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Report

Mutated *MESP2* Causes Spondylocostal Dysostosis in Humans

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Spondylocostal dysostosis (SCD) is a term given to a heterogeneous group of disorders characterized by abnormal vertebral segmentation (AVS). We have previously identified mutations in the *Delta-like 3* (*DLL3*) gene as a major cause of autosomal recessive spondylocostal dysostosis. *DLL3* encodes a ligand for the Notch receptor and, when mutated, defective somitogenesis occurs resulting in a consistent and distinctive pattern of AVS affecting the entire spine. From our study cohort of cases of AVS, we have identified individuals and families with abnormal segmentation of the *entire* spine but no mutations in *DLL3*, and, in some of these, linkage to the *DLL3* locus at 19q13.1 has been excluded. Within this group, the radiological phenotype differs mildly from that of *DLL3* mutation–positive SCD and is variable, suggesting further heterogeneity. Using a genomewide scanning strategy in one consanguineous family with two affected children, we demonstrated linkage to 15q21.3-15q26.1 and furthermore identified a 4-bp duplication mutation in the human *MESP2* gene that codes for a basic helix-loop-helix transcription factor. No *MESP2* mutations were found in a further 7 patients with related radiological phenotypes in whom abnormal segmentation affected all vertebrae, nor in a further 12 patients with diverse phenotypes.

The spondylocostal dysostoses (SCD [MIM 277300]) are a heterogeneous group of disorders with severe axial skeletal malformation characterized radiologically by multiple vertebral segmentation defects (MVSD). In addition, the ribs are frequently malaligned, with points of fusion and sometimes a reduction in number. The diverse SCD phenotypes have been reviewed elsewhere (Turnpenny and Kusumi 2003). Sporadic cases occur more commonly than familial ones and are more likely to be associated with multiple congenital abnormalities, whereas monogenic forms more commonly demonstrate autosomal recessive (AR) rather than autosomal dominant inheritance. We previously demonstrated linkage in some families with AR SCD to chromosome 19q13.1 (Turnpenny et al. 1999), a region syntenic to the *Dll3* homologue on mouse chromosome 7. Mutations in murine *Dll3* result in the "Pudgy" phenotype (Kusumi et al. 1998), and we subsequently identified mutations in the human *Delta-like 3* (DLL3 [MIM 602768]) gene (Bulman et al. 2000). We and others have characterized 17 familial mutations in *DLL3*, including nonsense, missense, frameshift, and splice-site mutations, all of which are likely to lead to functional null alleles either by premature truncation of the protein or by the substitution of essential amino acid residues (Bulman et al. 2000; Sparrow et al. 2002; Bonafé et al. 2003; Turnpenny et al. 2003).

Here we have studied a consanguineous family of Lebanese Arab origin with two offspring affected with SCD (fig. 1). Affected individuals presented with truncal shortening and short necks but no other abnormalities. On radiological examination, thoracic vertebrae bear a resemblance to those seen in SCD due to mutated *DLL3*, but the lumbar vertebrae appear more angular and irregular. The vertebral morphology, with regional differences throughout the spine, is highlighted by MR imaging (fig. 2). By use of genomic DNA extracted from blood samples from all five available family members, flanking microsatellite markers excluded linkage to the

