Identification of an Autosomal Dominant Mutation in the *COL2A1* Gene Leading to Spondyloepiphyseal Dysplasia Congenita in a Greek Family

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Established Facts

- Spondyloepiphyseal dysplasia congenita is a group of skeletal dysplasias associated with collagen, type II, and alpha 1 (*COL2A1*) mutations.
- Heterozygous mutations in *COL2A1* affect skeletal growth resulting in severe short stature with bone deformities, but they may also present with extraskelatal abnormalities.
- The c.1609G>A (p.Gly537Ser) mutation in *COL2A1* has been previously described in a male patient as a sporadic case, resulting in mild kyphoskoliosis, myopia, and a post-adolescent height of 117.5 cm at the age of 23 years.

Novel Insights

- We present a patient with a c.1609G>A (p.Gly537Ser) variant in *COL2A1*, with familial inheritance.
- The mutation resulted in a different clinical phenotype than previously reported, with less severe short stature and no extraskelatal defects, indicating genotype-phenotype discordance.
- Describing additional cases helps to understand the inheritance pattern and provides proper genetic counseling services for the parents and the patient.

Keywords

Children · Short stature · Skeletal disorders · Spondyloepiphyseal dysplasia

Abstract

A boy and his father with severe short stature, progressively evolving body asymmetry, and skeletal abnormalities are presented. A next-generation sequencing exome study was performed, and the patient was found heterozygous for the c.1609G>A (p.Gly537Ser) mutation in the *COL2A1* gene. This

mutation is considered a pathogenic variant and has been previously registered in the Human Gene Mutation Database (HGMD) in association with spondyloepiphyseal dysplasia (accession: CM052184). It has been described in a patient as a sporadic case and resulted in a severe phenotype. Segregation studies, in order to determine the inheritance pattern, identified the same mutation in our patient's father. The variant was transmitted in an autosomal dominant pattern. In conclusion, we describe a patient with hereditary spondyloepiphyseal dysplasia congenita, caused by a c.1609G_A (p.Gly537Ser) mutation in the *COL2A1* gene, which resulted in a milder phenotype.

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Spondyloepiphyseal dysplasia congenita (SEDC) is a heterogeneous group of skeletal dysplasias, associated with collagen, type II, and alpha 1 (*COL2A1*) mutations [Li et al., 2014]. It is characterized by disproportionate short stature (short trunk), abnormal epiphyses, flattened vertebral bodies [Anderson et al., 1990], platyspondyly, and odontoid hypoplasia [Jung et al., 2004; Zhang et al., 2011].

Skeletal and vertebral disorders are present, such as midface hypoplasia, a short neck, pear-shaped small chest, pectus carinatum, scoliosis and thoracic hyperkyphosis, micromelia (abnormally small limb/s), and congenital talipes equinovarus (clubfoot). Patients may also present with extraskeletal manifestations, including a cleft palate, retinal detachment, myopia, and hearing loss, which are often detected in other type II collagenopathies [Murray et al., 1989; Veeravagu et al., 2013; Xu et al., 2014].

SEDC is an autosomal dominantly inherited chondrodysplasia [Gelse et al., 2003] as a result of mutations in the gene encoding *COL2A1*. This gene, which contains 54 coding exons [Gelse et al., 2003; Sangsin et al., 2016], encodes the $\alpha 1$ chain of procollagen type II. Type II collagen is composed of 3 collagen $\alpha 1$ chains (*COL2A1* $\times 3$) and is a basic component of tissues such as the hyaline cartilage [Kusano et al., 2017]. Most *COL2A1* mutations occur in the triple-helical region of $\alpha 1$ (II) chains [Barat-Houari et al., 2016].

Until today, at least 57 different mutations in the *COL2A1* gene have been described to have a causal association with SEDC, leading to a variety of phenotypes [Xiong et al., 2018]. The accurate relationship between the *COL2A1* gene mutations and the corresponding phenotypes remains unclear, as well as the relationship between genotypes and extraskeletal (ocular and otolaryngological) phenotypes [Nishimura et al., 2005; Huang et al., 2015].

Fig. 1. Pelvis and lower limb X-rays of the patient with skeletal dysplasia at the age of 9 months showing shortening of the iliac bone, a wide triangular cartilage, flattening of the acetabular roof, absent ossification of the femoral head, coxa valga (a), and tibia vara (b).

Fig. 2. Pelvis X-ray of the father at the age of 41 years showing abnormal development of the femoral head and acetabulum, slight coxa vara and plana of the left hip without any signs of arthrosis.

Case Report

The patient is the firstborn son of nonconsanguineous Caucasian parents, delivered at term by cesarean section, with a normal birth weight of 3,390 g (+1 SD), short birth length of 45 cm (−2.5 SD), and a normal head circumference of 36.2 cm (−0.2 SD). At 9 months of age, he was referred for evaluation of poor growth. There was a positive family history for short stature in association with body asymmetry and skeletal deformities of the father (150 cm, <−3 SD) and the paternal grandmother.

