Case Report

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Identification of a novel heterozygous mutation of *ACAN* in a Korean family with proportionate short stature

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Aggrecan is a proteoglycan in the extracellular matrix of growth plate and cartilaginous tissues. Aggrecanopathy has been reported as a genetic cause not only for severe skeletal dysplasia but also for autosomal dominant short stature with normal to advanced bone age. We report a novel heterozygous mutation of *ACAN* in a Korean family with proportionate short stature identified through targeted exome sequencing. We present a girl of 4 years and 9 months with a family history of short stature over three generations. The paternal grandmother is 143 cm tall (–3.8 as a Korean standard deviation score [SDS]), the father 155 cm (–3.4 SDS), and the index case 96.2 cm (–2.9 SDS). Evaluation for short stature showed normal growth hormone (GH) peaks in the GH provocation test and a mild delayed bone age for chronological age. This subject had clinical characteristics including a triangular face, flat nasal bridge, prognathia, blue sclerae, and brittle teeth. The targeted exome sequencing was applied to detect autosomal dominant growth palate disorder. The novel variant c.910G>A (p.Asp304Asn) in *ACAN* was identified and this variant was found in the subject's father using Sanger sequencing. This is the first case of Korean familial short stature due to *ACAN* mutation. *ACAN* should be considered for proportionate idiopathic short stature, especially in cases of familial short stature.

Key words: Short stature, Aggrecans, *ACAN*.

Introduction

Aggrecan is a critical component for cartilage structure and joint function. Aggrecan is encoded by *ACAN* (MIM 155760, NM_013227.3) located on chromosome 15q26.1 and composed of 19 exons [1]. Since 2014, when whole exome sequencing found heterozygous *ACAN* mutations in three families with idiopathic short stature (ISS) and premature growth cessation [2], aggrecanopathy has been divided into five clinical entities: recessive spondyloepimetaphyseal dysplasia, aggrecan type (SEMD, OMIM 612813); spondyloepiphyseal dysplasia, Kimberley type (OMIM 608361); macrocephaly with multiple epiphyseal dysplasia and distinctive facies (OMIM 607131); autosomal dominant osteochondritis dessecans, short stature, and early onset osteoarthritis (OMIM 165800); and various ISS [1,2]. Only a single family with recessive SEMD has been reported, and they

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were characterized by extreme short stature (final adult height 66-71 cm) and severe skeletal dysplasia [3], whereas autosomal dominant aggrecanopathies showed various final heights and skeletal manifestations [1].

ACAN mutations have been identified in the ISS group, and they are associated with clinical characteristics including mild facial dysmorphism (prognathia, low nasal bridge, low-set ear, and macrocephaly), early growth cessation, and advanced bone age [1,2]. Functional haploinsufficiency of aggrecan leads to impairment of growth plate chondrogenesis by early invasion of blood vessels and osteoblasts and premature hypertrophic chondrocyte maturation in growth plate, and it may be associated with early growth cessation [1]. A previous Korean genome wide association study suggested that five loci including *ACAN*, *SPAG17*, *KBTBD8*, *HHIP*, and *HIST1H1D* genes were significantly associated with ISS [4]. Currently, only three Chinese families and four Japanese families with short stature due to heterozygous *ACAN* mutation have been reported in Asia [5-8].

Until now, there have been no reports of short stature patients with *ACAN* mutation in Korea. Herein, we report the first case of Korean familial short stature with a novel *ACAN* variant identified by targeted exome sequencing.

