A glycine to aspartic acid substitution of COL2A1 in a family with the Strudwick variant of spondyloepimetaphyseal dysplasia

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Summary

Background: Spondyloepimetaphyseal dysplasia (SEMD) is one of a clinically heterogeneous group of skeletal disorders, characterized by defective growth and modelling of the spine and long bones. Common clinical features include disproportionate short stature, malformed vertebrae and abnormal epiphyses or metaphyses. Some cases have been associated with mutations in the COL2A1 gene.

Aim: To determine whether the autosomal dominant Strudwick-type SEMD in a three-generation family, showing specific phenotypical features such as chest deformity, limb shortening, myopia and early-onset degenerative osteoarthrosis, might be caused by a novel COL2A1 mutation.

Design: Genetic testing and clinical examination of family members.

Methods: Direct sequencing of PCR-amplified genomic DNA from the COL2A1 gene.

Results: A point mutation within exon 20 of the COL2A1 gene was identified that substituted a glycine for an aspartic acid residue at codon 262.

Discussion: All previously reported autosomal dominant mutations causing SEMD have substituted an obligate glycine within the triple helix, in particular at codons 292, 304 and 709 in the three reported Strudwick-type patients.1 Additionally, a recurrent glycine substitution at codon 154 has been identified in two unrelated Finnish cases with radiological features consistent with the Strudwick subtype.2,3 Our sixth helical glycine substitution extends the mutational spectrum and genotype/phenotype correlations of Strudwick-type SEMD.

Introduction

Spondyloepimetaphyseal dysplasia (SEMD) is one of a clinically heterogeneous group of rare, inherited chondrodysplasias, all of which are characterized by abnormal growth of the developing cartilaginous skeleton. The chondrodysplasias comprise more than 150 distinct clinical disorders, ranging from lethal short-limbed dwarfism to relatively mild premature osteoarthrosis with associated short stature. The spondyloepiphyseal dysplasia (SED) spectrum ranges from achondrogenesis type II (or Langer-Saldino dysplasia), through hypochondrogenesis, Kniest syndrome and spondyloepiphyseal dysplasia congenita (SEDC), to milder forms of spondyloepiphyseal dysplasia (SED), which may be combined with precocious osteoarthritis, and Stickler syndrome4 (Figure 1). The SEDs are particularly characterized by limb-shortening combined with epiphyseal disorder of the spine and limbs,5

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and the milder phenotypes are often undetected until adulthood.

Although the current classification is both clinical and radiological, there is a great deal of clinical variability both within and between the subtypes. The earliest clinical features of SEMD Strudwick type (OMIM reference 184250) comprise unusually short stature, malformation of the vertebrae and distortion of the epiphyses, which overlaps with SEDC. However, the two subtypes may be differentiated in early childhood by the development of irregular, ‘dappled’ metaphyses, a radiological feature that is diagnostic for SEMD.6

The clinical pathology of SEMD is consistent with defects of cartilage protein components.7 Biochemically abnormal type II collagen is detectable in the cartilage of patients with either SED or SEMD,8 which led to the identification of the first mutation within the gene coding for type II cartilage (COL2A1), segregating in a family affected by SEDC.9 Although many private mutations in the COL2A1 gene have subsequently been identified, there is no clear correlation between the helical position and type of mutation, and severity of the clinical phenotype. This situation is further complicated by variable reports of clinical or radiological details and by an ascertainment bias towards clinically severe phenotypes.