Physical evaluation revealed short stature, pear-shaped chest, and a short trunk. Skeletal survey of the boy showed relative shortening of the iliac bone, a wide triangular cartilage, flattening of the acetabular roof, absent ossification of the femoral head, coxa valga,

Fig. 3. Thoracic spine X-ray of the father at the age of 41 years. Platyspondyly and complete absence of the normal sagittal curvature of the spine are shown.

and tibia vara (Fig. 1). Spinal X-rays did not show significant deformities of the vertebral bodies at this age.

The radiologic examination of his 41-year-old father revealed abnormal development of the femoral head and acetabulum as well as slight coxa vara and plana, particularly of the left hip, without any signs of arthrosis (Fig. 2), and platyspondyly with complete absence of the normal sagittal curvatures of the spine resulting in marked hypokyphosis (“flat back,” Fig. 3). Bilateral Madelung deformity of the wrist was also present.

As the boy’s growth rate and insulin-like growth factor 1 (IGF-1) were very low, growth hormone (GH) stimulation tests were performed. GH deficiency was diagnosed on the basis of a glucagon- and clonidine-stimulated GH peak below 10 ng/mL. MRI of the pituitary gland was normal. Treatment with recombinant human GH was initiated at the age of 3.5 years. He had an initially accelerated catch-up growth during the first year of therapy, but the growth rate subsequently declined, despite the fact that he achieved IGF-1 levels within normal range. Thus, treatment was discontinued after 2 years of GH therapy.

At the age of 8.5 years, the patient’s height was 110 cm (<–3 SD), arm span was 119 cm (0 SD), the lower segment length was 60.5 cm, and US/LS ratio was 0.8 (mean for this age 1.1). Clinical examination revealed relative flattening of the thoracic and lumbar spine. X-ray imaging and CT scan (Fig. 4) of the vertebral column showed osteochondral lesions of most thoracic and lumbar vertebrae, located predominantly at the superior end plate of the vertebral body. Depending on the extent of the epiphyseal end plate

Fig. 4. CT of the spine (3D and sagittal reconstruction) of the patient at the age of 8.5 years confirming osteochondral lesions of the thoracic and lumbar vertebrae, predominantly of the superior end plate of the vertebral body with simultaneous narrowing and irregular shape of the adjacent intervertebral discs contribute to the overall shortening of the spine. Posterior spondylolisthesis of the L5 vertebra over the S1 vertebra without clinical instability is also evident.

involvement, the vertebral bodies were wedge shaped (anterior part of the end plate affected), or acquired the morphology of vertebra plana (whole end plate affected). Narrowing and an irregular shape of the intervertebral discs were observed adjacent to the vertebral body deformities. The final result was a significant overall shortening of the spine, relative flattening of the normal sagittal curvatures, and a clinically insignificant scoliosis. Furthermore, posterior spondylolisthesis of the L5 vertebra over the S1 vertebra, without clinical instability or neurologic signs suggestive of spinal stenosis, was observed.

Two years later, remission of the osteochondral lesions of the vertebral end plates and relative flattening of the vertebral bodies were evident on radiological examination. The intervertebral discs presented a more or less normal morphology (Fig. 5). Lower limb axial alignment was almost normal, except of a valgus orientation of the tibial plafond.

Table 1. Genetic variant of SEDC identified in this study

Genomic position/ genotype	Gene/transcript	Nucleotide change/ aminoacid change	dbSNP ID/ExAC frequency	Exon/effect	Zygosity/variant frequency	In silico pred.	Categorization
Chr12:48379582 C/T	COL2A1 NM_001844.4	c.1609G>A p.Gly537Ser	–/–	Exon 25/ missense	Het/42%	8	Pathogenic

Het, heterozygous; SEDC, spondyloepiphyseal dysplasia congenita.

Fig. 5. Remission of the osteochondral lesions of the spine, flattening of the vertebra, hypokyphosis, hypolordosis, and mild scoliosis of the patient at 11 years of age.

At present, the patient is 12 and 10/12 years old; he is going through puberty, and his current height is 129 cm (<–3 SD). Ophthalmologic examinations and hearing tests are normal.

Molecular Survey

A next-generation sequencing exome study was carried out on DNA extracted from peripheral blood in order to identify genomic variants in 211 genes known to be associated with bone dysplasia and short stature.

SureSelect XT Human All Exon V5 (Agilent Technologies) was used for preparation of the library. It captures >19,000 genes, >350,000 exons, >85% of the alterations responsible for genetic diseases, and the splicing flanking (5 bp) regions. Its size is approximately 50 Mb. Sequencing was performed with the HiSeq 2500 System (Illumina) sequencer. The reads obtained were filtered based on quality parameters, and aligned to the reference genome (GRCh37) using the BWA (version 0.7.12) alignment program.

