

Review

ACAN mutations as a cause of familial short stature

Sumito Dateki¹

¹*Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*

Abstract. Aggrecan, encoded by *ACAN*, is a major proteoglycan component of the extracellular matrix in the growth plate and articular cartilage. Aggrecan provides the hydrated gel structure important for the load-bearing properties of joints and plays a key role in cartilage and bone morphogenesis. At least 25 pathological *ACAN* mutations have been identified in patients with highly variable phenotypes of syndromic or non-syndromic short stature. This review provides an overview of the current understanding of *ACAN* and the clinical and genetic findings concerning aggrecan-associated diseases.

Key words: aggrecan, *ACAN*, short stature, bone age, disc herniation

Introduction

Linear bone growth is determined by the division and growth of matrix-producing chondrocytes at the growth plate. The rate of growth plate chondrogenesis is regulated by many systems including multiple hormones (GH, thyroid hormone, and sex steroids), paracrine factors, extracellular matrix molecules, and intracellular proteins (1). Short stature can be caused by decreased chondrogenesis due to mutations in any gene that directly or indirectly affects growth plate chondrocytes and the process of growth plate chondrogenesis (1).

Aggrecan, encoded by *ACAN*, is a major

proteoglycan component in the extracellular matrix of the growth plate and articular cartilage. Aggrecan provides the hydrated gel structure important for the load-bearing properties of joints (2). At least 25 pathological *ACAN* mutations have been identified in patients with highly variable phenotypes of syndromic or non-syndromic short stature (Table 1) (3–11). This review article provides an overview of the current understanding of *ACAN* and the clinical and genetic findings concerning aggrecan-associated diseases.

Structure and Function of Aggrecan

Aggrecan consists of a 250-kDa protein core with approximately 100 chondroitin sulfate and 30 keratan sulfate glycosaminoglycan chains that attach to a large domain located between three globular domains (N-terminal G1 and G2 domains and a C-terminal G3 domain) (Fig. 1). The globular domains mediate interactions with other components of the extracellular matrix. The G1 domain is responsible for interaction with hyaluronan, and the G3 domain binds

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Corresponding author: Sumito Dateki, MD, Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

E-mail: sdateki1@nagasaki-u.ac.jp

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Table 1. Summary of molecular and clinical findings in patients with *ACAN* mutations

Mutation #	<i>ACAN</i> mutation						Phenotype	Reference
	cDNA	Protein	Type	Exon	Domain	Inheritance		
1	c.61G>T	p.Glu21*	Nonsense	2	-	Dominant	ISS	(7)
2	c.223T>C	p.Trp75Arg	Missense	3	G1	Dominant	ISS, OA	(7)
3	c.272delA	p.Arg93fs*41	Frameshift	3	G1	Dominant	ISS	(4), (7)
4	c.492C>G	p.Tyr164*	Nonsense	4	G1	Dominant	ISS, OA	(7)
5	c.532A>T	p.Asn178Tyr	Missense	4	G1	Dominant	ISS	(7)
6	c.903G>C	p.Trp301Cys	Missense	6	G1	Dominant	ISS, OA	(7)
7	c.916A>T	p.Ser306Cys	Missense	6	G1	Dominant	ISS	(7)
8	c.1047_1048dellinsAC	p.Tyr349*	Nonsense	6	G1	Dominant	ISS	(7)
9	c.1443G>T	p.Glu415*	Nonsense	7	IGD	Dominant	ISS, OA	(7)
10	c.1425delA	p.Val478fs*14	Frameshift	8	G2	Dominant	ISS, OA	(7)
11	c.1526C>A	p.Ser509*	Nonsense	8	G2	Dominant	ISS, OA	(7)
12	c.1608C>A	p.Tyr536*	Nonsense	9	G2	Dominant	ISS, OA	(6)
13	c.1744delT	p.Phe582fs*69	Frameshift	10	G2	Dominant	ISS, disc herniation	(11)
14	c.2026+1G>A	exon skipping	Splice	10	G2	Dominant	ISS	(4), (7)
15	c.3986dupC	p.Gly1330fs*221	Frameshift	12	CS	Dominant	SED Kimberley type	(8)
16	c.4657G>T	p.Glu1553*	Nonsense	12	CS	Dominant	ISS, OA	(7)
17	c.4762_4765del	p.Gly1588fs*26	Frameshift	12	CS	Dominant	ISS	(6)
18	c.5391delG	p.Gly1797fs*52	Frameshift	12	CS	Dominant	ISS	(5), (7)
19	c.7064T>C	Leu2355Pro	Missense	14	C3	Dominant	ISS, OCD, OA	(4), (7)
20	c.7090T>C	p.Gln2364*	Nonsense	14	C3	Dominant	ISS, OA	(6)
21	c.7141G>A	Asp2381Asn	Missense	15	C3	Recessive	SEMD aggregan type	(3)
22	c.7153G>A	p.Glu2385Lys	Missense	15	C3	Dominant	ISS, OA	(7)
23	c.7203G>A	p.Trp2401*	Nonsense	16	C3	Dominant	ISS, OA	(7)
24	c.7249G>A	Val2417Met	Missense	16	C3	Dominant	ISS, OCD, OA	(7), (9)
25	c.7276G>T	p.Glu2426*	Nonsense	16	C3	Dominant	ISS	(7)