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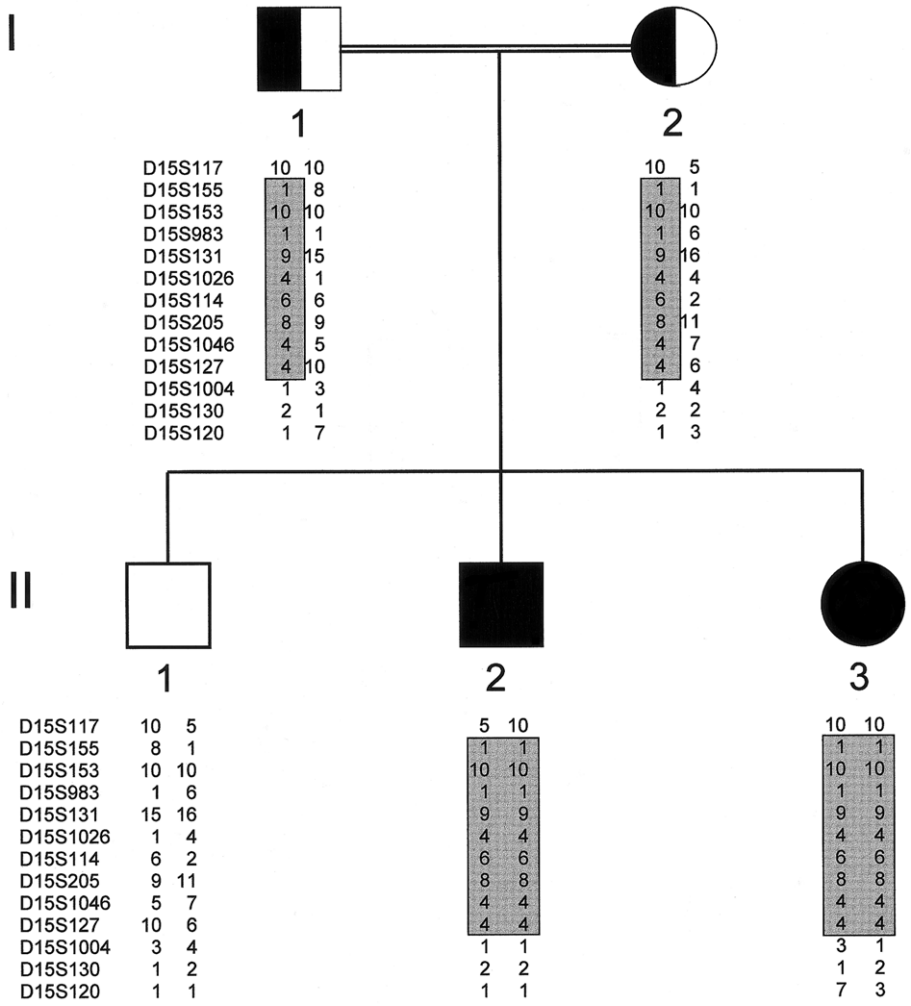

Figure 1 Pedigree of the family affected with SCD, *MESP2* type, demonstrating disease-linked haplotype. Affected members II.2 and II.3 are indicated by fully blackened symbols.

19q13.1/*DLL3* locus. Subsequent genomewide homozygosity mapping, performed using the Perkin Elmer Biosystems Linkage Mapping Set (version 1) with affected genomic DNA from individual II.2, revealed 84 homozygous markers scattered throughout the genome, of which 6 were concentrated in a block on 15q (D15S153 to D15S120) and 5 were concentrated in a block on 20q (D20S117 to D20S186). Subsequent mapping, performed using the second affected individual II.3 and the unaffected sibling II.1, excluded linkage to the 20q region but demonstrated linkage to the 15q markers—namely, D15S153, D15S131, D15S205, and D15S127. Subsequent fine mapping, performed using additional markers, demonstrated a 36.6-Mb region (according to The Human Genome Working Draft) on 15q21.3-15q26.1 between markers D15S117 and D15S1004 with a maximum two-point LOD score of 1.588 at $\theta = 0$ for markers D15S131, D15S205, D15S1046, and D15S127 (table 1). The region between markers D15S117 and D15S1004 contains >50 genes and is syntenic to mouse chromosome 7, which contains the *Mesp2* gene (MESP2 [MIM 605195]). The *Mesp2*-knockout mouse manifests altered rostro-caudal polarity, resulting in axial skeletal defects (Saga et al. 1997). The mouse gene was located using the Blat algorithm at The Human Genome Working Draft with MesP2 cDNA accession no. NM_008589, and the human gene was located, again using the Blat and Comparative Genome algorithms of The Human Genome Working Draft. Analysis of the predicted human gene, *MESP2,* reveals a two-exon gene spanning ~2 kb of 15q26.1. Direct sequencing of *MESP2* gene in the two affected individuals, by use of the primers M1 1F (5′-GAC ACC TCT CTG CAA CC TG-3′), M1 1R (5′-CCT GGA GTA GAT AAG CTG GG-3′), M1 2F (5′-CCA GCC ATA CCA TGG CAA C-3′), and M2 2R (5′-CCA AGC TAC AGG

