

The second report on spondyloepimetaphyseal dysplasia, aggrecan type: a milder phenotype than originally reported

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List of key features

Abnormalities of the metaphyses, epiphyses, and vertebral bodies

Disproportionate short stature

Short trunk and short limbs

Macrocephaly

Introduction

Spondyloepimetaphyseal dysplasias (SEMDs) are a heterogeneous group of skeletal dysplasia defined by the combination of vertebral, epiphyseal, and metaphyseal involvement (Cormier-Daire, 2008). SEMD, Aggrecan type (SEMD-ACAN; Online Mendelian Inheritance in Man, accession number 612813) is an autosomal recessive form of SEMD. The disorder was proposed by Tompson and colleagues, who reported three affected siblings with a new type of SEMD characterized by severe micromelic short stature and craniofacial abnormalities, including relative macrocephaly, severe midface hypoplasia, relative prognathism, and low-set ears. The radiological hallmarks were reported to be platyspondyly with rectangular vertebral bodies, widened metaphyses and small, irregular epiphyses of the long bones, severe brachydactyly with extra-carpal bones, and clefts of the cervical spine. The siblings had a homozygous mutation in *ACAN*, which encodes the aggrecan core protein.

Aggrecan is a member of the lectican family of proteoglycans and a major component in the cartilage extracellular matrix, playing a pivotal role in skeletal development (Aspberg, 2012). Mutations in *ACAN* are known to cause skeletal dysplasia phenotypes in humans and other species (Li *et al.*, 1993; Watanabe *et al.*, 1994, 1997; Krueger *et al.*, 1999). In humans, heterozygous variants of *ACAN* are responsible for spondyloepiphyseal

dysplasia, Kimberley type, and autosomal dominant familial osteochondritis dissecans (Gleghorn *et al.*, 2005; Stattin *et al.*, 2010). The first family with SEMD-ACAN is inherited as an autosomal recessive trait, and then no additional cases have been reported since the original report. Here, we report a man with SEMD-ACAN caused by heterozygous, missense mutations in *ACAN*. The skeletal phenotype was much milder than that reported originally.