Case

A girl aged 4 year and 9 month-old girl presented with proportionate short stature. Her height was 96.2 cm (−2.9 as a Korean standard deviation score [SDS]), and her body weight was 14 kg (−2.5 SDS). The head circumference was 50 cm (−0.1 SDS). She was born at 39 weeks and 3 days through normal vaginal delivery with a birth weight of 2.66 kg. The height of this subject's parents were also short: paternal height was 155 cm (−3.4 SDS) and maternal height was 150 cm (−2.2 SDS). The age of maternal menarche was 14 years, and paternal final height was reached at 15 to 16 years. This subject showed clinical characteristics including a triangular face, midfacial hypoplasia, low nasal bridge, prognathia, brittle teeth, blue sclera, and brachydactyly (Fig. 1A). Therefore, collagenopathy such as osteogenesis imperfecta was not excluded at first, even though there was no history of fracture or radiologic abnormalities for skeletal survey. No abnormalities were identified in the complete blood count, chemistry, inflammatory markers, urinalysis, or skeletal survey, particularly in the pelvis and spine (Figs. 1B and C). Endocrine evaluation showed no abnormal results, including thyroid function, insulin-like growth factor-1, and insulin-like growth factor binding protein-3. At a chronological age of 4 years and 9 months, her bone age was 4 years and 2 months (Fig. 1D). Her