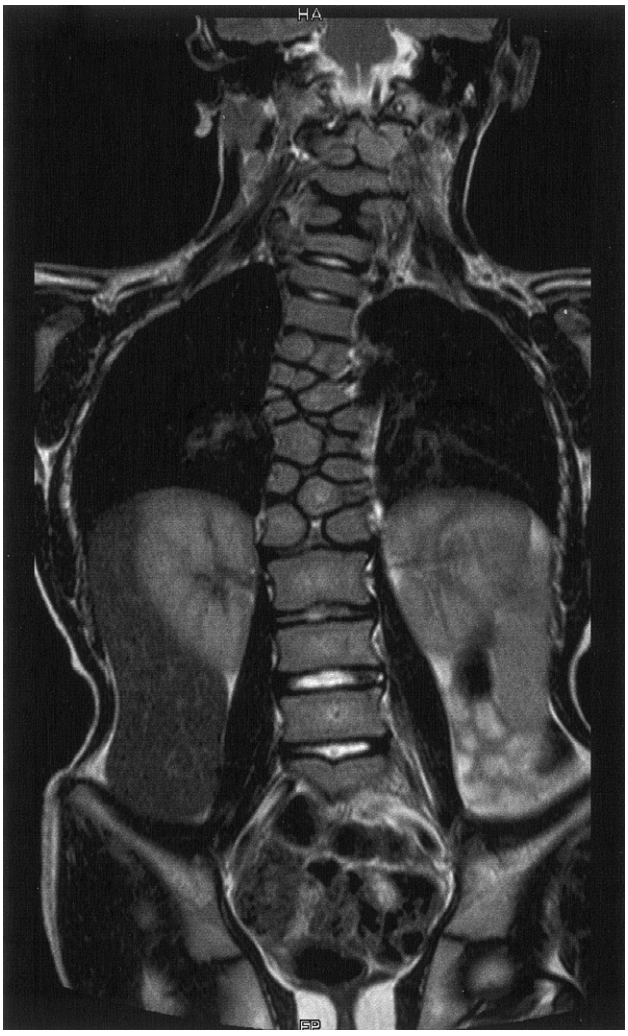

Figure 2 Anteroposterior MRI of the spine of individual II.2. The morphology of the thoracic spine and the upper cervical spine is severely disrupted, with multiple hemivertebrae, whereas the remaining regions show less severe disruption.

ACT GAT TC-3′), demonstrated a homozygous 4-bp (ACCG) duplication mutation in exon 1, termed "500–503dup" (fig. 3). The parents, I.1 and I.2, were shown to be heterozygous, and the unaffected sibling, II.1, was shown to be homozygous normal. Thus, the duplication segregates with the disease in this family. In addition, fluorescent PCR excluded this mutation from 68 ethnically matched control chromosomes. In analysis of the genomic structure of the *MESP2* gene, we note that there is a discrepancy between the Sanger Centre and NCBI human genomic assembly databases. In the latter, there is an additional short intron located after base 502 of the *MESP2* coding region. This does not appear in the Ensembl gene prediction, and, due to a lack of consensus splice sites within this proposed intronic sequence, we do not believe this intron exists. Clarification of this issue would necessitate transcript isolation and sequence analysis. This would be a difficult and ethically challenging task, since *MESP2* is only expressed for a brief period during embryogenesis, in a highly restricted domain in the presomitic mesoderm. However, the presence or absence of such an intron does not effect our conclusions about the effects of the 4-bp insertion on MESP2 protein production. The insertion is located overlapping the NCBI proposed 5′ splice site and would interrupt splicing, leading to a frameshift at the same point in the MESP2 protein.