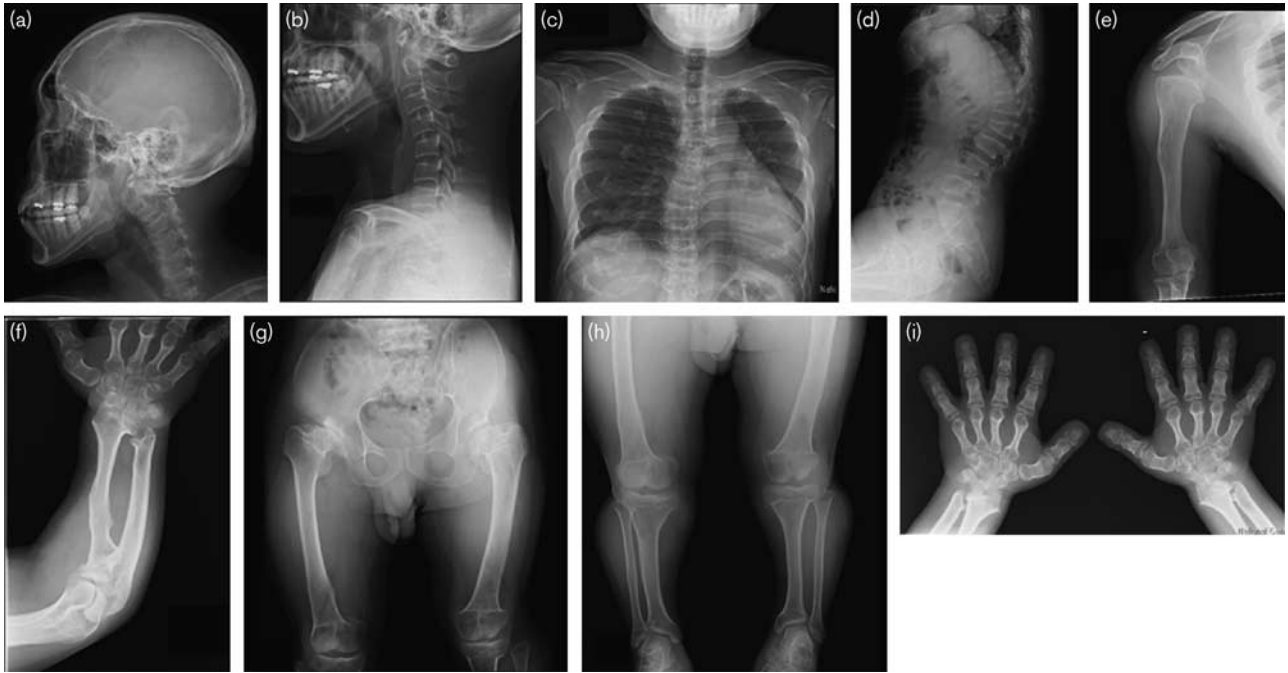
Patient report

The patient is a 45-year-old man. He was born at full term to nonconsanguineous parents. His birth weight and length were reported to be within the normal range. His mother, father, two sisters, and brother were all normal in height. At 6 years of age, his short stature attracted attention, and he was diagnosed with ‘osteogenesis imperfect.’ He underwent surgical correction for genu valgum. Other than his short stature and essential hypertension that began to manifest in adulthood, he has been healthy and living independently. At 42 years of age, he sought medical advice and genetic counseling for his condition. At our first examination, his height was 118.3 cm (−9.1 SD), his weight was 38.9 kg (−2.3 SD), and his occipital frontal circumference was 59.4 cm (+2 SD). He showed acromesomelic shortening of the limbs and relative macrocephaly. He had no midface hypoplasia, prognathism, or low-set ears.

A radiological examination confirmed that the skeletal phenotype fell in the wide category of SEMD (Fig. 1). The spine showed moderate platyspondyly with cervical lordosis and thoracolumbar kyphosis. The vertebral bodies were rectangular in shape, other than the wedge-shaped deformity of L1. The vertebral endplates were irregular. The acetabula were shallow. Degenerative joint disease of the large joints, particularly of the hip, was significant. The long bones were short and relatively

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Fig. 1



Radiographic findings of the patient. (a) Skull, lateral view. (b) Cervical spine, lateral view. (c) Thoracic spine, frontal view. (d) Lumbar spine, lateral view. (e) Right humerus. (f) Right forearm. (g) Pelvis and femurs. (h) Lower legs. (i) Hands and fingers.

broad, with metaphyseal flaring. Generalized brachydactyly was remarkable.

Molecular genetic studies

Genomic DNA from the patient was sent to Samsung Medical Center in Seoul, Korea, and analyzed by whole-exome sequencing. For the generation of standard exome capture libraries, we used the Agilent SureSelect Target Enrichment protocol for the Illumina paired-end sequencing library (version B.3, June 2015) together with 200 ng input formalin-fixed paraffin-embedded DNA. The SureSelectHuman All Exon V4 probe set was used. The captured DNA was amplified. The final purified product was then quantified using qPCR according to the qPCR Quantification Protocol Guide and qualified using the TapeStationDNAscreentape (Agilent, Santa Clara, California, USA). We then sequenced using the HiSeq 2000 platform (Illumina, San Diego, California, USA).

