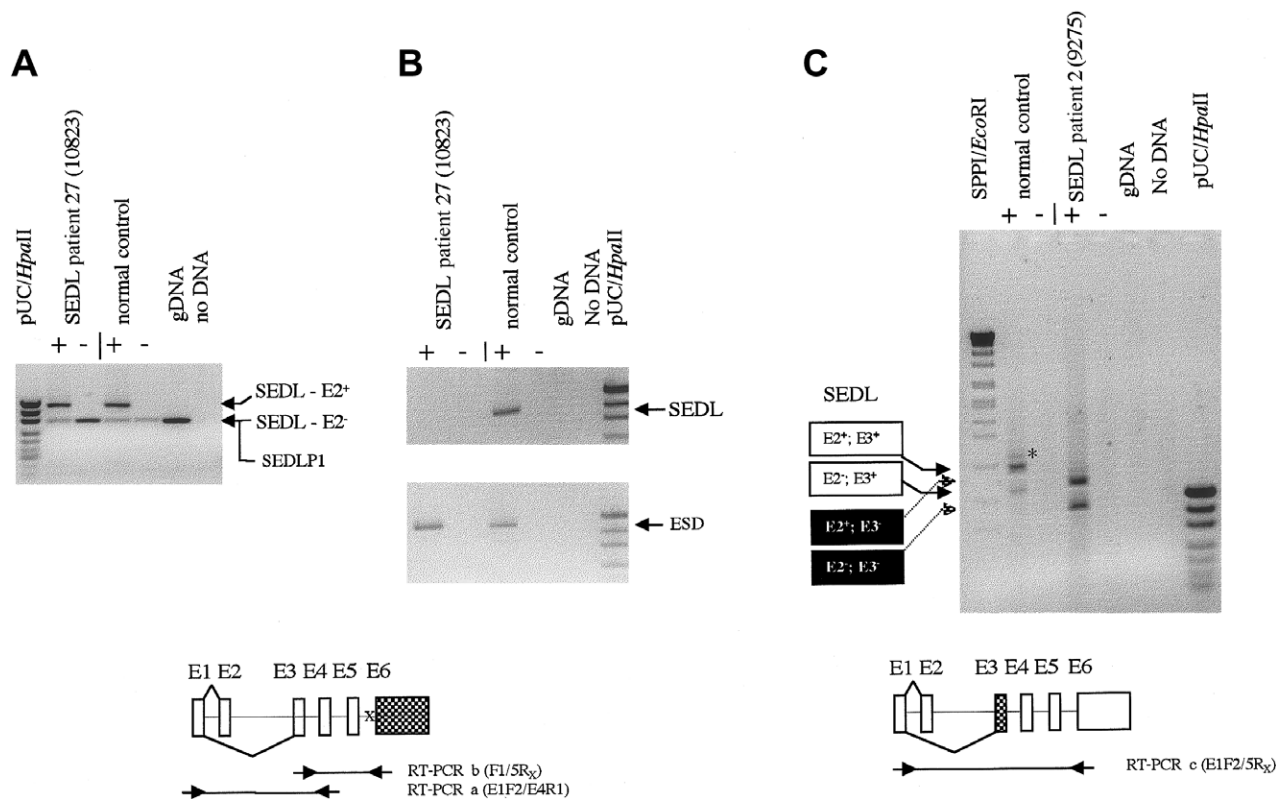


Figure 2 RT-PCR analysis of the *SEDL* gene in patient 27 (insertion/deletion of intron 5) and patient 2 (deletion of exon 3). Total RNA from blood lymphocytes was reverse transcribed with Superscript II reverse transcriptase (see Subjects, Material, and Methods section) and was PCR amplified with *SEDL* primers. Schematic diagrams indicate the positions of the RT-PCR primers used, the *SEDL* mutations tested, and the occurrence of the alternative splicing of exon 2. “gDNA” denotes normal genomic DNA used as a control; plus (+) and minus (-) signs denote presence and absence, respectively, of reverse transcriptase during the reverse transcription step of the RT-PCR; the asterisk (*) denotes presence of heteroduplexes; and pUC/*Hpa*II and SPPV/*Eco*RI are molecular-weight markers. **A**, Primers spanning *SEDL* exons 1–4. These primers amplify a 451-bp *SEDL*-gene product, when exon 2 is spliced in, or a 309-bp product, when exon 2 is spliced out. These two primers also amplify genomic DNA of the chromosome 19 (*SEDL*P1) pseudogene (309-bp product). This product was present in lymphocyte RNA from patient 27. **B**, Primers spanning exons 3–6 of *SEDL*. When these primers were used, no *SEDL* PCR product was amplified from patient 27’s lymphocyte RNA, indicating alternative 3’ processing of the *SEDL* RNA, as a consequence of the insertion/deletion mutation. Esterase D (ESD) RT-PCR is used as a control of RNA quality. **C**, RT-PCR amplification using primers spanning exons 1–6 from patient 2’s lymphocyte RNA. This patient carries a deletion of exon 3. Exon 3 is missing from its spliced *SEDL* transcript, demonstrated as a shift of 112 bp (exon 3 size) of both *SEDL* exon 2⁺ and exon 2⁻ isoforms.

