

# Aggrecan Mutations in Nonfamilial Short Stature and Short Stature Without Accelerated Skeletal Maturation

Christina Tatsi,<sup>1</sup> Alexandra Gkourogiani,<sup>2</sup> Klaus Mohnike,<sup>3</sup> Diana DeArment,<sup>4</sup> Selma Witchel,<sup>4</sup> Anenisia C. Andrade,<sup>2</sup> Thomas C. Markello,<sup>5</sup> Jeffrey Baron,<sup>1</sup> Ola Nilsson,<sup>2,6</sup> and Youn Hee Jee<sup>1</sup>

<sup>1</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, Bethesda, Maryland 20892; <sup>2</sup>Karolinska Institutet, Department of Women's and Children's Health, SE-171 77 Stockholm, Sweden; <sup>3</sup>Department of Pediatrics, Otto-von-Guericke-University, 39104 Magdeburg, Germany; <sup>4</sup>Division of Pediatric Endocrinology, Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, University of Pittsburgh, Pittsburgh, Pennsylvania 15224; <sup>5</sup>Undiagnosed Diseases Program, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892; and <sup>6</sup>Department of Medical Sciences, Örebro University, 702 03 Örebro, Sweden

Aggrecan, a proteoglycan, is an important component of cartilage extracellular matrix, including that of the growth plate. Heterozygous mutations in *ACAN*, the gene encoding aggrecan, cause autosomal dominant short stature, accelerated skeletal maturation, and joint disease. The inheritance pattern and the presence of bone age equal to or greater than chronological age have been consistent features, serving as diagnostic clues. From family 1, a 6-year-old boy presented with short stature [height standard deviation score (SDS),  $-1.75$ ] and bone age advanced by 3 years. There was no family history of short stature (height SDS: father,  $-0.76$ ; mother,  $0.7$ ). Exome sequencing followed by Sanger sequencing identified a *de novo* novel heterozygous frameshift mutation in *ACAN* (c.6404delC: p.A2135Dfs). From family 2, a 12-year-old boy was evaluated for short stature (height SDS,  $-3.9$ ). His bone age at the time of genetic evaluation was approximately 1 year less than his chronological age. Family history was consistent with an autosomal dominant inheritance of short stature, with several affected members also showing early-onset osteoarthritis. Exome sequencing, confirmed by Sanger sequencing, identified a novel nonsense mutation in *ACAN* (c.4852C>T: p.Q1618X), which cosegregated with the phenotype. In conclusion, patients with *ACAN* mutations may present with nonfamilial short stature and with bone age less than chronological age. These findings expand the known phenotypic spectrum of heterozygous *ACAN* mutations and indicate that this diagnosis should be considered in children without a family history of short stature and in children without accelerated skeletal maturation.

Copyright © 2017 Endocrine Society

This article has been published under the terms of the Creative Commons Attribution Non-Commercial, No-Derivatives License (CC BY-NC-ND; <https://creativecommons.org/licenses/by-nc-nd/4.0/>).

**Freeform/Key Words:** aggrecan, short stature, advanced bone age, exome sequencing

Aggrecan, a chondroitin sulfate proteoglycan, is an important component of the extracellular matrix of cartilage, including growth plate, articular, and intervertebral disc cartilage [1]. Aggrecan abnormalities disrupt growth plate chondrocyte differentiation and impair endochondral ossification at the growth plate [2–5].

In humans, mutations in *ACAN*, the gene that encodes aggrecan, result in short stature due to impaired function of growth plate cartilage and result in joint disease due to impaired function of articular and intervertebral disc cartilage. Homozygous mutations cause a severe skeletal dysplasia termed spondyloepimetaphyseal dysplasia, aggrecan type (MIM#612813)

Abbreviations: CS1, chondroitin sulfate 1; G3, Globular 3; GH, growth hormone; SDS, standard deviation score.

[6]. Heterozygous mutations can result in spondyloepiphyseal dysplasia, Kimberley type (MIM#608361) and osteochondritis dissecans with short stature and early-onset osteoarthritis (MIM#165800) [7, 8]. More recently, heterozygous mutations in *ACAN* have been identified as the cause of autosomal dominant isolated short stature [9, 10]. The inheritance pattern and the presence of bone age equal to or greater than chronological age have previously been consistent features and are considered important diagnostic clues [10].

Here we describe two unrelated children with *ACAN* mutations and novel clinical features. The first child had nonfamilial short stature due to a novel *de novo* frameshift mutation in *ACAN*, and the second child had a bone age less than chronological age due to a novel nonsense mutation. These findings expand the known phenotypic spectrum of heterozygous *ACAN* mutations to include children with nonfamilial short stature and with isolated short stature and a bone age less than chronological age.

