

# Spondyloperipheral Dysplasia Is Caused by Truncating Mutations in the C-Propeptide of COL2A1

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The term “spondyloperipheral dysplasia” (SPD) has been applied to the unusual combination of platyspondyly and brachydactyly as observed in a small number of individuals. The reported cases show wide clinical variability and the nosologic status and spectrum of this condition are still ill defined. Zabel et al. [1996: *Am J Med Genet* 63(1):123–128] reported an individual with short stature and SPD who was heterozygous for a frameshift mutation in the C-propeptide domain of COL2A1. To explain the additional finding of brachydactyly that is not an usual feature of the type II collagenopathies, it was postulated that the nature of the mutation induced precocious calcification and premature fusion of metacarpal and phalangeal growth plates. The C-propeptide of collagen II had previously been found to promote calcification (“chondrocalcin”). We have ascertained two further individuals with clinical and radiological findings of a type II collagenopathy in infancy who developed brachydactyly type E like changes of fingers and toes in childhood. In both individuals, heterozygosity for novel, distinct mutations in the C-propeptide coding region of COL2A1 were found. Although all three mutations (the one previously reported and the two novel ones) predict premature termination, their location close to the 3'-end of the mRNA probably protects them from nonsense-mediated decay and allows for synthesis of mutant procollagen chains. However, loss of crucial cysteine residues or other sequences essential for trimerization prevents these chains from associating and participating in procollagen helix formation, and thus leads to accumulation in the ER-consistent with EM findings. The mechanism leading to precocious fusion of phalangeal epi-

physes remains to be explored. The consistency of clinical, radiographic, and molecular findings in these three unrelated individuals confirms SPD as a distinct nosologic entity. The diagnosis of SPD is suggested by the appearance of brachydactyly in a child who has clinical and radiographic features of a collagen II disorder. © 2004 Wiley-Liss, Inc.

**KEY WORDS:** collagen; collagenopathy; chondrodysplasia

## INTRODUCTION

Kelly et al. [1977] described a skeletal dysplasia with platyspondyly and brachydactyly E-like changes (short metacarpals and metatarsals, short distal phalanges in the hand and feet). They termed the condition spondyloperipheral dysplasia (SPD) [Kelly et al., 1977]. Additional cases were reported by [Sybert et al., 1979; Vanek, 1983; Sorge et al., 1995; Zabel et al., 1996]. The condition is still ill defined and appears to be very variable in expression with some patients presenting additional findings such as shortening of long bones [Kelly et al., 1977], degenerative changes in the proximal femora [Kelly et al., 1977; Vanek, 1983; Zabel et al., 1996], limited elbow extension [Kelly et al., 1977; Zabel et al., 1996], midface hypoplasia [Kelly et al., 1977; Sorge et al., 1995; Zabel et al., 1996], myopia [Zabel et al., 1996], deafness [Sorge et al., 1995], and mental retardation [Sorge et al., 1995]. In one family, some family members showed only brachydactyly type E [Sybert et al., 1979].

Zabel et al. [1996] reported a patient with platyspondyly, coxa vara, flat capital femoral epiphyses, midface hypoplasia, myopia, and brachydactyly E-like changes. This combination of features evoked a type II collagen disorder, although brachydactyly is characteristically absent in the type II collagenopathies [Spranger et al., 1994]. Sequencing of the COL2A1 gene in this patient revealed a 5 bp duplication in the C-propeptide domain, causing a frameshift and a premature stop codon in exon 51. This mutation was unusual as mutations in the type II collagenopathies generally cluster in the helical domain. The findings suggested that the peculiar features in SPD might be specifically related to the position of the mutation in the C-propeptide domain of COL2A1, particularly as that propeptide had been found to promote calcification (“chondrocalcin”; [Poole and Rosenberg, 1986; Van der Rest et al., 1986]). However, the observation of a mutation in the C-propeptide of COL2A1 in a family with Stickler syndrome and normal hands and feet is not compatible with this model [Ahmad et al., 1995]. Here, we describe two further individuals with SPD and truncating mutations in the C-propeptide domain of COL2A1 and propose a pathogenetic model to explain this characteristic genotype-phenotype correlation.