individuals (table 1). Four mutations occurred in more than one unrelated family or individual and affect 43% (13/30) of patients with confirmed cases of *SEDL*. A total of 21 different pathogenic *SEDL* gene–sequence alterations have now been identified that affect the *SEDL* ORF (fig. 1). None of these changes were present in control chromosomes. Six patients in the present study had no detectable mutation in the *SEDL* ORF or in the sequences adjacent to each exon/intron boundary. Although noncoding mutations affecting regulation of *SEDL* cannot be excluded, these patients may not have X-linked *SEDL*.

Eight single-base substitutions were found, three of which caused missense amino acid substitution and two of which resulted in nonsense mutations; of the remain-

ing three, two occurred at splice-acceptor sites, and only one occurred at a splice-donor site. Except for the latter, each point mutation occurred only once in this cohort of patients with *SEDL*. Deletion mutations were the most common pathogenic mutation affecting the *SEDL* gene, accounting for 17 (56%) of the 30 cases. Although recurrence of three deletions was observed, 12 (57%) of the 21 different mutations disrupting *SEDL* are deletions of two or more bases. Five dinucleotide and two pentanucleotide deletions of the coding sequence affect frameshift in the ORF of the gene and lead to premature termination of translation, whereas three intronic deletions are likely to disrupt splicing. RNA was available, from patient 27, to assess the consequence of a complex deletion/insertion in intron 5. RT-PCR showed normal