G1, globular domain 1; IGD, interglobular domain; G2, globular domain 2; CS, chondroitin sulfate attachment domain; G3, globular domain 3; ISS, idiopathic short stature; OA, osteoarthritis; SED, spondyloepiphyseal dysplasia; OCD, osteochondritis dissecans; SEMD, spondyloepimetaphyseal dysplasia. This Table is modified from that of Dateki *et al.* (11).

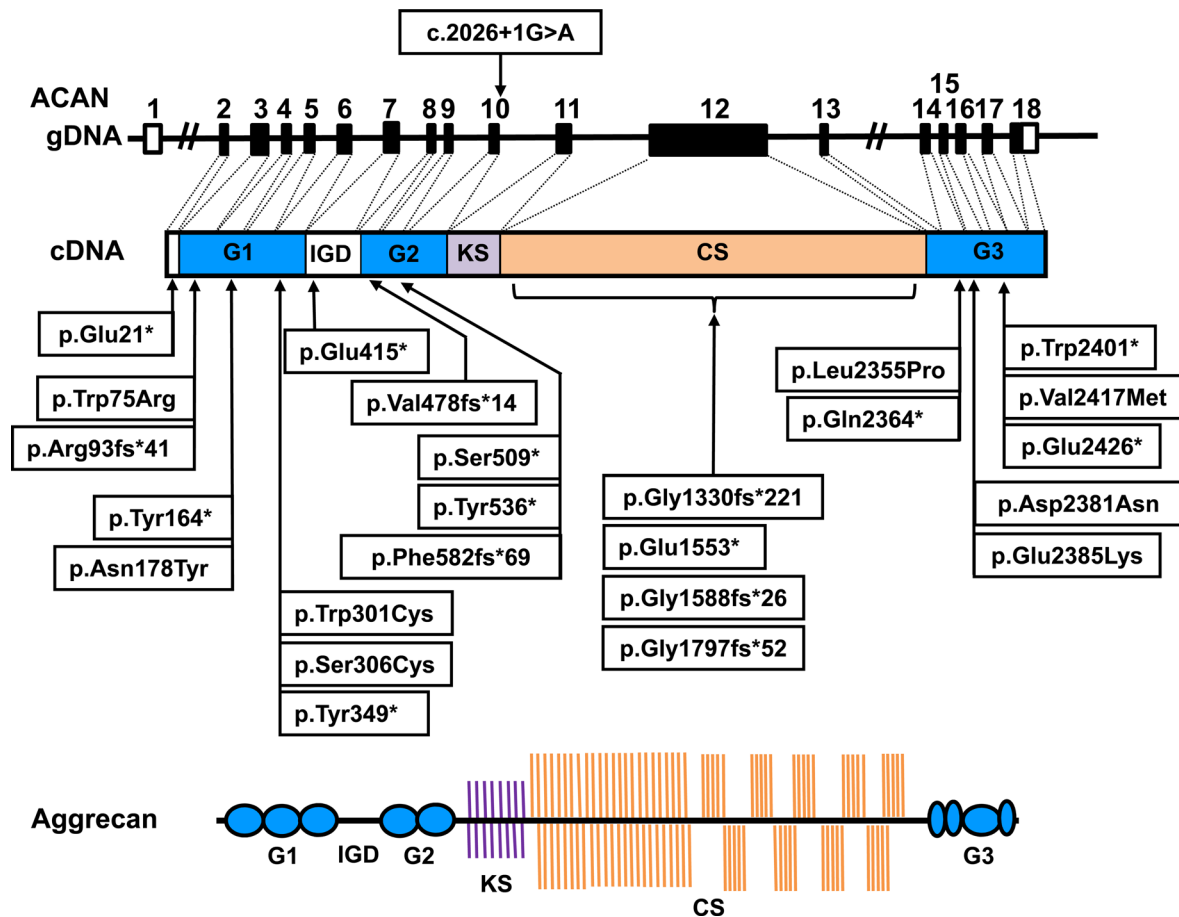


Fig. 1. The structure of *ACAN* and the position of mutations. The black and white boxes on genomic DNA (gDNA) denote the coding regions on exons 1–18 and the untranslated regions, respectively. The mutations identified in previous studies are shown (NM_013227.3, NP_037359.3). G1, globular domain 1; IGD, interglobular domain; G2, globular domain 2; KS, keratin sulfate attachment domain; CS, chondroitin sulfate attachment domain; G3, globular domain 3. The figure is modified from that of Dateki *et al.* (11).

tenascin and fibulin through its C-type lectin repeat domain. The C-type lectin domain may also be involved in the secretion of aggrecan from chondrocytes (2).

Extensive sulfation of keratan sulfate/chondroitin sulfate chains and aggregation with hyaluronan generate a substantial fixed negative charge that renders the aggregates highly hydrated, and the resulting swelling pressure confers load-bearing properties on cartilage. In addition, aggrecan regulates the expression of key growth factors and signaling molecules during the development of cartilaginous tissue

and plays a key role in cartilage and bone morphogenesis (12, 13).

Animal Model of Aggrecan-associated Diseases

The cartilage matrix deficiency (cmd) mouse is the first known animal model of aggrecan-associated diseases (14). The mice harbor a 7-bp deletion in exon 5 of the aggrecan gene that leads to a premature termination codon in exon 6. The phenotype of homozygous cmd mice is characterized by dwarfism and a cleft