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## CLINICAL REPORT

### Patient 1 (Fig. 1)

The patient, the first child of healthy, non-consanguineous parents (father 47 years, mother 24 years), was born after an uneventful pregnancy at 41 weeks of gestation. The birth weight (2,670 g) was slightly, the birth length (41 cm) markedly below the 10th centile. Head circumference (34 cm) was normal. A mildly flattened face, short limbs, narrowing of the thorax, ulnar deviation of the hands, and bilateral clubfeet were noted at birth. Evaluation of a photograph taken at that age (not shown) shows facial features compatible with a collagen II disorder (flat face with midface hypoplasia, short, upturned nose with depressed nasal bridge). Radiographic evaluation revealed shortening of long bones with metaphyseal enlargement, platyspondyly, and a bell-shaped thorax. The middle phalanx of the 5th finger was short. The child was tachypneic after birth and respiratory difficulties persisted over the next 18 months until they gradually resolved. The foot deformities were treated with casts and two surgical interventions on the right foot. In the newborn period, hands and feet did not show overt clinical changes. However, the patient recalls that around 4 years of age her great toe began to stand out significantly from the other toes that were much shorter. At 6 years of age the patient developed myopia, which progressed rapidly over the next 15 years. She currently needs correction of 11.75 dpt on the left eye and 7.75 dpt on the right eye. Her intraocular pressure has been elevated for the past 3 years. The patient's growth remained below the 3rd centile and she reached her final height of 131 cm at the age of 16 years. She developed a marked lumbar hyperlordosis and repeatedly complained of pain in the back, hips, and knees. At 23 years of age, an arthrodesis T12-L2 was performed with limited benefit. Currently, the patient still suffers from intermittent pain in the back, hips, and knees. Her facial appearance is characterized by midface hypoplasia and a short, upturned nose. Apart from the complications described, the individual is healthy and leads an active life. She has a medical degree and is working full-time.

### Patient 2 (Fig. 2)

This individual was born at term after an uneventful pregnancy to healthy non-consanguineous parents (father

33 years, mother 31-years-old). The family history is non-contributive. The patient measured 43 cm and shortening of the legs, bilateral clubfeet, and cleft palate were noted at birth. The clubfeet were treated with splints for 3 months and the patient learned to walk at the age of 2 years. The cleft palate was closed at 6 years of age. At 7 years of age, the patient started to complain of hip pain and developed a waddling gait. He responded well to physical therapy and had only intermittent pain in the hips and knees over the following years. He developed myopia and had a retinal detachment at 19 years of age that was treated surgically. At examination at 21 years of age, he was 141.5 cm tall and complained of mild pain in the right hip. He had marked thoracic kyphosis, lumbar lordosis, type E-like brachydactyly in the hands, and short toes 2 to 5 with a prominent first toe. The subject is otherwise healthy and not limited in his daily activities. He is an active surgeon and is working full-time.

## MOLECULAR STUDIES

After informed consent was obtained from both individuals, blood was drawn and DNA was isolated from peripheral blood leukocytes. Exons 49–52 and flanking intronic sequences of COL2A1 were amplified by PCR (Table I) and the resulting fragments were sequenced bidirectionally on an ABI 3100 Genetic Analyzer according to the manufacturer's protocols (Applied Biosystems, Foster City, CA).

In patient 1, we identified a heterozygous 1 bp deletion (4337delG) in exon 52. This resulted in a frameshift at codon 1446 and a premature stop codon 25 amino acids further downstream. This mutation was not present in the patient's parents and was equally absent from 100 control chromosomes.

In patient 2, we identified a heterozygous nonsense mutation (4314C > A) in exon 51, which resulted in a stop codon at amino acid 1438 (C1438X). This mutation was not present in the patient's parents and was equally absent from 100 normal control chromosomes. In addition, we detected a homozygous G > A substitution at nucleotide position 3613. This leads to a change in the amino acid sequence (G1405S), but is a known polymorphism (dbSNP: 2070739). This polymorphism was also present in heterozygous form in both parents. (Exon and nucleotide numbering based on RefSeq NM\_001844, starting at the ATG translation initiation codon).