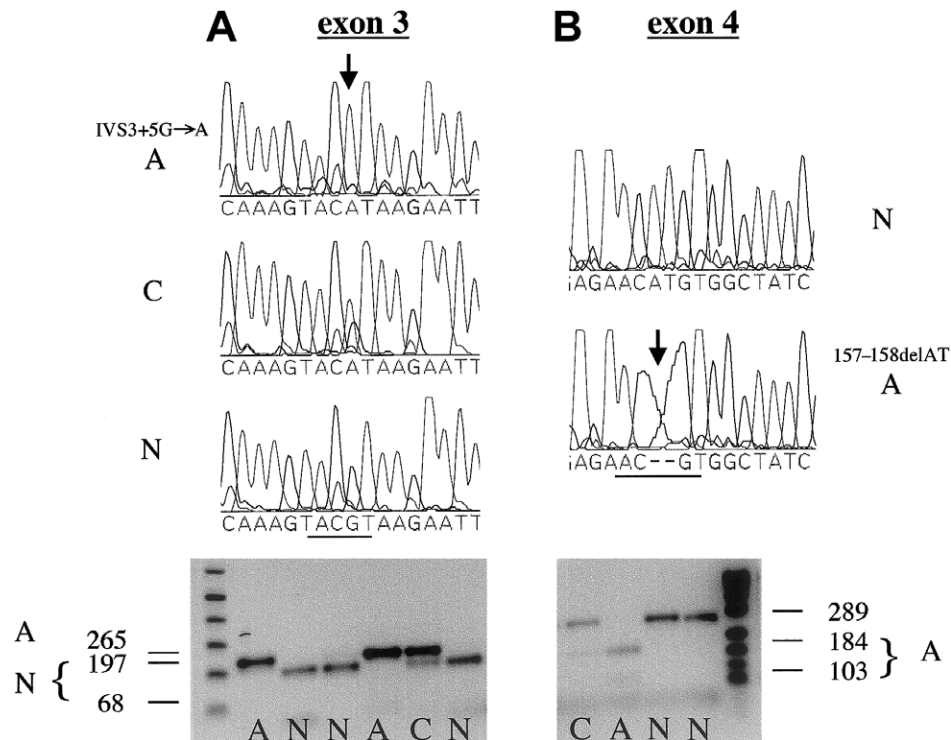


Figure 3 Sequence chromatograms of two common mutations. Restriction digestion of exon 3 and exon 4 PCR products with *TaiI* detects half of the recurrent mutations, accounting for >56% of all *SEDL* cases. A, IVS3+5G→A mutation, which abolishes a *TaiI* site (underlined) so that the 265-bp exon 3 PCR product is resistant to cleavage into 197-bp and 68-bp fragments. B, 157–158delAT mutation in exon 4, which creates a *TaiI* site cleaving the 289-bp product into 103-bp and 184-bp fragments. Carriers can be identified. A = affected, C = carriers, and N = normal control.

transcription of exons 1–4 but no transcript with primers spanning exons 3–6 (fig. 2), indicating alternative 3' processing due to a lack of splicing of exon 6.

Larger deletions were suggested in two patients by the lack of PCR amplification products. In one of these, patient 2, the gene was deleted for exon 3 but was not found to be deleted when tested with primers (SEDL2F and 1BR) within *SEDL* exon 2, limiting the deletion to exon 3, which contains the ATG start site (results not shown). Southern blot hybridization of an *EcoRI* digest revealed deletion of exon 3, with no evidence of a junction fragment, implying complete deletion of this exon (data not shown). RT-PCR experiments on lymphocyte RNA of patient 2 with *SEDL* exon 1-specific (E1F2) and exon 6-specific (*SEDL*5R_x) primers confirmed complete absence of exon 3 from the processed *SEDL* transcript (fig. 2c). In the other patient, patient 30, the deletion of exon 6 involves the entire 504-bp 6AF-6BR PCR product but does not extend to the polymorphic marker DXS16 distal to *SEDL*. To more precisely characterize this deletion and, particularly, to investigate whether this deletion extends beyond the *SEDL* gene, long-range PCR across exons 5 and 6 was performed. A junction fragment showing a deletion of ~1.3 kb was

amplified from the patient's DNA and sequenced. Sequence analysis revealed that in this subject, patient 30, 1,330 bp of gDNA was deleted. This deletion occurred between two *Alu* sequences, one lying within intron 5 and the other being the first *Alu* sequence of exon 6 of the *SEDL* gene (results not shown). We speculate that the most likely cause of this deletion was an intrachromosomal *Alu-Alu* sequence-mediated recombination with subsequent deletion of the inter-*Alu* sequences.