Sequence variations with respect to the reference genome were detected using the GATK (version 2.3.9) algorithm.

For this case we used the Ion Reporter 5.0 version.

This analysis focuses on the identification of variants included in coding or splicing regions. These changes include missense, nonsense and stop-loss mutations as well as nucleotide insertions or deletions found in a frequency higher than 40% of the sequences obtained. The list of identified variants was evaluated on the basis of the information contained in the databases of described polymorphisms (<http://www.ncbi.nlm.nih.gov/SNP/>, <http://www.1000genomes.org>, and <http://evs.gs.washington.edu/EVS>) in view of identifying benign variants which usually exist among the general population and which have not been associated with any disease.

Furthermore, the functional effect of the genomic variants classified as pathogenic was evaluated by different predictive methods including the analysis package ALAMUT (<http://www.interactive-biosoftware.com>). Finally, after evaluation, the identified mutations were checked in the OMIM database for their associated syndromes. The genetic variant identified in this study is listed in Table 1.

The variant identified in heterozygosity in the patient is considered a pathogenic variant and has been previously registered in HGMD associated to spondyloepiphyseal dysplasia (accession: CM052184), which we confirm by Sanger sequencing (Fig. 6).

Segregation studies in order to determine the inheritance pattern of the identified variant were done, and the same variant was identified in the father, but not the mother.

Results and Discussion

In this study, we describe a patient and his father, who carry a previously described mutation, c.1609G>A (p.Gly537Ser), resulting in a glycine to serine substitution in the triple helical domain of the type 2 procollagen chain. Glycine to serine substitutions seem to be quite common and result in variable phenotypic outcomes. This variant was first isolated in a patient who is included in the review by Nishimura et al. [2005]. It was described as a sporadic case. However, in this report, the same mutation was inherited by an autosomal dominant pattern from father to son.

Depending on the severity of coxa vara, SEDC has been subdivided into SEDC-S (severe) and SEDC-M (mild) [Wynne-Davies and Hall, 1982; Wynne-Davies et

Fig. 6. Sanger sequencing of the patient. The red arrow indicates the heterozygous pathogenic variant c.1609G>A, confirming the exome sequencing results. The father is carrier of the same variant.

al., 1985]. Based on this classification, the c.1609G>A (p.Gly537Ser) mutation is considered as a variant related to SEDC-S. Surprisingly, our patient presented with coxa valga (Fig. 7). His father's hips, at the age of 41 years, were normal regarding to valgus or varus (Fig. 2) and did not show significant pathology. However, 10 years later, early-onset hip arthrosis resulted in bilateral total hip replacement.

Ocular abnormalities such as myopia seem to be more prevalent in SEDC-S than in the other phenotypes [Nishimura et al., 2005]. Other variants related to SEDC-S are described to cause a cleft palate and hearing impairment. The c.1609G>A (p.Gly537Ser) mutation is described to be related to myopia [Nishimura et al., 2005]. Neither the patient nor his father presents any of the aforementioned abnormalities.

Comparing the growth patterns of the patient presented here and the previously reported patient, the same mutation resulted in extremely low adult height, 117.5 cm, of the latter [Nishimura et al., 2005]. Our patient has a better prognosis, given that he measures 129 cm at the age of 12 and 10/12 years and is in mid-puberty. However, his final adult height is expected to be lower than his father's. This could be the result of the early normal onset of his puberty or the clinical heterogeneity of his disease.

Although Nishimura et al. [2005] indicated that this variant was related to extremely short stature and extraskelletal disorders such as myopia, this was not apparent in the individuals we studied. They showed relatively milder phenotypes of SED, and there were no extraskelletal findings, which proves that phenotypes can be diverse among individuals carrying the same mutation, even within the same family.

There are limited data on the effect of GH treatment on children with short stature and skeletal dysplasias

Fig. 7. X-ray of the patient at 11 years reveals coxa valga.

[Hagenäs and Hertel, 2003; Kanazawa et al., 2003]. Initiation of such treatment in this patient was based on the persistently low IGF-1 levels and the very low response of GH on the GH stimulation tests. The patient was monitored very closely, and no side effects were detected. GH therapy did not improve growth velocity, despite the fact that IGF-1 reached the targeted levels.

In conclusion, we described a patient with SEDC caused by a *COL2A1* heterozygous mutation c.1609G>A (p.Gly537Ser) inherited by his father. The recognition of this variant as being pathogenic, in the patient and his father as well as the autosomal dominant pattern of inheritance, contributes to the understanding of variability between genotype and phenotype expression of these syndromes.

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Disclosure Statement

The authors have no conflicts of interest to disclose concerning this publication.

Statement of Ethics

Informed consent was obtained from the patient and all members of his family included in the study. The study was approved by the institutional Ethics Committee of the Children's Hospital "P. & A. Kyriakou," Athens, Greece.

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