palate, and the animals die shortly after birth because of respiratory failure. In contrast, the heterozygous mutant mice are apparently normal at birth; however, they develop proportional dwarfism with age-associated misalignment of the cervical and thoracic spine and usually die early from starvation followed by spastic paralysis. A histological examination revealed a high incidence of vertebral disc herniation and degeneration in the mutant mice. Electron microscopy of the disc cartilage in the mutant mice showed abnormal rough collagen fibrils with increased diameter, appearance of periodic banding patterns and banding formation. These findings indicate that a reduced level of aggrecan and an abnormal collagen network in the disc cartilage of the mutant mice cause disc degeneration and the resultant early-onset severe disc herniation.

ACAN Mutations in Humans

Autosomal-dominant spondyloepiphyseal dysplasia Kimberley type (SEDK) is characterized by short stature, shortening of the trunk and limbs, and early-onset osteoarthritis (OA) (15). In 2002, a linkage study identified a novel locus responsible for this disorder on chromosome 15q26 in a multigenerational South African family (16). *ACAN* was considered a strong candidate gene for SEDK because it maps to the same region and because an *Acan* mutation results in a form of chondrodysplasia in a mouse model (14). In a family with SEDK reported by Anderson *et al.* (1990), Gleghorn *et al.* (2005) screened for *ACAN* mutations and identified heterozygosity for a single-base pair insertion within the variable repeat region of exon 12 (OMIN 155760.0001) in affected individuals (8, 15).