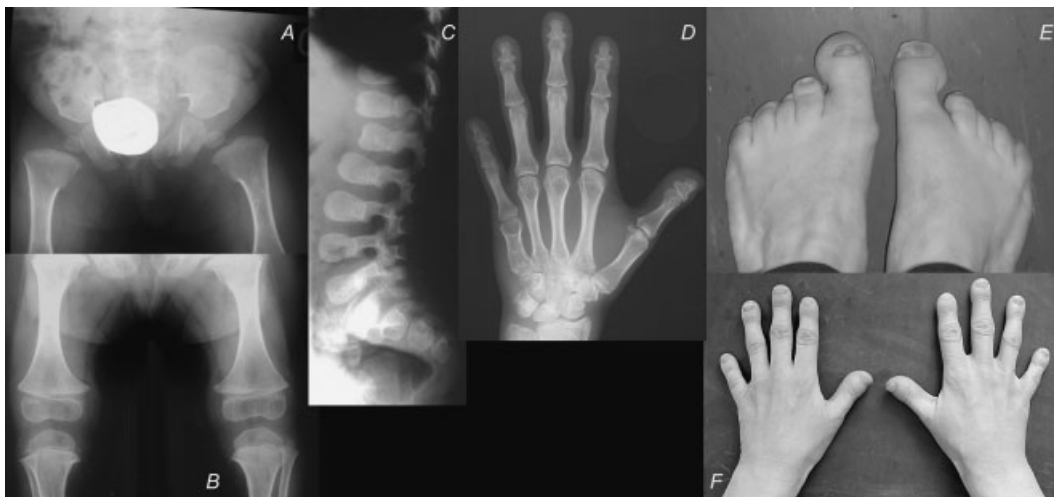


Fig. 1. Radiographic and clinical features of patient 1. **Panels A–C** show radiographic changes around age 2 years that are those of a type II collagen disorder such as SEDC or Kniest dysplasia: Delayed ossification of pubic and ischial bones and of the femoral head, moderate metaphyseal widening, mild epiphyseal irregularities at the knee joints, mild platyspondyly with pear-shaped vertebral bodies. **Panels D–F** are taken at adult age: The hand radiograph shows shortening of the fifth metacarpal as well as of all distal phalanges and mild shortening of the ulna. The clinical photographs show brachydactyly as well as the characteristic pattern of a relatively long great toe with short toes 2 to 5.

