Recurrent Dominant Mutations Affecting Two Adjacent Residues in the Motor Domain of the Monomeric Kinesin KIF22 Result in Skeletal Dysplasia and Joint Laxity

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Spondyloepimetaphyseal dysplasia with joint laxity, leptodactylic type (lepto-SEMDJL, aka SEMDJL, Hall type), is an autosomal dominant skeletal disorder that, in spite of being relatively common among skeletal dysplasias, has eluded molecular elucidation so far. We used whole-exome sequencing of five unrelated individuals with lepto-SEMDJL to identify mutations in *KIF22* as the cause of this skeletal condition. Missense mutations affecting one of two adjacent amino acids in the motor domain of KIF22 were present in 20 familial cases from eight families and in 12 other sporadic cases. The skeletal and connective tissue phenotype produced by these specific mutations point to functions of KIF22 beyond those previously ascribed functions involving chromosome segregation. Although we have found *Kif22* to be strongly upregulated at the growth plate, the precise pathogenetic mechanisms remain to be elucidated.

Heritable disorders of skeletal growth and development have revealed a surprising variety of underlying molecular mechanisms, bringing this clinical and diagnostically difficult field to the front of molecular genetics research.[1,2] Genes responsible for these disorders might code for extracellular structural proteins, enzymes responsible for the synthesis or degradation of matrix components, hormones and signal transmission factors, nuclear transcription factors, intracellular cytoskeletal proteins, structural proteins of the endoplasmic reticulum, noncoding RNAs, and most recently, genes involved in ciliary assembly and transport. Here we report that mutations in *KIF22* (aka *KID* [MIM 603213]), which encodes a monomeric kinesin,[3–5] are the cause of spondyloepimetaphyseal dysplasia with joint laxity, leptodactylic type (lepto-SEMDJL; aka SEMD, Hall type [MIM 603546]). This implicates this class of molecules in the pathogenesis of human skeletal dysplasias and suggests a hitherto unknown role for KIF22 in skeletal growth and homeostasis.

Lepto-SEMDJL is characterized by a flat face, perinatal onset of short stature with shortening of both the trunk and the limbs, generalized joint laxity with multiple dislocations, and progressive scoliosis and limb deformity.[6] The radiographic pattern is that of a spondyloepimetaphyseal dysplasia with moderately flattened vertebral bodies, striated metaphyses, and small and fragmented epiphyses with delayed maturation. The most distinctive features for differential diagnosis are the slender metacarpals and phalanges ("leptodactyly," meaning slender fingers) and the progressive degeneration of carpal bones; however, the latter two features are evident only in older children and young adults. The soft consistency of cartilage in the airways leads to laryngotracheomalacia with proneness to respiratory obstruction and inspiratory stridor in infancy and childhood.[7–9] Although the majority of cases

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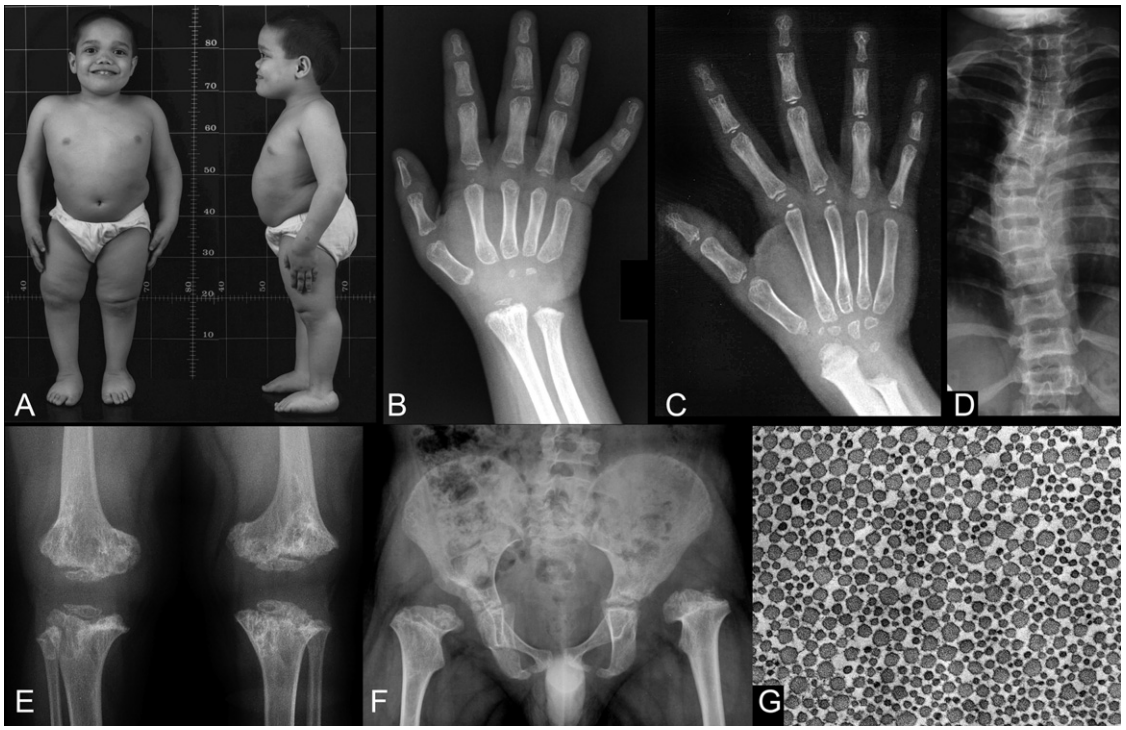