The mutations of the splice-donor site in intron 3 (IVS3+5G→A) and the deletion of five consecutive bases in exon 5 (271–275delCAAGA) are the most common (table 1). The recurrent G→A transition in the intron 3 splice donor abolishes a *TaiI* restriction site. The isoschizomer of *MaeII*, *TaiI*, is useful as a diagnostic tool for screening for the recurrent splice mutation of exon 3, as well as for the 157–158delAT deletion in exon 4 (fig. 3). In families with the recurrent IVS3+5G→A splice-donor mutation, the *TaiI* site is destroyed in intron 3, and *TaiI* digestion of the 265-bp PCR products will cut only normal sequences. In patient 10, as in patient 11, a *TaiI* site is created by the deletion of the nucleotides AT at positions 157 and 158 in exon 4 and could be used to confirm segregation of the mutation with the

phenotype in the family and for subsequent carrier detection. Cosegregation of the disorder with other deletion mutations was confirmed, in each of the remaining families, by fragment-size variation of PCR products on 5% polyacrylamide gels or by SSCA analysis (results not shown).

Haplotype Analysis

Haplotypes around the *SEDL* gene were used to determine whether recurrent mutations of the *SEDL* gene could be due to ancestral mutation events. Haplotype analysis in at least one pair of patients with each recurrent mutation gave no evidence to support IBD (table 3). Many patients have diverse ethnic origins (table 1), although those with the recurrent splice-donor mutation in intron 3 are all of European origin. The 5-bp deletion in exon 5 occurs in people of differing ethnicity. Two of the dinucleotide deletions described in the first three Australian families reported (Gedeon et al. 1999) were also detected in two French families. Those sharing the 191–192delTG mutation have entirely different haplotypes around the *SEDL* gene, rendering a founder ancestor highly unlikely.

Correlation of Genotype with Phenotype

Objective assessment of the severity of the *SEDL* phenotype is difficult, since intrafamilial variation is not unusual; however, there does appear to be a trend of decreasing severity, within the group of patients described, in the order of their mutations, 5'→3', along the gene (table 2). The severity of the condition in patient 2, compared with that in patient 28 or in asymptomatic

patient 30, is suggestive of some genotype/phenotype correlation. It appears that mutations affecting exons 5 and 6 in this subset of patients result in a milder clinical condition, with little or no hip pain and no evidence of kyphosis or scoliosis. Toward the 5' end of *SEDL*, mutations in exons 3 and 4 result in kyphosis and scoliosis, with both severe pain evident earlier in life and a more debilitating set of complications. Although no obvious correlation with phenotype was apparent, that may be a reflection of the ascertainment bias for patients included in the present study; on the other hand, patient 22 carries the pentanucleotide deletion in exon 5 and is one of three brothers, described by Icton and Horne (1986), who have had different clinical courses, from a lack of symptoms to disabling arthritis.

Of the six patients with no identified *SEDL* mutation, three had no family history of the disease. One of these had hip pain provoked by exercise, and degenerative changes and characteristic platyspondyly of the spine. Given the absence of an identifiable *SEDL* mutation, an alternative diagnosis, of mild Kniest syndrome with no eye or hearing problems, is possible. In the three others clinically assessed as having *SEDL*, two had unknown family history and one was a member of a sibship of three affected males in whom, although x-rays were not wholly typical, the clinical diagnosis had never been doubted.

Discussion

In our initial study of *SEDL*, three Australian families carried truncating mutations (Gedeon et al. 1999). The present study describes the 21 novel mutations account-

Table 3

Genotypes at Seven Polymorphic Loci Flanking the *SEDL* Locus

MUTATION AND PATIENT DNA NUMBER ^a	GENOTYPE AT							
	DXS7109	DXS1224	DXS16	(<i>SEDL</i>) ^b	AFMa124wc1	DXS8022	AFM126ye11	DXS987
Controls:								
1347-02	139	159	3,4		3	176, 178	A, B	206, 216
1331-02	141	159, 167	2		2	160, 178	A, B	...
IVS3+5G→A:								
SED1	139	159	4		3	172	A	220
10008	139	159	4		1	176	A	...
10191	139	165	5		3	160	B	...
157–158delAT:								
6513	139	159	5		1	180	B	206
SED6	139	159	5		...	178	B	206
191–192delTG:								
4937	141	167	9		3	168	B	206
SED7	139	165	4		2	178	A	216
271–275delCAAGA:								
SED3	139	159	6		1	168	A	216
10010	141	159	5		3	176	A	...