The *MESP2* gene is predicted to produce a transcript of 1,191 bp encoding a protein of 397 amino acids with a predicted molecular weight of 41,744 Da and isoelectric point (pI) of 7.06. The human MesP2 protein has 58.1% identity with mouse MesP2, and 47.4% identity with human MesP1. Human MesP2 amino terminus contains a basic helix-loop-helix (bHLH) region encompassing 51 amino acids divided into an 11-residue basic domain, a 13-residue helix I domain, an 11-residue loop domain, and a 16-residue helix II domain. The loop region is slightly longer than that found in homologues such as *paraxis*. The length of the loop region is conserved between mouse and human MesP1, MesP2, and Thylacine 1 and 2, as well as chick mesogenin. In addition, both MesP1 and MesP2 contain a unique CPXCP motif immediately carboxy-terminal to the bHLH domain. The amino- and carboxy-terminal domains are separated in human MesP2 by a GQ repeat region also found in human MesP1 (2 repeats) but expanded in human MesP2 (13 repeats). Mouse MesP1 and MesP2 do not contain GQ repeats, but they do contain two QX repeats in the same region: mouse MesP1 QSQS; mouse MesP2 QAQM. Hydrophobicity plots indicate that

Table 1

Two-Point LOD Scores between ARSCD Type 2 and Chromosome 15q Markers

	Z AT θ =						
MARKER	.00	.05	.10	.20	.30	.40	.50
D15S117	−∞	−.282	−.085	.020	.273	.015	.000
D15S155	1.125	.991	.857	.594	.353	.151	.000
D15S153	.034	.026	.019	.095	.004	.001	.000
D15S983	1.125	.991	.857	.594	.353	.151	.000
D15S131	1.588	1.420	1.248	.895	.550	.239	.000
D15S1026	1.125	.991	.857	.594	.353	.151	.000
D15S114	1.125	.991	.857	.594	.353	.151	.000
D15S205	1.588	1.420	1.248	.895	.550	.239	.000
D15S1046	1.588	1.420	1.248	.895	.550	.239	.000
D15S127	1.588	1.420	1.248	.895	.550	.239	.000
D15S1004	−∞	−1.137	−.653	−.266	−.103	−.025	.000
D15S130	−∞	−.282	−.085	.020	.273	.015	.000
D15S120	−∞	−1.929	−1.148	−.488	−.200	−.059	.000

NOTE.—All values are calculated under the assumption of 100% penetrance, a disease allele frequency of 0.0001, and a disease haplotype frequency of 0.1.

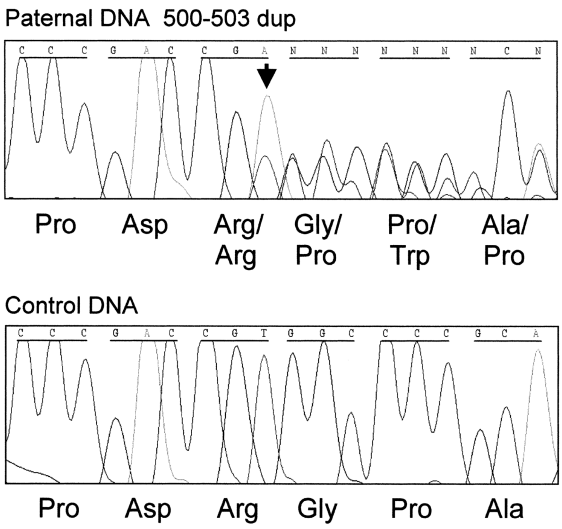

Figure 3 Detection of the mutation 500–503dup by direct nucleotide sequencing. The sequence of a heterozygous individual (as indicated by the arrow) (*top*) is compared with the normal homozygous sequence (*bottom*).

MesP1 and MesP2 share a carboxy-terminal region that is predicted to adopt a similar fold, although MesP2 sequences do contain a unique region at the carboxy-terminus.