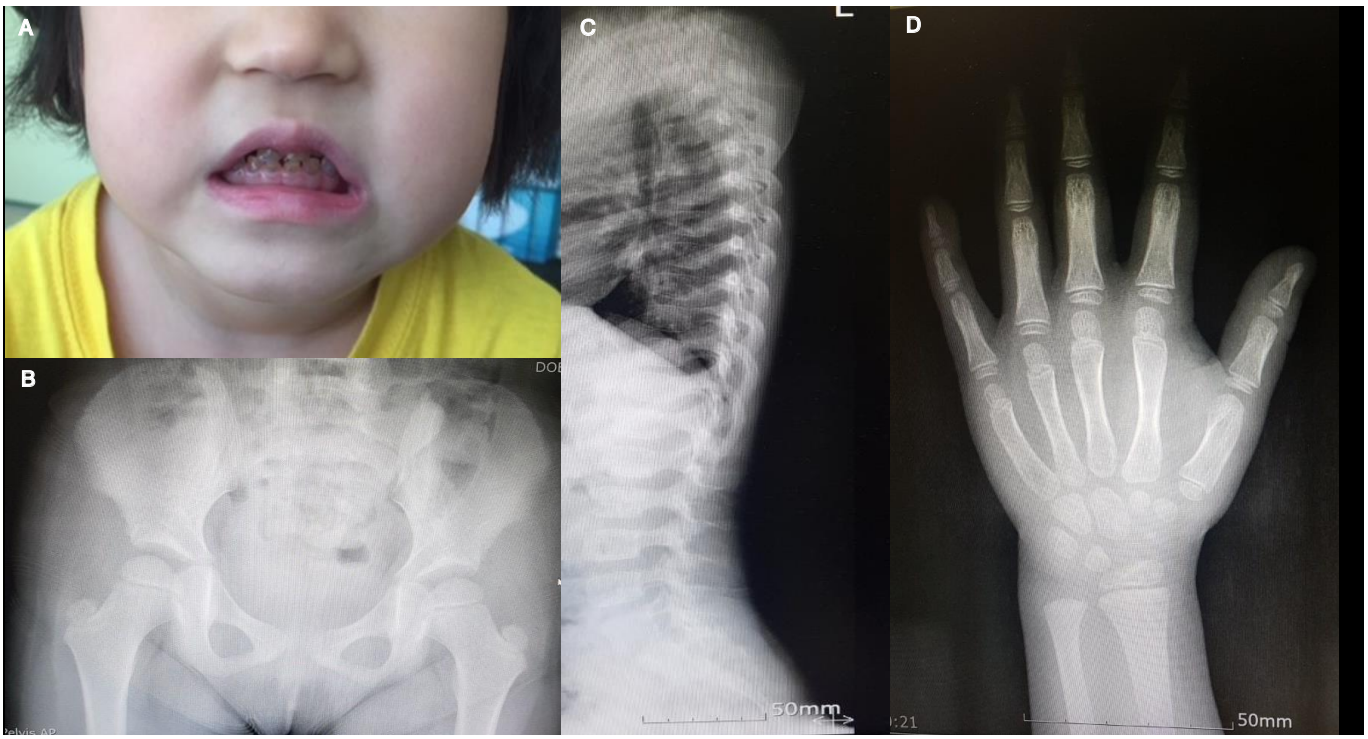

Fig. 1. Radiologic finding and clinical photo of patient. (A) She had prognathia, low nasal bridge, and brittle teeth. There is no definite abnormality in the pelvis or spine (B, C) and bone age based on Greulich-Pyle method (D) was 4 years and 2 months.

karyotyping was normal (46, XX), and growth hormone (GH) provocation tests (L-dopamine and insulin) showed peak GH levels of 6.4 ng/mL and 19.9 ng/mL, respectively.

Infomed consent was obtained from her parents before genetic testing. We performed targeted exome sequencing using the Trusight One Sequencing Panel (Illumina Inc., San Diego, CA, USA). The extensive targeted exome sequencing revealed that a novel heterozyous variant with c.910G>A (p.Asp304Asn) in exon 6 of *ACAN* gene (Fig. 2A). This region is highly conserved and this variant is not found in 1000 Genomes Project, the Exome Variant Server, the Exome Aggregation Consortium, or the dbSNP database. This variant is predicted to be deleterious through *in silico* prediction, including SIFT (scale-invariant feature transform), Mutation Taster, and polyphen-2. Segregation analysis was done, and the short statured father was found to have this variant (Fig. 2B). Although additional familial testing for the paternal sister (146 cm, –3.1 SDS) and grandmother (height 143 cm, –3.8 SDS) was recommended, further familial testing could not be undertaken. A subject's father aged 43 years had back and hip pain; however, as they refused further evaluation for bone status, osteochondritis dissecans in this family could not be assessed.

GH therapy was started for this subject, but the treatment was not continued due to poor compliance on the part of the parents. Short-term follow-up and monitoring for bone age and growth velocity is planned for this subject.

Discussion

We identified a novel heterozygous variant (c.910G>A;