We detail the clinical and radiological features of a three-generation family with SEMD Strudwick type, caused by a helical glycine to aspartic acid substitution in COL2A1. To our knowledge, molecular defects have been characterized in only five other individuals with this particular SEMD phenotype1–3 and in one large kindred with autosomal recessive SEMD.10

**Methods**

**Clinical description**

The proband (IV:2, Figure 2) first presented at the age of 9 years with persistent aching of his left knee, generalized joint laxity and a family history of short stature. On clinical examination, he was significantly short for his age (below the third centile). He had a major bony abnormality of his chest, with an excessively prominent sternum, rhizomelic upper segments, limb-shortening and rocker-bottom feet. Orthopaedic examination showed pelvic obliquity, with the left side of the pelvis being lower due to slight shortening of the left leg. This was partly due to a genu valgum deformity, possibly combined with some overgrowth of the left tibia. The proximal left tibia had a recurvatum of 30° and valgus alignment of the knee, with a 20% flexion reduction. Consequent to the pelvic tilting, he had a compensatory thoracolumbar scoliosis. In addition there was generalized ligamentous laxity. Like his mother and grandmother, he was myopic.
Radiological investigation of the spine, epiphyses and metaphyses was typical of SEMD. There was platyspondyly with no alteration of the interpedicular distance (i.e. the spine was shortened but not narrowed). His long bone metaphyses showed flaring of the metaphyseal corners and slight widening of the metaphyseal plates (Figure 3). The proximal tibial epiphyses were particularly abnormal in the region of the tibial-tuberosity, leading to the recurvatum deformity of the knee. The possibility of an anterolateral growth plate tether was eliminated by a MRI scan, which also identified an absent anterior cruciate ligament. His hips showed epiphyseal as well as the metaphyseal changes, and the iliac wings were very broad.

His mother (III:2) and maternal grandmother (II:2) also had marked short stature and rhizomelic upper segmental limb shortening. In addition, both had early-onset degenerative joint disease (predominantly affecting the hip joint) and the grandmother (II:2), aged 55 years, had already undergone a bilateral total hip replacement, while the mother (III:2), who also has prematurely diseased hips, is currently awaiting a bilateral total hip replacement at age 29 years (Figure 4a). In addition, she has spinal shortening (platyspondyly) and wedging of her vertebral bones (Figure 4b). Retrospective family review suggested that the deceased maternal grandfather (I:1) was also affected.

Figure 3. A standing X-ray of the proband’s lower limbs, showing marked loss of bone formation in the proximal femoral epiphyses. There are patchy sclerotic changes of the metaphyses. The left knee has a valgus deformity in the proximal tibial metaphysis associated with a lucent area. The proximal tibial epiphyses are wedge-shaped. The abnormal features affect both epiphyses and metaphyses.

Figure 4. a. Pelvis and hips of proband’s mother (III:2). A view of both hips with legs both in the neutral position and in abduction. The femoral epiphysis has an oval shape with loss of the weightbearing subchondral bone outline. The acetabulum has a similar oval shape with subchondral sclerosis secondary to early degenerative change. The arc of hip abduction is restricted. b. Antero-posterior and lateral projections of the thoracolumbar spine show platyspondyly, especially of T12-L2, associated with vertebral end plate irregularities and marked anterior wedging of the vertebral bodies in the lower thoracic spine.
This family shares many of the features described in the prototype SEMD Strudwick patient after whom this subtype is named; specifically the unusually short stature, large head (relative to the size of the body), genu valgum, prominent chest and asymmetry due to scoliosis.\(^6\) The patient described by Murdoch and Walker\(^6\) had a number of additional features not present in our family, including midface haemangioma, cleft palate, inguinal hernia, clubfoot and pes planus. However, the radiological features are strikingly similar in both cases and allow the definitive diagnosis of SEMD Strudwick. A summary of the clinical features present in our family together with those recorded for other subtypes of SEMD are listed in Table 1.

**Table 1** Comparison of the physical attributes of spondyloepimetaphyseal dysplasia (Strudwick type) to those of other SEMD variants

<table>
<thead>
<tr>
<th>Proband</th>
<th>SEMD Strudwick OMIM 184250</th>
<th>SEMD Type II OMIM 602111</th>
<th>SEMD JL OMIM 271640</th>
<th>SEMD with multiple dislocations OMIM 603545</th>
<th>SEMD Pakistani OMIM 603005</th>
<th>SEMD SEDC OMIM 183900</th>
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<td>AD</td>
<td>AR</td>
<td>AR</td>
<td>AD</td>
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<tr>
<td>Epiphyseal</td>
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</table>

\(^a\)All cases of SEMD with multiple dislocations are sporadic.