Sequence analysis of 20 ethnically matched and 10 nonmatched individuals revealed the presence of a variable length polymorphism in the GQ region of human MesP2, beginning at nucleotide 535. This region contains a series of 12-bp repeat units. The smallest GQ region detected contains two type A units (GGG CAG GGG CAA, encoding the amino acids GQGQ), followed by two type B units (GGA CAG GGG CAA, encoding GQGQ) and one type C unit (GGG CAG GGG CGC, encoding GQGR). The polymorphism comprises a variation in the number of type A units, with 2, 3, or 4 being present in the sequenced individuals. The fluorescent PCR used to confirm the familial mutation in the 68 control chromosomes revealed 23 alleles of 2 repeats, 44 alleles of 3 repeats, and 1 allele of 4 repeats, yielding a polymorphism information content (PIC) value of 0.3707, thus demonstrating its usefulness in linkage analysis. The 20 nonethnically matched chromosomes consisted of 7 alleles of 2 repeats, 12 alleles of 3 repeats, and 1 allele of 4 repeats. Therefore, allelic frequencies are not statistically significantly different between these two populations ($P = .41$).

Here we have shown that a frameshift mutation in the human homologue of the mesoderm posterior 2 gene, *MESP2,* results in AR SCD in man, and we suggest that this association be designated "SCD, MESP2-type" (or "SCD type 2," to be distinguished from SCD type 1 by mutated *DLL3*). MesP2 is a member of the bHLH family of transcriptional regulatory proteins essential to a vast array of developmental processes (reviewed in Massari and Murre 2000). Murine *Mesp1* and *Mesp2,* located on chromosome 7, are separated by ~23 kb. They are positioned head to head and are transcribed from the interlocus region (Saga et al. 1996). At least two enhancers are involved in the expression of these genes in mouse (Haraguchi et al. 2001), one in early mesoderm expression and the other in presomitic mesoderm (PSM) expression. In addition, a suppressor responsible for the rostrally restricted expression in the PSM has been identified (Haraguchi et al. 2001). These enhancers are essential to the specific and coordinated expression of the MesP proteins. The expression of *MesP1* and *Mesp2* is first detected in the mouse embryo at the onset of gastrulation (~6.5 d *post coitum* [dpc]) and is restricted to early nascent mesoderm (Saga et al. 1996; Kitajima et al. 2000). At this stage, the expression domain of *Mesp1* is broader than that of *Mesp2,* and lineage analysis of *Mesp2* expressing cells shows that they contribute to cranial, cardiac, and extra-embryonic mesoderm (Saga et al. 1999). Expression is then downregulated, as *Mesp* transcripts are not detected later in development (Saga et al. 1999). A second site of *Mesp* expression is detected at 8.0 dpc immediately prior to somitogenesis. A pair of MesP-expressing bands appear on each side of the embryonic midline, at the anterior part of the PSM where the somites are anticipated to form (Saga et al. 1997, 1999). During somitogenesis *MesP1* and *MesP2* continue to be expressed in single bilateral bands in the anterior PSM. MesP2 expression within the PSM continues until about the time when somite formation ceases (~13.5 dpc) (Saga et al. 1997), after which *Mesp* expression is rapidly downregulated, as transcripts are not detected in newly formed somites.

Alignment of MesP2 homologues from human, mouse, *Xenopus,* and chick demonstrates a highly divergent carboxy-terminus between species (fig. 4), and it is unknown whether functional domains are similarly arranged in all Mesp2 orthologues, though this is well characterized for the *Xenopus* Mesp2 orthologue, Thylacine (Sparrow et al. 1998). If the carboxy-terminus in human MesP2 is required for transcriptional activation, then the mutant form of the protein described here, lacking the carboxy-terminus, would lose this function. Mouse pups lacking MesP2 die within 20 min of birth, presenting with short tapered trunks and abnormal segmentation affecting all but a few caudal (tail) vertebrae (Saga et al. 1997). This resembles the phenotype of *Dll3*-null mice, in which the rib and vertebral architecture is disturbed along the entire axis.