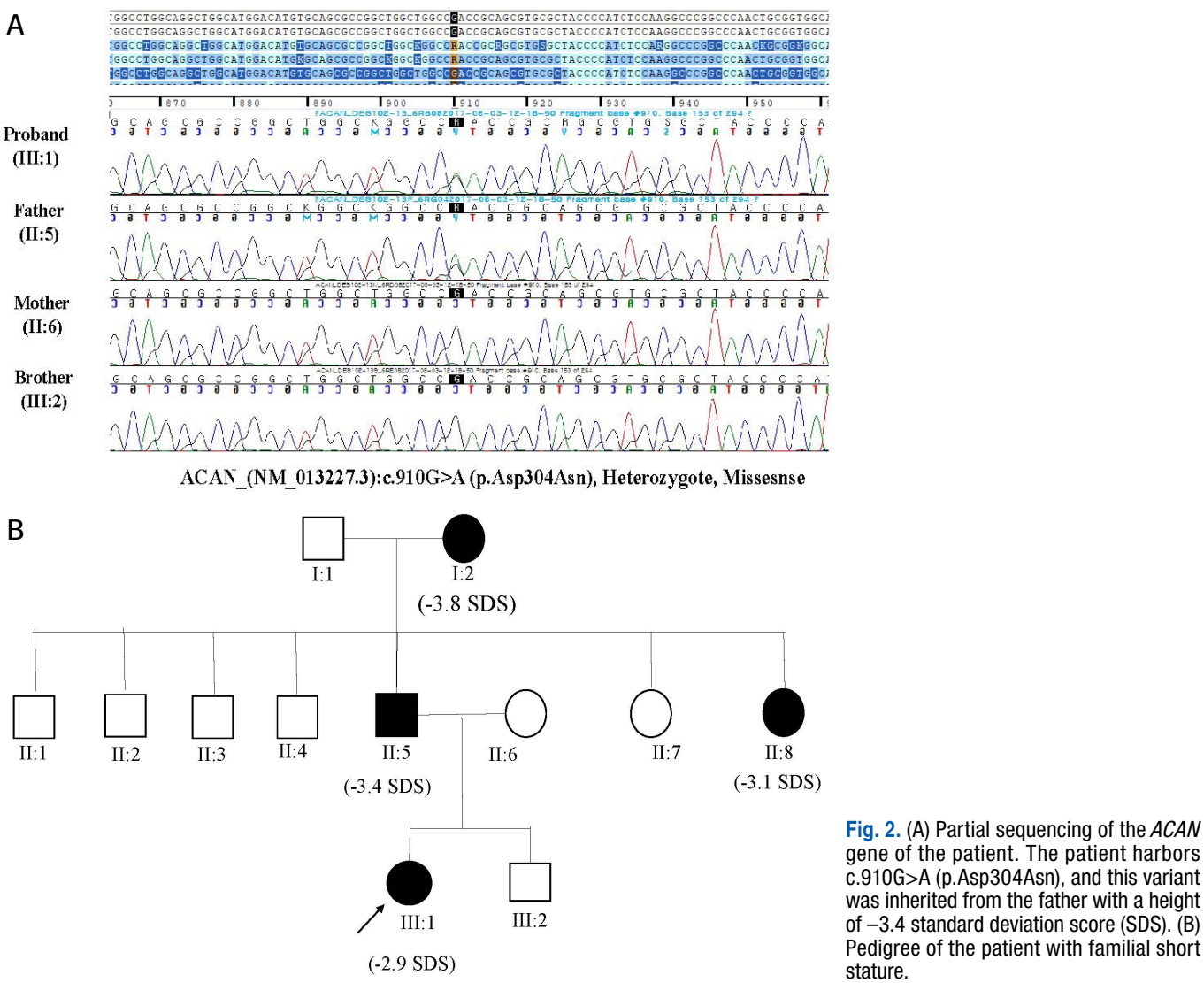

Fig. 2. (A) Partial sequencing of the *ACAN* gene of the patient. The patient harbors c.910G>A (p.Asp304Asn), and this variant was inherited from the father with a height of –3.4 standard deviation score (SDS). (B) Pedigree of the patient with familial short stature.

p.Asp304Asn) in Korean family with short stature and mild delayed bone age. To date, there have been 55 families with 54 *ACAN* heterozygous variants in the literature and in this study [2,5,7-14]. The previous *ACAN* mutations were distributed across intron 1 to exon 17, and almost all variants were located in globular domains G1 to G3. Each location seems to be associated with the patient's phenotype [2,14]. Our patient showed a novel *ACAN* variant in exon 6 regarding the globular domain G1; however, only four different *ACAN* mutations, including p.Trp301Cys, p.Ser306Cys, p.Tyr349Cys, and p.Tyr349*, in exon 6 have been reported in the literature (Table 1) [6,9,13]. The heights of those patients ranged from –2.7 to –4.1 SDS, and almost all of them showed similar facial features with delayed to advanced bone age. Only one patient had *de novo* mutation, and three families showed joint disease, including in affected adults (Table 1). A mouse model for the *ACAN* mutant revealed that both heterozygous and homozygous 7 bp deletion in exon 5 leading a premature termination codon in exon 6 showed a significant reduction in aggrecan mRNA levels [15]. Mice harboring homozygous deletion in exon 5 of *ACAN* showed severe dwarfism and cleft palate at birth and died early due to respiratory failure, whereas mice with the heterozygous mutant showed postnatal proportional short stature and malalignment of the spine [15].