^a Haplotypes can be compared between individuals with the same *SEDL* mutation and against those of normal female CEPH controls.

^b (*SEDL*) is shown for the purpose of location only.

ing for the X-linked *SEDL* phenotype in 30 of 36 independently ascertained patients. These mutations, which are spread throughout the gene, include point mutations, splice alterations, an insertion, several deletions, and a complex deletion/insertion. Four mutations were found more than once in this cohort of patients, indicating clustering at more-mutable sites within the gene. None of the familial mutations could be traced back to their origins. In those isolated cases in which the patient's mother was available for testing, she was confirmed to be a carrier.

The most common type of *SEDL*-gene disruption was deletion, representing 40% of the types of detected *SEDL* mutations. Several different deletions of 2–5 bp and two large deletions within the coding sequence caused frameshifts and accounted for 14/30 (46%) of *SEDL* cases. Three deletions within introns affected normal splicing and resulted in the removal of large portions of coding sequence. Three of four identified recurrent mutations were deletions. More than half of all confirmed *SEDL* cases were due to a deletion, with almost one quarter due specifically to dinucleotide deletions. This number is up to 10-fold greater than the average deletion frequency (5%–10%) in disease-causing genes (Wicking and Williamson 1991) and approaches that quoted for Duchenne muscular dystrophy (Koenig et al. 1987; Forrest et al. 1988). This is an unusually high deletion frequency, particularly in a gene encoding only a small protein of 140 amino acids. The five copies of *SEDL* mapped to the Y chromosome (Gecz et al. 2000) may provide a possible mechanism to explain the high frequency of deletion mutations affecting this gene through homologous recombination and slipped mispairing.

Nucleotide-base substitutions were a relatively less common type of mutation contributing to the pathogenesis of *SEDL*. Equal numbers of transitions and transversions were present. All five nonsynonymous base substitutions, however, alter the amino acids that are highly conserved across species. D47 and L61 are the most highly conserved across mouse, rat, fruit fly, yeast, and worm, whereas S73, Q91, and V130 are retained in the mouse and rat (Gedeon et al. 1999). The *SEDL*-gene function is not clear, but knowledge of the role of the yeast ortholog in endoplasmic reticulum-to-Golgi transport, and the subcellular localization of normal *sedlin* constructs, supports the possibility that the *SEDL* protein may be involved in intracellular transportation of molecules (Gecz et al. 2000).

In the present study, we observed eight small deletion/insertion mutations of the ORF that cause frameshifts in a maximum of 13 independent events over a target sequence of only 420 bp. By comparison, Gianelli et al. (1999) observed 17 independent events of such types of mutation in the 1,362-bp factor IX-coding sequence.

The uncommonly high frequency of deletion mutations in *SEDL* would explain this disparity. All eight mutations result in a shift to the same alternate *SEDL* reading frame that accordingly terminates in one of three new downstream stop positions, at D87X, T100X, or E116X. The most 3' of these arises because of the 320–321insT mutation, which causes silent changes at amino acids 107 and 108, maintaining the Ile(I) and Lys(K) sequences—with the first putative protein variation introduced by the frameshift, not detectable until F109V, culminating in E116X. At the protein level, there may be some functional dependency on the highly conserved amino acids 111–115. This amino acid sequence, MNPFY, is conserved in mouse, rat, worm, and yeast, with the NPFY being retained also in fruit fly (Gedeon et al. 1999). Of course, the early truncation of the protein will also affect the putative Golgi-targeting domains within the COOH terminus. Subcellular-localization studies of constructs of a naturally occurring nonsense mutation in exon 5 (C271T) and of 157–158delAT showed misplacement of the protein from the vesicular tubular complex VTC/Golgi to the cell nucleus (Gecz et al. 2000). On the basis of this finding, it can be predicted that the remaining mutations that lead to a polypeptide truncated at D87X—for example, 191–192delTG and 241–242delAT—would also show a loss of function of the protein, through misplacement of the *SEDL* protein within the cell.