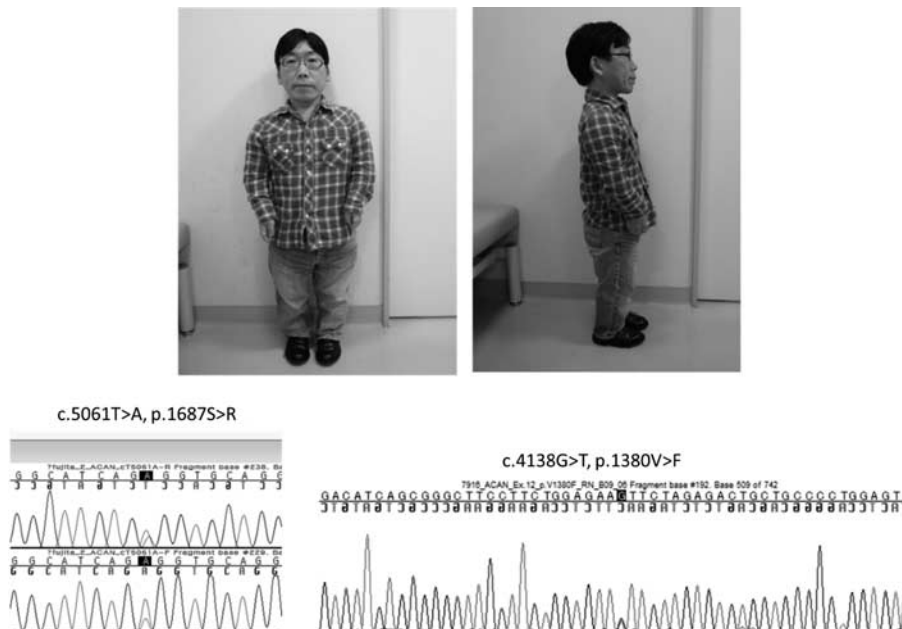
We attempted to narrow down the causative gene on the basis of the assumption that this disease was inherited in an autosomal recessive manner. As shown previously (Cho *et al.*, 2016), we eliminated nonpathogenic variants with our own script according to the following conditions: (i) variants showing an allele frequency of more than 1% in the ESP6500 or 1000 Genomes Project; (ii) variants found in our in-house controls ($n = 452$); (iii) synonymous amino acid changes; and (iv) a low quality of reads (read number <20,

quality scores <30, or minor allele frequency <20%). A total of 52 autosomal recessive homozygous variants and 36 autosomal recessive heterozygous variants were thus selected through the above process. On the basis of the clinical and radiological findings of the patient, most of the genes were excluded, but the *ACAN* gene held our attention as it is known to be a causative gene of SEMDs. The variants in the *ACAN* gene were both missense mutations (c.4138G>T, p.1380V>F, and c.5061T>A, p.1687S>R) and had been tested for mutational effects using the amino acid substitution prediction tool, Mutation Taster (<http://www.mutationtaster.org/>). Neither of these missense mutations was registered in the ESP6500 or 1000 Genomes Project, and both of the amino acids mutated in p.1380V>F and p.1687S>R were conserved in some other species, suggesting that they might be pathogenic. Given that no other genes associated with skeletal diseases were found in the above list, we speculated that these *ACAN* mutations might have affected the patient's SEMD presentation. The variants were validated by subsequent Sanger sequencing (Fig. 2). The patient's father and mother had already died, and his siblings refused genetic tests; hence, we could not obtain samples from them.

Discussion

SEMD-ACAN is a newly recognized autosomal recessive form of SEMD. Only one affected family has been reported to date, and here, we have reported the second

Fig. 2



Photographs of the patient and validation of *ACAN* variants by Sanger sequencing. We have received documented permission that his photos can be used in publication in scholarly journals from the patient. The variants (c.5061T>A, p.1687s>R and c.4138G>T, p.1380V>F) in *ACAN* were validated by Sanger sequencing.

case. Three affected individuals in the original family showed very severe short stature (66–71 cm), craniofacial abnormalities (relative macrocephaly, severe midface hypoplasia with absent nasal cartilage, relative prognathism, and low-set, posteriorly rotated ears) as well as a short neck, barrel chest, and lumbar lordosis. The body proportion was micromelic despite the presence of spondylar dysplasia. Limb shortening was acromesomelic. Brachydactyly was very severe, with short, broad thumbs, horizontal nails, and telescoping interphalangeal joints. The radiological hallmarks included moderate platyspondyly with rectangular vertebral bodies, wide metaphyses and small, irregular epiphyses, multiple clefts of the cervical spine, and bradydactyly with accessory carpal ossification centers. The clinical and radiological manifestations in the present patient recapitulated those of the original report; however, he showed a much milder short stature, a normal face, and a few different manifestations. The features that were identical and different between the original family and the present individual are summarized in Table 1.