The autosomal recessive *ACAN* mutation has only been reported in a single family (3). The disorder, termed spondyloepimetaphyseal dysplasia aggrecan type, was characterized with extreme short stature (a final height of 66–71 cm)

with a short neck, a barrel chest, brachydactyly, broad thumbs, and craniofacial abnormalities including macrocephaly, low-set rotated ears, prognathism, and severe mid-face hypoplasia. Genetic studies of the affected family members revealed a homozygous missense mutation (p.Asp2381Asn) in the G3 C-type lectin domain of *ACAN* (Fig. 1). Heterozygous carriers of the mutation in the family exhibited a mild proportionate short stature phenotype (mean adult height between -2 and -4 SDs).

The heterozygous *ACAN* mutations exhibit a broad phenotypic spectrum of non-syndromic short stature associated with advanced bone maturation, osteochondritis dissecans (OCD), early-onset OA, and mild dysmorphic features including mid-facial hypoplasia, brachydactyly, broad great toes, and lumbar lordosis, with no genotype-phenotype correlations (4–11). The phenotype is highly variable, even in patients from the same family.

At least 25 pathological *ACAN* mutations, consisting of 10 nonsense mutations, 6 frameshift mutations, 8 missense mutations, and 1 splice exon-skipping mutation have been reported (3–11). The locations and types of the mutations in literature are shown in Table 1 and Fig. 1, which are modified from those of Dateki *et al.* (11). Seventeen (68%) of the 25 mutations lead to premature stop codons, which result in early truncation of the aggrecan protein and probably nonsense mediated mRNA decay, implying that haploinsufficiency of *ACAN* is the main mechanism underlying the heterozygous aggrecan-related diseases (17).

Clinical Manifestations in Patients with Heterozygous *ACAN* Mutations

Short stature

The short stature in patients with *ACAN* mutations is typically associated with an advanced bone age and early cessation of growth. Individuals with *ACAN* mutations have mild short stature with advanced bone age at a pre-

pubertal stage that leads to premature growth cessation after the start of puberty, resulting in a severely short adult height (4–7). Children with *ACAN* mutations are mostly proportionate, though the arm span is longer than the height in some patients (11). Premature hypertrophic chondrocyte maturation and early invasion of blood vessels and osteoblasts in the growth plate have been proposed as the mechanism for the advanced bone age, early epiphyseal fusion, and premature growth cessation in patients with *ACAN* mutations (12, 13). In general, a short stature is most commonly associated with a delayed bone age in endocrine disorders (GH deficiency, hypothyroidism, and Cushing syndrome), malnutrition, chronic diseases, and idiopathic short stature (1). Conversely, the combination of short stature and advanced bone age is much less common. Thus, when clinicians see patients with such a combination, an *ACAN* mutation may be the most likely cause of pathogenesis.

Early-onset lumbar disc herniation

We recently reported a female Japanese patient with a heterozygous *ACAN* mutation (p.Phe582fs*69) who had early-onset herniation of multiple lumbar discs from L1/2 to L5/S1 (Fig. 2) (11). In addition, Gkouroggianni *et al.* reported several patients with *ACAN* mutations suffering from back pain and intervertebral disc disease (7). In this regard, the aggrecan-deficient *cmd* mice exhibit degeneration of vertebral discs leading to misalignment of the vertebral spine and early disc herniation (14). The spinal phenotype of aggrecan-deficient mice implies that *ACAN* mutations, which lead to reduced aggrecan levels in the cartilage and degeneration of disc cartilage, can cause early-onset and multiple spinal disc herniation and may be involved in the genetic predisposition to disc herniation in humans.