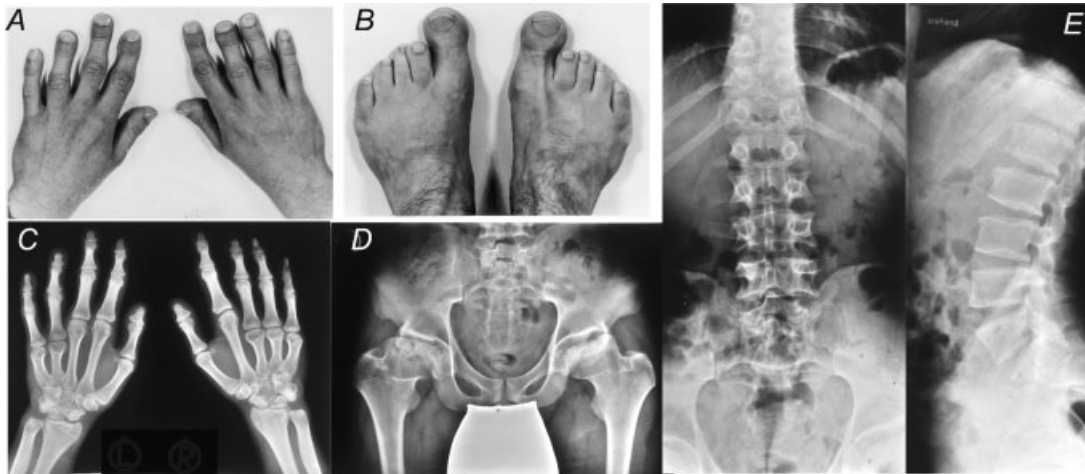


Fig. 2. Radiographic and clinical features of patient 2 at adult age. **Panels A–B:** Brachydactyly is seen in the hands, while the feet show the typical disproportion between the great toe and toes 2 to 5. The hand radiographs (**panel C**) show shortening of metacarpals 4 and 5 (left) and 3 to 5 (right) as well as shortening of the distal phalanges. The pelvis radiograph (**panel D**) shows moderate antetorsion of the pelvis and degenerative changes of the femoral heads. The spine (**panel E**) shows moderate platyspondyly with modest irregularities of the vertebral endplates (T12 to L2).

## DISCUSSION

The two individuals reported here had similar findings: clubfeet, midface hypoplasia, early onset high grade myopia, platyspondyly, epiphyseal dysplasia, and brachydactyly E-like changes developing in childhood.

Some of these features are compatible with acromesomelic dysplasia (AMD) (platyspondyly, brachydactyly, prominent first toes, midface hypoplasia). However, brachydactyly in AMD is usually more generalized and does not show the pattern of brachydactyly type E. Mesomelic shortening is more pronounced in AMD and there is less epiphyseal involvement, especially at the femoral heads; finally, club feet, cleft palate, and high grade myopia are not features of AMD.

The two individuals also have several features evocative of a type II collagen disorder (platyspondyly, epiphyseal dysplasia resembling SEDC or Kniest, myopia, and cleft palate) but brachydactyly is unusual for a type II collagenopathy. Both patients' phenotype is remarkably similar to the individual with SPD described by Zabel et al. [1996] in whom a truncating mutation of the C-propeptide of collagen II was found. Targeted sequencing of the exons corresponding to the C-propeptide of collagen II identified truncating mutations in the two patients reported here. These findings confirm that within the type II collagenopathies, there is a genotype-phenotype correlation between SPD and truncating mutations in the C-propeptide. SPD resembles other, more frequent type II collagenopathies like SEDC and Kniest dysplasia in infancy, until premature fusion of phalangeal epiphyses during childhood leads to brachydactyly.

The C-propeptide of the fibrillar collagens plays a crucial role in triple helix formation [Kielty and Grant, 2002]. Newly synthesized procollagen chains associate via their C-propeptides

to form homo- or heterotrimeric complexes. This initial association is stabilized by intra- and interchain disulfide bonds in the C-propeptides and is thought to assure the correct alignment of the procollagen chains for assembly. Once the complex is formed, triple helix formation initiates at the carboxy-terminal end (corresponding to the region encoded by exons 48–49) and proceeds in amino-terminal direction. Alterations of the C-propeptide would therefore be expected to interfere with triple helix formation. Several studies have shown this to be the case.

By exchanging the C-propeptides of  $\text{pro}\alpha 1(\text{III})$  and  $\text{pro}\alpha 2(\text{I})$ , Lees et al. [1997] identified a discontinuous motif of 15 amino acids in  $\text{pro}\alpha 1(\text{III})$  that is required for procollagen self-association. Similar motifs were then found in all other fibrillar collagen molecules. The corresponding motif in COL2A1 is preserved in the patient of Zabel et al. [1996] and the two patients reported here.

Doyle and Smith [1998] analyzed the role of the conserved cysteine residues in the procollagen C-propeptide that form intra- and interchain disulfide bonds. The cysteine residues in mouse  $\text{pro}\alpha 2(\text{I})$  chains were replaced with alanines by site-directed mutagenesis. Chains lacking single intra- or interchain disulfide bond retained their ability to assemble while loss of both intrachain disulfide bonds prevented the formation of stable type I collagen molecules. The SPD mutations observed in patient 2 and the patient reported by Zabel et al. [1996] result in the loss of two intrachain bonds, while in patient 1, loss of only one disulfide bond is predicted. According to Doyle and Smith [1998] these changes would be expected to abolish chain association in the first two but not in the latter patient.