Figure 1. Morphologic Features of Lepto-SEMDJL
(A, B, D, E and F) are all from subject 2 (family 1) at age 7.
(A) In this boy, stature is markedly below the normal range, with short-trunk type disproportion. There is frontal bossing with flattening of the face and a sunken nasal bridge. There is left hip subluxation (F), leg length difference, and right genu varum (A). Joint laxity is indicated by the scoliosis and the flat feet.
(B) The hand radiographs of this boy; there is a very marked delay in the maturation of all epiphyseal centers and of the carpal bones, as well as metaphyseal irregularities at the distal radius and ulna.
(C) The hand X-ray of an unrelated boy, age 10. Also in this individual, there is a marked delay in all secondary ossification centers and there is shortening of the distal ulna. The proximal phalanges and the metacarpals are slender; this feature, leptodactyly, that becomes apparent only over time, is characteristic for this bone dysplasia.
(D) The moderate platyspondyly and the scoliosis (ligamentous laxity).
(E) The marked dysplasia of the metaphyses at the knee (distal femur, proximal tibia) and at the same time the small and dysplastic epiphyses.
(F) A similar pattern at the proximal femurs with shortening of the femoral necks and the presence of epiphyses that are barely visible and markedly small for age. The acetabula are not well developed; they are less well developed on the left than on the right; the left hip is subluxated because of the acetabular dysplasia and associated ligamentous laxity.
(G) An electron microscopy image of a tendon biopsy section (subject 6 in Table 1) at right angle to the collagen fiber (magnification, approximately 5000×). The diameter of the fibers shows a significant variability.

have been sporadic in their families, dominant inheritance has been documented.[8,10–12] The condition is likely to be both under- and misdiagnosed because the specific radiographic findings appear only in late childhood.

The pathogenesis of lepto-SEMDJL has remained obscure. Disturbed formation of the extracellular matrix was suggested by the observation of highly abnormal collagen fibers in a tendon biopsy of an affected individual (Figure 1). This, and some phenotypic overlap with two other conditions characterized by generalized bone dysplasia and joint laxity, namely spondyloepiphyseal dysplasia congenita (a dominant collagen 2 disorder [MIM 183900]) and pseudoachondroplasia (a dominant disorder associated with mutations in cartilage oligomeric matrix protein [MIM 177170]) had led to the investigation of these genes in a few cases, with negative results.

We studied a cohort of 32 affected individuals with lepto-SEMDJL from 20 families of different ethnic origins (Table 1). The study was approved by the cantonal ethic committee of Lausanne, Switzerland. In 20 individuals from eight families, the condition was inherited in an autosomal dominant manner, whereas there was no family history of disease in 12 individuals. Clinical and radiographic features common to all affected individuals are summarized in Figure 1. A significant proportion of cases presented laryngotracheomalacia. We performed whole-exome sequencing by using DNA from five unrelated lepto-SEMDJL individuals (subjects 2, 6, 7, 30, and 32 in Table 1) and 14 unrelated controls to identify gene variants that were present in affected individuals and absent in controls. Exome capture utilized the SureSelectXT Human All Exon 50Mb kit (Agilent Technologies) following the manufacturer's protocol, except that we used adapters