OCD and early-onset OA

ACAN mutations can affect not only the

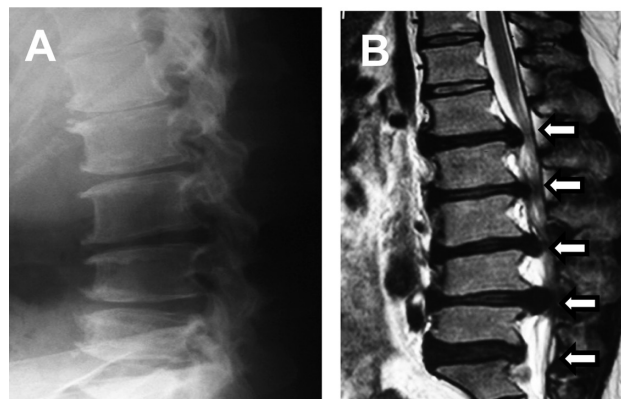


Fig. 2. Spinal phenotype of an *ACAN* mutation. The radiographic image shows vertebral spicules and severe lumbar deformity (A), and the T2-weighted magnetic resonance image shows multiple lumbar disc herniation (white arrows) (B) in a female patient with a heterozygous *ACAN* mutation (11).

growth plate cartilage but also the articular cartilage. The related articular cartilage phenotypes have been reported as OCD and early-onset OA. OCD is characterized by the separation of an articular cartilage and subchondral bone fragment from the articular surface. To date, only two mutations in *ACAN* (p.Leu2355Pro and p.Val2417Met) have been associated with familial OCD (4, 9). Since both missense mutations were located at the C-terminal C-type lectin domain in *ACAN*, dominant negative effects of the mutant proteins have been proposed for the specific articular phenotype.

Early-onset OA has also been reported in patients with *ACAN* mutations (6, 7, 9). The phenotype is associated with missense, truncating, and nonsense mutations located in various regions of *ACAN*, indicating that there is no genotypic correlation for the phenotype.

Other facial and skeletal features

ACAN mutations have been associated with mild dysmorphic findings including mid-facial hypoplasia, flat nasal bridge, relative macrocephaly, frontal bossing, brachydactyly, broad thumbs, and lordosis (7). The facial and

skeletal phenotypes are variable, even in the same family. This may be explained by the notion that haploinsufficiency of developmental genes is usually associated with a wide range of penetrance and expressivity depending on other genetic and environmental factors (18), though the actual underlying factors remain to be identified.

Treatment of Patients with *ACAN* mutations

An advanced bone age at the pre-pubertal stage and premature growth cessation after the start of puberty has been noted in patients with *ACAN* mutations (4–7, 11). In this regard, blocking puberty through means such as GnRH analog therapy might be an option for patients with *ACAN* mutations. Recently, the effectiveness of combined GH and GnRH analog treatment for achieving an appropriate adult height has been reported in several cases with *ACAN* mutations (4, 6, 11). Gkourogianni *et al.* found that the average height SD score of GH-treated adult individuals with *ACAN* mutations ($n = 5$) was -2.5 , while that of untreated adult individuals ($n = 65$) was -3.0 (7). Furthermore, Van der Steen *et al.* reported that patients with *ACAN* mutations who received GH treatment in combination with GnRH analog treatment for 2 yr from the onset of puberty were 5–8 cm taller at their adult height than their same-sex family members with the same *ACAN* mutation (6). In our study, the estimated final height of the elder brother who received combined GH and GnRH analog treatment was higher than that of the younger brother, who only received the GnRH analog (158.5 cm *vs.* 145.6 cm) (11). Collectively, these observations suggest a modest response to GH and GnRH analog treatment for adult height in patients with *ACAN* mutations.

Conclusion

We reviewed current knowledge regarding aggrecan-associated diseases. *ACAN*

haploinsufficiency is a newly discovered cause of short stature with accelerated bone age. Further studies are needed to determine the incidence of *ACAN* mutations in patients with idiopathic short stature and to clarify the effectiveness and safety of GH and GnRH analog treatment for patients with *ACAN* mutations.

Conflicts of Interest: The authors declare no conflicts of interest in association with this study.

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