This family shares many of the features described in the prototype SEMD Strudwick patient after whom this subtype is named; specifically the unusually short stature, large head (relative to the size of the body), genu valgum, prominent chest and asymmetry due to scoliosis.\(^6\) The patient described by Murdoch and Walker\(^6\) had a number of additional features not present in our family, including midface haemangioma, cleft palate, inguinal hernia, clubfoot and pes planus. However, the radiological features are strikingly similar in both cases and allow the definitive diagnosis of SEMD Strudwick. A summary of the clinical features present in our family together with those recorded for other subtypes of SEMD are listed in Table 1.

**COL2A1 PCR amplification and direct sequencing**

Genomic DNA was prepared from peripheral blood samples collected from three affected family members (II:2, III:2 and IV:2) and an unaffected maternal aunt (II:4). The entire COL2A1 gene was amplified essentially as described by Richards et al.,\(^11\) except that 10 non-overlapping products were analysed rather than five overlapping products. Products were purified using a QIAquick PCR purification kit (Qiagen) and each individual exon was then directly sequenced using the Thermosequenase Cycle Sequencing kit (Amersham) as recommended by the manufacturers. The products were electrophoresed through a 6% denaturing polyacrylamide gel and autoradiographed.

**Restriction enzyme analysis**

Genomic DNA from all four family members was amplified using PCR primers specific for COL2A1 exon 20.\(^12\) The PCR products were incubated with the restriction enzyme Cac8I according to the manufacturers instructions (NEB). The restriction fragments were separated on a 10% polyacrylamide gel and visualized using silver staining.
Results

Direct sequence analysis of COL2A1 revealed a single point mutation within exon 20, which was present in the three family members affected with SEMD Strudwick (II:2, III:2 and IV:2), but not in an unaffected aunt (II:4). The mutation resulted in a single amino acid substitution, altering codon 262 from a glycine (GGC) to an aspartic acid (GAC) (Figure 5), and destroyed a Cac8I restriction enzyme site (Figure 6).

Discussion

This family has the rare Strudwick variant of SEMD, caused by a glycine to aspartic acid substitution within exon 20 of the COL2A1 gene, representing only the sixth reported COL2A1 mutation associated with a SEMD Strudwick phenotype.1–3 All autosomal dominant examples have substituted an obligate glycine within the triple helix, specifically at codons 292, 304 and 709 in the three classic SEMD Strudwick patients.1 Additionally, a recurrent substitution at codon 154 has been found in two unrelated Finnish cases with radiological features consistent with the Strudwick subtype.2,3

As the spectrum of mutations within COL2A1 becomes associated with different clinical entities, the understanding of the genetic basis of SEMD will continue to evolve.
general genotype-phenotype correlations are emerging. Milder disorders, such as Stickler syndrome, appear to be mostly, but not exclusively, associated with exon-skipping mutations or small deletion or insertions, causing premature termination and subsequent degradation of abnormal messenger RNA prior to translation. Conversely, as is the case for the other fibrillar collagens, the more clinically severe disorders arise from the inclusion of abnormal polypeptides, with single amino acid substitutions, into the developing collagen triple helix and hence the mature extracellular matrix (ECM). Here, both the position and type of substitution are likely to affect the clinical outcome.