Table 1. Clinical Features, Origin, Inheritance, and Mutations in *KIF22* in All Individuals with Lepto-SEMDJL

Family	Subject	Origin	Short Stature	Skeletal Dysplasia	Laryngeal Stenosis	Joint Laxity	*KIF22* Mutation (cDNA)	KIF22 Mutation (Protein)	Inheritance or De Novo
1	1,2,3,4	Italy	+	+	–	+	c.443C>T	p.Pro148Leu	autosomal dominant
2	5,6	UK	+	+	–	+	c.443C>T	p.Pro148Leu	autosomal dominant
3	7,8,9	USA	+	+	–	+	c.446G>A	p.Arg149Gln	autosomal dominant
4	10,11	Italy	+	+	–	+	c.446G>A	p.Arg149Gln	autosomal dominant
5	12,13,14	Japan	+	+	–	+	c.446G>A	p.Arg149Gln	autosomal dominant
6	15,16	UK	+	+	–	+	c.446G>A	p.Arg149Gln	autosomal dominant
7	17,18	Belgium	+	+	+	+	c.446G>A	p.Arg149Gln	autosomal dominant
8	19,20	USA	+	+	not known	+	c.446G>A	p.Arg149Gln	autosomal dominant
9	21	Lebanon	+	+	not known	+	c.446G>A	p.Arg149Gln	de novo
10	22	France	+	+	+	+	c.446G>A	p.Arg149Gln	de novo
11	23	Greece	+	+	–	+	c.446G>A	p.Arg149Gln	de novo
12	24	UK	+	+	+	+	c.446G>A	p.Arg149Gln	de novo
13	25	Germany	+	+	not known	+	c.446G>A	p.Arg149Gln	de novo
14	26	USA	+	+	not known	+	c.446G>A	p.Arg149Gln	de novo
15	27	USA	+	+	not known	+	c.446G>T	p.Arg149Leu	de novo
16	28	USA	+	+	not known	+	c.443C>T	p.Pro148Leu	de novo
17	29	Brazil	+	+	+	+	c.443C>T	p.Pro148Leu	de novo
18	30	Germany	+	+	+	+	c.443C>T	p.Pro148Leu	de novo
19	31	Japan	+	+	+	+	c.443C>T	p.Pro148Leu	de novo
20	32	Italy	+	+	not known	+	c.443C>T	p.Pro148Leu	de novo

with 3 bp barcodes[13] to allow multiplexing of samples during capture. Captures were performed in seven independent reactions with two to four samples per reaction with 300–500 ng DNA per sample and then combined into one molarity-balanced library on which we performed 75 bp paired-end sequencing in seven lanes of one Illumina HiSeq2000 flow cell. Sequence reads were debarcoded with Novobarcode (Novocraft Technologies), aligned to the reference genome (hg19) with BWA,[14] and culled of PCR duplicate reads with SAMtools.[15] Variant bases were called with SAMtools/BCFtools, and annotated with ANNOVAR,[16] filtered by presence in dbSNP132, 1000 g, and our 14 unaffected control samples, and prioritized according to putative functionality; splice site and coding nonsilent variants were given the highest priority.

We identified one gene, *KIF22*, in which one of two heterozygous missense mutations (p.Pro148Leu [c.443C>T] or p.Arg149Gln [c.446G>A]) was present in all five lepto-SEMDJL cases and absent in 2,500 exomes from the National Heart Lung and Blood Institute Exome Sequencing Project (accessed August 2011). The mutations alter highly conserved residues within the KIF22 motor domain near an ATP-binding site (Figure 2). Sanger sequencing of *KIF22* exon 4 in all 32 lepto-SEMDJL affected individuals and available relatives revealed that all affected persons were heterozygous for either p.Pro148Leu, p.Arg149Gln, or p.Arg149Leu [c.446G>T] allele (Table 1) and that these mutations were not present in unaffected relatives. We tested both parents (and unaffected siblings when available) of each affected individual and complete cosegregation of mutations with the phenotype was identified. Sequencing of exon 4 in 480 unrelated control samples of European descent (Sigma-Aldrich) revealed no mutations (data not shown).