Lim et al. [1998] constructed a human  $\text{pro}\alpha 2(\text{I})$  cDNA with a premature stop codon that eliminated the last 10 amino acids. When expressed in fetal rat liver epithelial cells together with  $\text{pro}\alpha 1(\text{I})$  collagen, the truncated  $\text{pro}\alpha 2(\text{I})$  chains failed to assemble into stable type I collagen molecules. The result suggested that a sequence critical to helix formation resides in the last 10 amino acids of  $\text{pro}\alpha 2(\text{I})$  collagen. Though this region contains one conserved cysteine residue, the observations of Doyle and Smith [1998] above suggest that other motifs, such as a chaperone binding site, are involved. The corresponding region in  $\text{pro}\alpha 1(\text{II})$  collagen is deleted in the patient of Zabel et al. [1996] and in both patients in this report.

TABLE I. Primers and Annealing Temperatures for Genomic Amplification of Exons 49–52 of COL2A1

Exon	Forward primer	Reverse primer	Temperature
49	aggggcactttcacacaatc	aggggatcctgttctccaag	59°C
50	gtgtctgtgctgtctgagc	gtttgaggagccatctctgc	56°C
51	tgaacctgaactcttgcca	caggcagetcttctctctgg	58°C
52	gttcagtttgggctctctgg	tgccctacagaacggaggatt	55°C

The disproportionate micromelia mouse (Dmm) carries a homozygous three-nucleotide deletion in the C-propeptide of Col2a1 [Pace et al., 1997]. Homozygous mutant mice have severe shortening of long bones, a shortened vertebral column, and a small rib cage. Heterozygotes have short legs and a blunt head. The mutation occurs in a highly conserved region between the last two cysteine residues of the C-propeptide and replaces two highly conserved lysine-tyrosine residues with asparagine. Secondary structure prediction suggests that this substitution is sufficient to change the local secondary structure from coil to strand, which might interfere with intrachain disulfide bonding [Fernandes et al., 2003]. In heterozygous Dmm mice, Pace et al. [1997] observed a drastic reduction of disulfide bonded  $\text{pro}\alpha 1(\text{II})$  dimers and an about 50% reduction of collagen II homotrimers with no evidence of overmodification. Electron microscopy demonstrated dilated cisternae of the endoplasmic reticulum in chondrocytes and a reduced number of collagen fibrils in the extracellular matrix. Using immunohistochemistry, Fernandes et al. [2003] showed that the amount of type II collagen in the extracellular matrix is reduced and that type II collagen chains accumulate inside the chondrocytes of heterozygous Dmm mice. The conserved motif that is mutated in the Dmm mouse is also present in the human *COL2A1* gene and is deleted in the patient of Zabel et al. [1996] and patient 1 in this report. In patient 2, the frameshift mutation leads to replacement of the motif with different amino acids.

Taken together, the experiments above suggest that the SPD mutations in the C-propeptide abolish one or more sequence features crucial to chain association and thus result in exclusion of altered procollagen chains from triple helix formation. In patients with SPD, who are heterozygous for a C-propeptide mutation, we would therefore expect a 50% reduction of type II collagen fibrils in the extracellular matrix. Indeed, Zabel et al. [1996] observed a reduced amount of type II collagen in the extracellular matrix of their patient. The SPD phenotype could thus result from haploinsufficiency. However, several studies have shown that haploinsufficiency of *COL2A1* results in the Stickler syndrome phenotype [Spranger et al., 1994; Van Der Hout et al., 2002]. In agreement with this view, Ahmad et al. [1995] reported a frameshift mutation in the C-propeptide of *COL2A1* in a family with Stickler syndrome. How can this discrepancy be explained?