As with the other major fibrillar collagen genes (COL1A1, COL1A2 and COL3A1), causative COL2A1 point mutations are widely scattered throughout the gene, and the majority result in substitutions of glycine residues being substituted by bulkier amino acids (Figure 7). However, a loose correlation between the location of the point mutation along the molecule and disease severity is emerging, in agreement with a general trend which has evolved from genotype-phenotype studies involving COL1A1, COL1A2 and COL3A1. Each collagen fibril is composed of three individual peptide chains that self-associate at the C-terminal domain and wind together to form the triple helix. Mutations within the C-terminal region disrupt the triple helix at an early stage of helix formation, and tend to produce a severe phenotype. Mutations nearer the N terminus of the molecule result in less disruption of helical winding, and are generally associated with milder phenotypes. The effects of COL2A1 mutations are beginning to follow the C-N gradient of severity, as the majority of glycine substitutions associated with lethal chondrodysplasias, such as achondrogenesis type II or hypochondrogenesis, are located within the C-terminal half of the molecule. A recent study of 12 achondrogenesis type II/hypochondrogenesis patients by Korrko et al. has, however, demonstrated that lethal COL2A1 mutations are more widely distributed along the molecule, arguing against a ‘pure’ gradient hypothesis. It is possible that ascertainment bias exists for the more severe phenotypes, and the identification of further mutations in milder phenotypes should provide conclusive evidence.

The position of the mutation is clearly not the only determinant of disease severity. For instance, one reported case of mild SED with precocious osteoarthritis was associated with a C-terminal glycine to serine substitution at position 976 of the triple helix, implying that the type and size of substitution also influences disease severity. In contrast, some glycine to aspartic acid substitutions in COL2A1 are closely clustered in the N-terminal region, yet have a wide spectrum of clinical severity ranging from lethal achondrogenesis II (G253D and G310D), through to Kniest dysplasia (G103D and G127D) and Stickler syndrome.

![Figure 7](https://academic.oup.com/qjmed/article-abstract/96/9/663/1522600/668)

**Figure 7.** Amino acid substitutions of COL2A1 arranged by disease, type and position of substitution. Note that no aspartic acid substitutions (*) prior to this report (G262D) have caused SEMD, but have caused achondrogenesis II/hypochondrogenesis, Kniest dysplasia and Stickler/Wagner syndrome.
In our mildly-affected family, this identical substitution at G262D is mostly clinically benign. Thus, previously the gradient of clinical severity correlated with mutational position, with the least severe phenotype (Stickler) occurring when the mutation was closest to the N terminus. Our family’s phenotype disturbs this correlation, falling between two lethal achondrogenesis II patients. Other SEMD mutations also fail to show clear positional or mutational-type correlations (Figure 7). Widely separated glycine substitutions at positions 292 and 709 both cause SEMD. Similarly, the valine and arginine substitutions at G154R and G292V are also surprising as equivalent mutations in COL1A1, COL1A2 and COL3A1 produce more severe phenotypes. A review of the clinical phenotypes associated with glycine to aspartic acid substitutions identified within other fibrillar collagen genes has revealed that all such substitutions within COL1A1 are invariably lethal\(^{21-23}\) (Figure 8). In contrast, a more variable clinical outcome accompanies glycine to aspartic acid substitutions of COL1A2 (http://www.le.ac.uk/genetics/collagen) which cause variable, non-lethal forms of osteogenesis imperfecta (OI), ranging from mild OI type I to the progressively deforming types III, III/IV and I\(^{24,25}\) as well as lethal OI type II. This is in agreement with previous evidence indicating that both the clinical and biochemical changes arising from base substitutions in \(\alpha2(I)\) are generally milder than those in \(\alpha1(I)\), perhaps because type I collagen is composed of two \(\alpha1(I)\) polypeptide chains but only one \(\alpha2(I)\) chain. Similarly, glycine to aspartic acid substitutions of type III collagen (encoded by COL3A1) frequently have a poor clinical outcome,\(^{26}\) although specific clinical complications (such as arterial or gastrointestinal rupture) have not yet been associated with different types of mutation or with specific mutations.\(^{27}\)

Ours is only the eighth reported glycine to aspartic acid substitution of COL2A1, and lies closest to the G253D substitution causing lethal achondrogenesis II. Although more mutations are required to fully clarify genotype/phenotype correlations, evidently focal cartilaginous disorganization is less generally disruptive than analogous mutations in bone and blood vessels.