We next tested whether the lepto-SEMDJL mutations affect protein abundance, posttranslational processing, or cytoskeletal architecture by using skin fibroblast cell lines from three unrelated affected individuals (subjects 1, 10, and 32 in Table 1) and controls as well as in tendon derived fibroblast-like cells of subject 6 and a control cell line. We observed no differences from control in KIF22 localization or cytoskeletal architecture as determined by anti-KIF22 or anti-tubulin immunofluorescence (data not shown). Because immunoblot performed with a commercial antibody against human KIF22 failed to reveal expression in both skin fibroblasts and tendon fibroblast-like cells from lepto-SEMDJL affected individuals and controls (data not shown), we looked for specific expression in cartilage growth plates of wild-type mouse tibia. Quantitative RT-PCR in microdissected mouse growth plates, performed

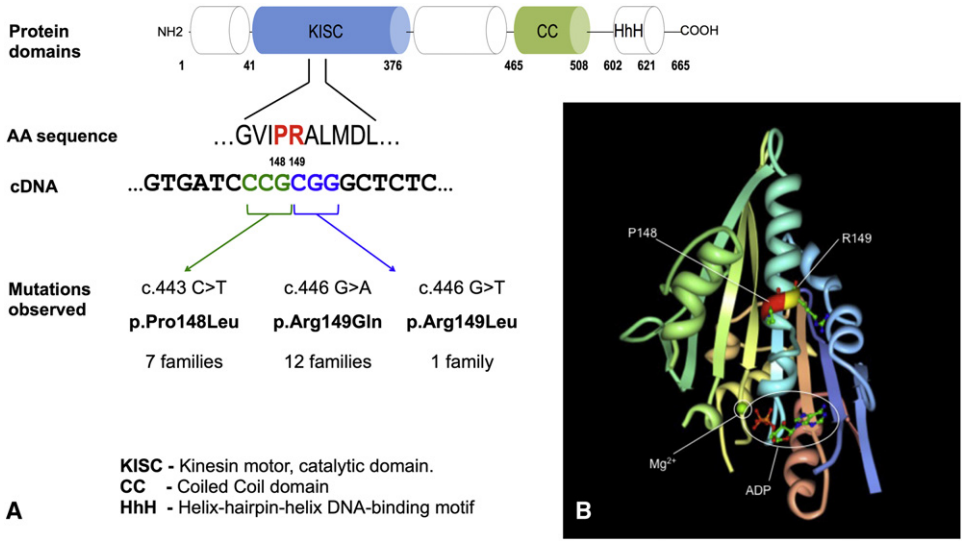

Figure 2. Schematic Representation of KIF22 Protein and Mutations Found in Individuals with Lepto-SEMDJL
(A) Functional domains of KIF22; the mutations found in lepto-SEMDJL patients are located on two adjacent amino acid residues in the motor/catalytic domain.
(B) Crystal structure of the motor domain of KIF22, generated with Research Collaboratory for Structural Bioinformatics-Protein Data Bank (RCSB-PDB), viewer Protein Workshop (see Web Resources; deposition: 2007-11-22; release: 2007-12-04; last modified 2011-07-13). The two contiguous residues mutated in lepto-SEMDJL (Pro148 and Arg149) are located close to the ATP/ADP binding site.

as previously described[17] and with the housekeeping gene *Mrps16* as a reference, showed strong upregulation of *Kif22* in the proliferative zone of the growth plate (Figure 3 and Table S1, available online).

The observation that *KIF22* mutations were restricted to two adjacent codons in all examined lepto-SEMDJL individuals confirms the genetic homogeneity of this disorder and the specificity of the diagnostic criteria as outlined by Hall et al.[6] and Kim et al.[18] The findings also raise questions on the possible molecular mechanisms. Haploinsufficiency seems unlikely, because it would be extremely unusual to have independent occurrence in a large number of unrelated pedigrees clustering on two adjacent amino acids; instead, the two residues must have a functional role that is hitherto unknown. Previously ascribed functions for KIF22, based upon knockout and knockdown studies, involved spindle formation,[19] chromosomal movement,[19] microtubule stabilization,[5,20] genomic stability, and cellular replication.[21] However, the phenotype of lepto-SEMDJL does not have any feature that would seem related to these functions. Several kinesins play important roles in the transport of morphogens (KIF3B),[22] cell surface receptors (KIF17),[23] and matrix metalloproteinases (KIF5B, KIF3A, and KIF3B).[24–26] By analogy, KIF22 might also have an important trafficking role in chondrocytes or the motor domain missense mutations might cause KIF22 to interfere with other motor domain kinesins that function in cartilage. As an alternative explanation, the mutations at residues 148 and 149 might transspecify the protein, conferring to it some properties normally reserved to other members of the kinesin family. This latter hypothesis is supported by the observation that some kinesins do have Leu or Gln at position 149 (Supplemental Data). Finally, the mutations might produce a dominant negative effect if KIF22 heterodimerizes with other kinesins and/or interacts with other partner proteins.