Freddi et al. [2000] recently showed that mutant mRNA in cells from individuals with Stickler syndrome undergoes nonsense-mediated decay. In contrast, the dilated cisternae in the endoplasmic reticulum in the Dmm mouse, which were also observed in the patient of Zabel et al. [1996] suggest that mutant  $\text{pro}\alpha 1(\text{II})$  chains in patients with SPD accumulate in chondrocytes. Accordingly, mRNA or monomeric protein was detectable in the *in vitro* studies mentioned above [Lees et al., 1997; Doyle and Smith, 1998; Lim et al., 1998], even if the construct failed to trimerize. The SPD phenotype could thus result both from reduced amounts of type II collagen in the matrix and the presence of abnormal molecules in chondrocytes.

Nonsense mediated mRNA decay (NMD) is usually triggered by the presence of a premature stop codon located at least 50 nucleotides upstream of the last exon-exon junction [Schell et al., 2002]. Consistent with our hypothesis, the frameshift mutation reported by Ahmad et al. [1995] results in a premature stop codon at the beginning of exon 51, more than 200 nucleotides upstream of the last exon-exon junction in *COL2A1*. This mutation is therefore likely to cause NMD, consistent with the Stickler syndrome phenotype in this family. All other mutations reported in association with Stickler syndrome lie even further upstream and are hence equally likely to be subject to NMD. In contrast, the three SPD mutations lead to premature stop codons within the last

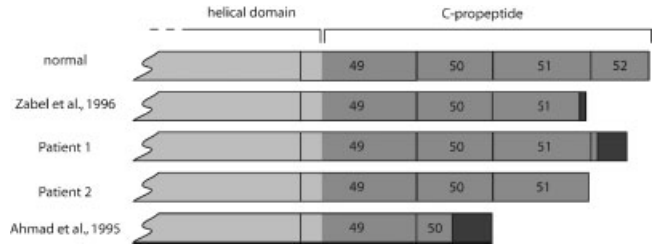


Fig. 3. Schematic comparison of truncated *COL2A1* proteins: End of helical domain shaded in light grey, C-propeptide domain shaded in medium grey, numbers indicate the corresponding exons. Sequence alterations due to frameshift are shaded in dark grey. The mutations in the patient of Zabel et al. [1996] and the in two patients from this report truncate the protein more distally than the mutation in a patient with Stickler syndrome reported by Ahmad et al. [1995]. While the latter should lead to nonsense-mediated mRNA decay (NMD), the former might escape this mechanism and thus cause the distinct spondyloperipheral dysplasia (SPD) phenotype.

20 nucleotides of exon 51 or in exon 52 (Fig. 3) and thus would be predicted to escape NMD.

To fit these data, we would suggest a model in which SPD mutations escape NMD by virtue of their position at the 3'-end of the mRNA, and cause absence or disruption of specific recognition sequences at the carboxy-terminus of procollagen II that exclude mutant chains from association into trimers and lead to accumulation of "free" chains within the ER (as opposed to the associated but misfolded procollagen molecules typical of the more common type II collagenopathies). This effect can be obtained by mutations that introduce premature stop codons downstream of the last 50 nucleotides of exon 51, either directly (patient 2) or via a frameshift mechanism (patient from Zabel et al. [1996] and our patient 1). In-frame deletions/duplications in the C-propeptide should presumably have the same effect. The in-frame deletion in the Dmm mouse seems to be an example of the latter.

The question of the pathogenesis of brachydactyly remains open. It is conceivable that an excess of free procollagen II chains may be responsible for this. A peptide with calcification-promoting effect isolated from cartilage and called chondrocalcin has later proven to be identical with the C-propeptide of collagen II [Poole and Rosenberg, 1986], and this had led Zabel et al. [1996] to speculate that a chondrocalcin-related effect might be implied in the pathogenesis of brachydactyly in SPD. This question might be addressed in the Dmm mouse.

Brachydactyly E-like changes also occur in pseudo- and pseudopseudo-hypoparathyroidism (OMIM 103580). The observation that some members of families with (as yet molecularly uncharacterized) SPD showed isolated brachydactyly E might suggest that screening for *COL2A1* C-propeptide mutations in isolated brachydactyly E might be worthwhile.

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