Unlike the mouse null MesP2 allele, the human mutant protein retains its bHLH region and, although truncated, might still dimerize, bind DNA, and thus act in

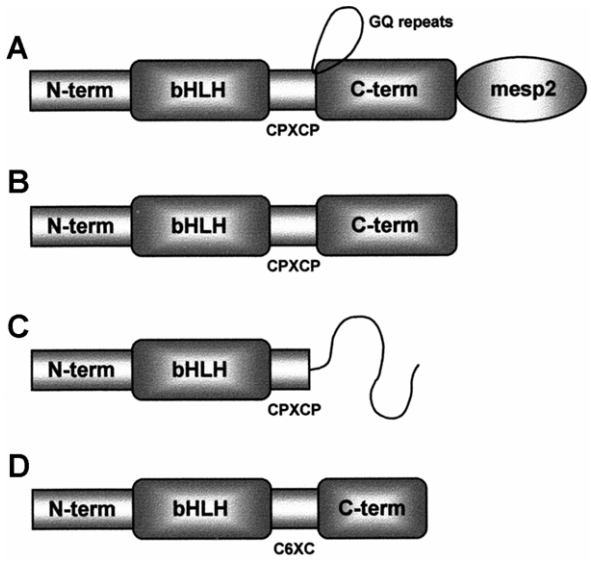

Figure 4 Comparison of MesP2 (*A*) and MesP1 (*B*), both containing a bHLH domain. MesP1 and MesP2 contain a unique CPXCP motif immediately C-terminal to the bHLH domain. *C*, Predicted product of the human MesP2 frameshift mutant. *Xenopus* and zebrafish sequences (*D*) are shorter at the C-terminus and have the residues C6XC in place of the CPXCP motif.

a dominant negative manner. However, the heterozygous parents in this family demonstrate no axial skeletal defects, from which we deduce that this *MESP2* mutation is recessive. Similarly, heterozygous mice are normal and fertile (Saga et al. 1997). It is possible that a single functional carboxy-terminus is sufficient to activate transcription. Alternatively, MESP1 may compensate for the absence of MESP2 function, just as MesP1 can rescue the axial skeletal defects in MesP2-deficient mice in a dosage-dependent manner (Saga 1998). It is also possible that the mutant *MESP2* transcript, containing a premature stop codon at 1099–1101 bp, is degraded by nonsense-mediated RNA decay, with no truncated protein being produced (see Culbertson 1999).

In murine somitogenesis, MesP2 has a key role in establishing rostrocaudal polarity by participating in distinct Notch-signaling pathways (Takahashi et al. 2003). *Mesp2* expression is induced by Dll1-mediated (DLL1 [MIM 606582]) Notch signaling (presenilin1-independent (PSEN1 [MIM 104311]) and Dll3-mediated Notch signaling (presenilin1-dependent), whereas inhibition of *Mesp2* expression is achieved through presenilin1-independent Dll3-Notch signaling. Since Mesp2 can inhibit *Dll1* expression, this complex signaling network results in stripes of *Dll1, Dll3,* and *Mesp2* gene expression in the anterior PSM. The extent to which this represents somitogenesis in man is unknown, except that the phenotypes of the *Mesp2* and *Dll3* mutant mice closely resemble SCD (Saga et al. 1997; Kusumi et al. 1998; Dunwoodie et al. 2002). Here we provide the first evidence that *MESP2* is critical for normal somitogenesis in man. Affected individuals in the family studied have short trunks and abnormal segmentation of all vertebrae, though the disruption to normal morphology appears most marked in the thoracic spine. The reasons for regional variations in disruption pattern throughout the spine are not known. Mutated *MESP2* appears to be a rare cause of SCD, since we have identified only one affected family from a panel of 20 cases that were *DLL3* mutation–negative, including 8 cases in whom abnormal vertebral segmentation affects the entire spine, 4 cases of spondylothoracic dysplasia, and a further 8 cases with diverse phenotypes.

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Electronic-Database Information

URLs for data presented herein are as follows:

Human Genome Working Draft, The, http://genome.ucsc.edu/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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