To date, few human monogenic diseases have been associated with mutations in any of the 45 currently annotated human kinesin genes. Recessive mutations in kinesin genes have been associated with acrocallosal syndromes, Joubert syndrome (*KIF7*),[27,28] and hereditary sensory and autonomic neuropathy type 2 (*KIF1A*).[29] Dominant, recurrent missense mutations in a methylated CpG dinucleotide in the C-terminal domain of *KIF21A* cause congenital fibrosis of extraocular muscles,[30,31] and a dominant missense mutation in the motor domain of *KIF1B* (p.Gln98Leu [c.293A>T]) has been associated with Charcot-Marie-Tooth type 2 disease in a single pedigree.[32] Similar to the p.Gln98Leu (c.293A>T) KIF1B mutation, the *KIF22* mutations found in lepto-SEMDJL affect the motor domain of the kinesin and might therefore result in a loss of its motor activity.[32] However, as outlined above, simple loss of function is unlikely to explain the clustering of mutations on the two adjacent amino acids. Because kinesins have not been associated with human cartilage biology so far, our findings also raise the possibility that KIF22 might be implicated in intracellular transport (and possibly, secretion) of an extracellular matrix protein and/or in cilia-associated transport mechanisms, two mechanisms that have been evoked in *Kif3a* and *Kif5b* conditional chondrocytic knockouts.[33,34] This hypothesis is supported by our expression data in mouse growth plates. Strong expression of *Kif22* in the proliferative zone of the growth plate with downregulation in hypertrophic chondrocytes is compatible with a broader role of KIF22 in the synthesis of extracellular

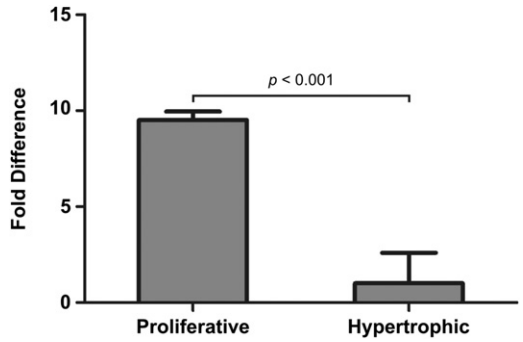

Figure 3. Quantitative RT-PCR Analysis of *Kif22* Expression in Wild-Type Mouse Growth Plate Cartilage Zones
qPCR was performed for *Kif22* on cDNA derived from proliferative and hypertrophic zones microdissected from 2-week-old wild-type mouse tibial growth plates (n = 3). qPCR was conducted with three technical replicates. *Kif22* expression was calculated relative to the housekeeper gene *Mrps16* and expressed as the fold difference in expression in the proliferative zone compared to the hypertrophic zone. Statistical significance was calculated with the Student's t test. Error bars represent standard deviation. Detailed quantitative expression data are reported in Table S1.

matrix rather than with a more restricted role in the hypertrophic zone, that would be a prelude to calcification and vascular invasion. Alternatively, this finding might be explained by higher expression of *KIF22* in proliferating cells because of its likely involvement in chromosomal movement during cell division.

The elucidation of the mechanism by which the specific mutations at codons 148 and 149 of *KIF22* result in a phenotype restricted to bone and connective tissue will require the design of knockin experiments with appropriate cellular or animal models. Given the relatively high frequency of lepto-SEMDJL, its dominant inheritance, and the diagnostic difficulty in infancy and early childhood, we communicate our findings in order to allow the clinical community and the affected families to benefit from them and to inform basic scientists involved in the study of KIF22 and other kinesins about these unexpected aspects of KIF22 physiology.

Supplemental Data

Supplemental Data include one figure and one table and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Ensembl, http://www.ensembl.org/index.html
Integrative Genomics Viewer, http://www.broadinstitute.org/igv/
National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/
National Heart Lung and Blood Institute (NHLBI) Exome Sequencing Project, http://snp.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
RCSB-PDB, http://www.rcsb.org/
UCSC Genome Browser, http://genome.ucsc.edu/

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