One-third of the *SEDL* cases are due to mutations that result in a putative splicing error. The recurrence of one of these in particular—IVS3+5G→A, affecting splicing of exon 3—is a transitional base substitution in the consensus splice donor. This changes the consensus strength from 84% to 5% at this nucleotide (Shapiro and Senepathy 1987). Experimental demonstration of this splicing error and of its effects on tissue histology will be discussed in detail elsewhere (Tiller et al. 2001 [in this issue]). Ethnic and haplotype differences support the recurrence of this disease-causing mutation (tables 1 and 3). The splice-acceptor-site mutations in introns 2 and 5 of *SEDL* may cause skipping of following exons (and of the initiator methionine in patient 1), retention of the intron in the final mRNA, or activation of a cryptic splice site, all potentially causing changes to the protein (Maquat 1996). The deletions in donor splice sites in intron 4 (i.e., 241–9 through –12 and 241–4 through –11) may have the same effects on the consensus splice site or may alter a branch site, affecting recognition of the splice junction by the spliceosome. Since only genomic DNA was available in most cases, the consequences of these splice-site mutations could not be proved experimentally.

Haplotype analyses in patients sharing each of the four common mutations demonstrated that the *SEDL* gene is subject to recurrent mutation rather than to an

ancestral founder mutation (table 3). The 157–158delAT haplotypes are the same at several flanking loci but are also the same as one of the control haplotypes. The various ethnic origins of four patients with the CAAGA pentanucleotide deletion in exon 5 also indicate recurrence rather than IBD of this mutation. A similar 5-bp deletion mutation has also been detected in a large family with *SEDL* from Arkansas (Mumm et al. 2000). The sequence in this region is such that the 5-bp deletion could be any one of five alternatives (AA-GAC, AGACA, GACAA, ACAAG, or CAAGA) that include the C base at position 271. Two other mutations—C271T and 272–273delAA—also disrupt the glutamine amino acid at position 91. One might speculate that the sequence in this region is relatively more vulnerable to pathogenic mutation than is the remainder of the gene.

Given the spectrum of mutations altering the expression of the *SEDL* gene, the phenotypic findings in these families with *SEDL* have shown a remarkable degree of homogeneity. The large-scale deletions involving exons at either end of the gene do not seem to have more-deleterious consequences; however, the additional features of kyphosis and scoliosis and the reporting of pain appear to be greater and to occur at an earlier age when mutations are at the 5' end of the ORF. On the other hand, in terms of relative severity, tolerance of exercise in two other Australian patients has been unimpeded by the condition. It appears that, among affected families, there is no clearly distinguishable clinical effect of the types of mutation, including missense and truncation mutations, despite the considerable allelic heterogeneity of *SEDL*. There do not seem to be any specific regions of functional significance in the *SEDL* gene that have a role in determining the severity of the primary clinical outcome. There is insufficient clinical variation to predict the phenotype based on the genotype, though this may reflect the ascertainment, for inclusion in the study, of patients with a specific phenotype commonly accepted as typical *SEDL*.

The *SEDL* phenotype might be explained by reduced endochondral bone growth at the epiphyses, particularly in the vertebral bodies. The autosomal forms of *SEDL* can largely be accounted for by mutations in the type II procollagen gene (*COL2A1*), which suggests some functional interaction between *sedlin* and collagen. Recurrent R519C mutations in *COL2A1* are known to be associated with mild, late-onset spondyloepiphyseal dysplasia (SED) and precocious generalized osteoarthritis (Ala-Kokko et al. 1990; Holderbaum et al. 1993; Williams et al. 1995; Bleasel et al. 1998). A spectrum of *COL2A1* mutations cause more-severe disorders of long-bone growth, through alteration of the assembly, interactions, and secretion of the collagen triple helix (Kuivaniemi et al. 1997). The R519C mu-

tation may alter this “end molecule,” such that an interacting surface is not exposed, or in a way that would slow secretion of the molecule (Byers 2000). Similarly, the *SEDL* mutations may prevent *SEDL*-protein interactions with other proteins in the TRAPP complex (Gecz et al. 2000), which may itself have a role in collagen biosynthesis and/or transport.