Aggrecan is a major proteoglycan in the articular cartilage and essential for the cartilage function and skeletal development (Aspberg, 2012). Proteoglycans consist of a core protein and covalently attached glycosaminoglycans side chains. *ACAN* encodes the core protein of aggrecan, which consists of three globular domains (G1, G2, and G3) and two glycosaminoglycan attachment domains [keratan sulfate and chondroitin sulfate (CS)], located

Table 1 A comparison of the clinical and radiographic findings between Tompson’s patients and our patient

	Tompson’s	Our patient
Height (cm)	66–71	118.3
Macrocephaly	○	○
Midface hypoplasia	○	×
Prognathism	○	×
Low-set ears	○	×
Short neck	○	×
Thoracic deformity	Barrel chest	Funnel chest
Lumbar lordosis	○	○
Mezomelia	○	○
Brachydactyly	○	○
Short, broad thumbs	○	○
Horizontal nails	○	×
Telescoping interphalangeal joints	○	×
Irregular epiphyses	○	○
Widened metaphyses	○	○
Platyspondyly	○	○
Multiple cervical vertebral clefts	○	×
Accessory carpal ossification centers	○	×

between the G2 and G3 domains (Watanabe *et al.*, 1997). CS domain is the largest domain of aggrecan and is decorated by about 100 chains of CS. Negatively charged chondroitin sulfate chains in the CS domain account for the major function of aggrecan as a structural proteoglycan, attracting counter ions and holding a large amount of water in the extracellular matrix. This is the biochemical basis for the viscoelastic properties of cartilage, allowing for load distribution in the joints (Aspberg, 2012).

Loss-of-function mutations in the aggrecan gene have been reported in humans, mice, cattle, and chickens (Vertel *et al.*, 1994; Watanabe *et al.*, 1994, 1997; Gleghorn *et al.*, 2005; Cavanagh *et al.*, 2007; Stattin *et al.*, 2010; Aspberg, 2012). In animals, homozygous mutations cause lethal skeletal dysplasia, whereas heterozygous mutations result in much milder phenotypes, such as only mild dwarfism (Aspberg, 2012). In humans, heterozygosity for a single-base-pair insertion causing a premature stop codon in the aggrecan gene induces spondyloepiphyseal dysplasia (Kimberley type), in which affected individuals show a short stature, stocky build, and early-onset osteoarthritis (Gleghorn *et al.*, 2005). It is tempting to assume that this phenotype may represent haploinsufficiency of *ACAN*.

Whole-genome association studies of normal variation in human height have implicated the *ACAN* region on chromosome 15 as a regulator of height (Weedon *et al.*, 2008). G3 domain is considered to be the most important (Tompson *et al.*, 2009). The G3 domain consists of four structural motifs: two epidermal growth factor-like repeats, followed by a C-type lectin domain (CLD) and a complement regulatory protein repeat. The CLD domain has a high affinity for extracellular matrix molecules, including tenascin-R, tenascin-C, fibulin-1, fibulin-2, and fibrillin-1, and they interact with each other in a calcium-dependent manner (Aspberg *et al.*, 1997; Rauch *et al.*, 1997; Olin *et al.*, 2001; Isogai *et al.*, 2002; Day *et al.*, 2004; Aspberg, 2012). A missense mutation in the CLD domain is also responsible for a five-generation family with autosomal dominant familial osteochondritis dissecans (Gleghorn *et al.*, 2005).

SEMD-*ACAN* was also caused by missense mutations in the CLD domain. The affected individuals in the original family had homozygosity for a missense mutation (c.6799G > A, p.D2267N) in the CLD domain, which was predicted to affect conformational binding loops of the CLD domain. In contrast, the present individuals had heterozygous mutations in the CS1 and CS2 domains, consisting of a large number of repeats. These different mutation patterns account for the phenotypic differences between the original and the present reports.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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