As more data accumulate, it is becoming clear that it is not solely the nature or location of each point mutation which influences its clinical outcome; other, epigenetic factors are also involved. One accepted hypothesis is that variation in the quantity and/or quality of type II collagen protein expressed in each affected tissue may directly alter the clinical phenotype. According to the ‘protein suicide’ hypothesis proposed by Prockop et al.,\(^{28}\) very much less normal collagen is secreted into the extracellular matrix than would be theoretically predicted from loss of a single allele. Because the mature procollagen polymer consists of three identical \(\alpha\) chains, only one-eighth of the assembled triple helical molecules are normal, even when the protein pool contains equal amounts of normal and mutant molecules. The remaining 7/8 are abnormal molecules with 1, 2 or 3 mutant components and might be retained or degraded intracellularly.

It is well documented that intra-familial phenotypic variability affects relatives harbouring identical collagen mutations, implying that other variables are likely to act as modifiers.\(^ {29}\) For example, a clinically silent glycine to serine substitution at position 530 of the COL5A1 triple helix, segregating independently of classical familial EDS

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**Figure 8.** Comparative distribution of glycine to aspartic acid mutations in COL2A1 compared with other collagen type I and III genes. *Severe/lethal or potentially lethal mutations.*
augments the degree of clinical severity when co-inherited with a disease-causing glycine to aspartic acid substitution at position 1489. Various other silent glycine variants (in heterozygotes) enhance the severity of COL7A1 mutations in epidermolysis bullosa.

Lastly, the context of the sequence surrounding the substitution, or mutations within special helical domains, significantly influence the effect of particular mutations. Comparisons of the clinical and biochemical effects of certain α1(II) and α2(II) substitutions clearly illustrate such a ‘domain theory’. Wenstrup et al. compared the effects of two glycine to cysteine substitutions on the thermal stability of type I collagen. A more N-terminal substitution (at position 259) resulted in type I collagen molecules that were significantly more unstable that those containing a more C-terminal substitution at position 646, implying that the type I triple helix contains discontinuous domains that differ in their contributions to helix stability. Similarly, Nuytinck et al. compared the clinical and biochemical findings in five unrelated patients with α2(II) glycine to serine substitutions and their data confirmed that other domain-related factors can determine the phenotypic outcome in type I collagen disorders. This comparison separated ‘lethal’ regions crucial for the structural and functional integrity of the molecule from other ‘non-lethal’ regions in which sequence variations have a less dramatic effect on molecular stability. Such regions differ in individual type I chains, and are chain-specific rather than molecule-specific. They suggest that specific regions might normally undergo micro-unfolding at body temperature, and that some mutations destabilize a critical co-operative block to prevent folding of the entire molecule. Mutations might also cluster within those crucial points of interaction of the helix with other components of the ECM. Lastly, variation in genetic background and differences in the levels of gene expression or variation in levels of other ECM proteins may modify disease severity, as there has been one report of a deficiency of decorin in a lethal case of OL, bearing an α1(II) glycine to serine substitution at position 415.

In conclusion, we describe the sixth reported glycine substitution causing the clinical phenotype of SEMD Strudwick, and only the eighth glycine to aspartic acid COL2A1 substitution. So far only 53 COL2A1 base substitutions have been published, and many more mutations are required to approach the level of genotype/phenotype correlations now provided for COL1A1, COL1A2 and COL3A1 (http://www.le.ac.uk/genetics/collagen). Screening and sequencing of the whole COL2A1 gene is necessary, and so far the mutational spectrum of COL2A1 does not allow such clear-cut predictions of genotype/phenotype as in other more densely plotted genes.

Acknowledgements

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References