Currently the diagnostic precision of “X-linked” *SEDL*, in the absence of clear radiographic features, rests on recognition of the mode of inheritance. Of six additional patients, who, on the basis of clinical descriptions, were tested for *SEDL* mutation, three had no family history and three had unknown family history beyond that of the nuclear family. Some had flattened vertebral bodies shown on radiographic examination, but none had a detectable mutation in the analyzed ORF or in the closely flanking intronic sequences. These patients may have mutations in regions of the gene that were not tested, such as in introns away from the exon-intron boundaries or in regulatory elements, or they may be autosomal phenocopies of *SEDL*. This suggests that not all cases of *SEDL* are clinically recognizable. Reexamination of the clinical data and mutation analysis of *COL2A1* might be instructive for these patients. The correct establishment of etiology and mode of inheritance is critical for determining the recurrence risks and risks to relatives. Molecular analysis of *SEDL* will now be a useful adjunct to clinical diagnosis and molecular analysis of *COL2A1*, to distinguish between the X-linked and autosomal forms, particularly in sporadic and atypical cases. The question of clinical heterogeneity caused by the *SEDL* gene remains, since the sample cohort included only those patients recognized clinically as having typical X-linked *SEDL*. Although heterogeneity may be sought where none exists, studies to determine the role of *SEDL* in premature osteoarthritis are in progress. Only one study has been published of a family with a severe X-linked variant of spondyloepimetaphyseal dysplasia (SEMD) with distinctive phenotype and severe, progressive mental retardation (Bieganski et al. 1999). Another family has been described with nonsyndromic X-linked SEMD that is clinically distinct from X-linked *SEDL* (Camera et al. 1994). SEMD may be part of the clinical picture defined by the *SEDL* gene, or these families may represent the heretofore-unrecognized genetic heterogeneity of X-linked *SEDL*. The analysis of atypical cases will permit estimation of the true prevalence of *SEDL*-gene mutations and will give greater insight into the function of the gene in bone growth.

That both the *SEDL* and adjacent *Cxorf5* genes escape X inactivation has been demonstrated in a first-generation study of X inactivation (Carrel et al. 1999). Recent microarray-expression profiling has confirmed that *SEDL* and the nearby *GPM6B* also escape X in-

activation (Sudbrak et al. 2001). These genes lie within an ~100-kb interval in Xp22 (Gecz et al. 2000). Such a nonrandom distribution may indicate another multigene cluster escaping inactivation, similar to that described in Xp11.2 (Miller and Willard 1998). The lack of clear evidence of the SEDL phenotype in carrier females suggests that expression of the normal gene product can compensate for expression of the defective gene. The classic description of SEDL suggests that bone growth and formation are normal in affected males during infancy and early childhood. Longitudinal studies of phenotype development may reveal a postnatal window of time during which therapeutic intervention may reduce or override the ill effects of mutations. The timely switching to up-regulate the endogenous expressed pseudogene on chromosome 19 (Gedeon et al. 1999) may provide a molecular means of therapy.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/index.html> (for SEDL mRNA [accession number NM_014563] and SEDL exons 1 [accession number AF157060], 2 [accession number AF157061], 3 [accession number AF157062], 4 [accession number AF157065], 5 [accession number AF157064], and 6 [accession number AF157065])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for SEDT [MIM 271600, MIM 184100], *SEDL* [MIM 300202], and SEDL [SEDT, X-linked] [MIM 313400])

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