Since heterozygous *ACAN* mutants affecting ISS have been introduced through next-generation sequencing, several cohort studies for prevalence of heterozygous *ACAN* mutation in short stature patients have been reported worldwide [5,6,10]. In a European cohort, four nonsense mutations and two missense variants in six families were identified in 428 families with short stature (1.4%) [10]. In Asia, a total of three patients (1.3%) with *ACAN* mutation showing typical facial features were detected among 218 Chinese non-syndromic short stature children, and these three patients showed typical facial features and familial short stature [5]. In Japan, in contrast, *ACAN* mutation was relatively frequently identified; four patients (4.8%) among 84 ISS patients were identified through targeted exome sequencing, and these four Japanese patients showed *de novo* short stature with normal and even delayed bone age [6].

Typical clinical manifestations have been described in affected patients: small for gestational age, early growth cessation, prognathia, flat nasal bridge, midfacial hypoplasia, relative macrocephaly, brachydactyly, short stature below –2.5 SDS, joint problems, and early advanced bone age [2,7,13]. However, various clinical manifestations have been reported even in family members who have same mutation, suggesting clinical heterogeneity [8,9,13]. Our patient showed brittle teeth which have not been described in previous reports. As a recent *in vitro* study showed that aggrecan was one of the important factors during chondrogenesis, like alkaline phosphatase, collagen type-I alpha-1, and collagen type-II alpha-1 [16], enamel abnormality may be associated with aggrecan haploinsufficiency. Although there was a recent report that familial short stature with advanced bone age was a sufficient clue to apply genetic testing for *ACAN* [11], their various phenotypes still emphasize the need for an extensive gene panel [10].

For final adult height, combinational therapy with recombinant human GH and gonadotropin-releasing hormone analogue (GnRHa) may be effective in pediatric patients [2,8,9,12].

Table 1. Clinical findings of patients with *ACAN* mutation in exon 6 in this study and the literatures

Subject	Mutation	Sex/Age	Height (SDS)	Clinical findings	Affected parents (height), affected family members	Reference
1	c.910G>A p.Asp304Asn	F/4.8 yr	–2.9	Midfacial hypoplasia, prognathia, frontal bossing, depressed nasal bridge, delayed bone age (4.1 yr)	father (–3.4 SDS), 3, joint disease: NA	In this study
2	c.1046A>G p.Tyr349Cys	F/6.8 yr	–3.7	Delayed bone age (6.1 yr)	None	[6]
3	c.1047_1048del p.Tyr349*	F/NA	–4.1	Prenatally identified proportionate short stature and facial dysmorphism (frontal bossing, midface hypoplasia, anteverted ears, brachydactyly)	mother (–5.9 SDS), 1, joint disease: NA	[9]
4	c.916A>T p.Ser306Cys	F/5.5 yr	–2.7	Advanced bone age (8 yr)	mother (–2.6 SDS), 6, joint disease (+)	[9]
5	c.903G>C p.Trp301Cys	F/NA	–3.0	Short limb at birth, midfacial hypoplasia, prognathia, frontal bossing, depressed nasal bridge	father (–4.4 SDS), 5, joint disease (+)	[9]
6	c.903G>C p.Trp301Cys	F/7 yr	–3.5	Frontal bossing, midfacial hypoplasia, depressed nasal bridge, coxa valga, slender femora, osteochondral knee mild defects, advanced bone age (-)	father (–4.4 SDS), 2, joint disease (+)	[13]

SDS, standard deviation score; NA, not available; -, none.

Adult height in these patients after combinational therapy was increased by 5 to 11.5 cm compared to previous predicted height or the height of the affected family member [2]. In future, randomized controlled trials and long-term follow-up will be required to prove the effectiveness of combination therapy with GH and GnRHa in short stature pediatric patients with heterozygous *ACAN* mutation.

In conclusion, we report the first Korean case of familial short stature with a novel variant of *ACAN*. Growth plate disorder should be considered, especially in cases of familial short stature. Careful management that takes account of premature bone cessation and new therapeutic approaches will be needed to enhance the patient's final height and prevent joint disease during early adulthood.

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