## 1. Materials and Methods

A description of the methods is provided in the supplemental material.

## 2. Results

### A. Case Reports

#### A-1. Family 1

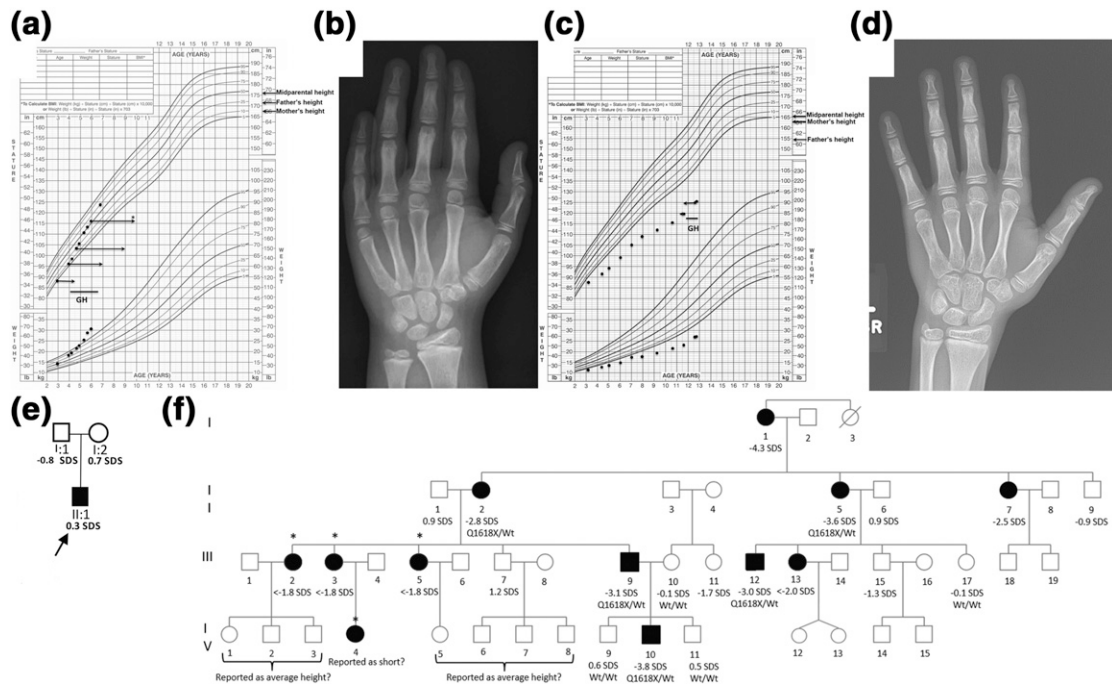
The proband is a prepubertal boy (6 years and 9 months of age) with nonfamilial, disproportionate short stature and advanced skeletal maturation. He was born at 41 weeks of gestation with birth length of 48 cm [−1.6 standard deviation score (SDS)] and birth weight of 2.8 kg (−1.7 SDS) to healthy parents of normal height [father: 171.4 cm (−0.76 SDS); mother: 167.8 cm (0.7 SDS)] [Fig. 1(e)]. There was no short stature in the extended family. Between 1 and 4 years of age, his height was close to the fifth percentile (−1.75 SDS) [Fig. 1(a)]. He was disproportionate, with sitting height index (sitting height/stature) of +2.3 SDS. He had a normal arm span and mild midface hypoplasia. His bone age was 4 years and 6 months at chronological age of 3 years and was 7 years at chronological age 4 years. Growth hormone (GH) stimulation tests (clonidine and arginine, unprimed) produced a peak GH value of 7 ng/mL. Additional endocrine and other laboratory evaluation (Supplemental Table 1) did not identify a cause for the short stature or the advanced bone age. He was treated with growth hormone (GH) (0.22 mg/kg/wk) since 4 years of age, with increasing height SDS (−1.35 to 0.62 SDS). During treatment, bone age minus chronological age increased from 3 to 4 years [Fig. 1(b); Table 1], and predicted adult height increased from 143 to 156.8 cm.

Using exome sequencing followed by Sanger sequencing, a novel heterozygous frameshift mutation in exon 12 (c.6404delC: p.A2135Dfs) of the *ACAN* gene was detected and confirmed in the proband. It was not present in the parents. This mutation is predicted to cause truncation of the aggrecan protein at amino acid 2135. It was not found in a large database (ExAC). Paternity was confirmed by single nucleotide polymorphism array.

#### A-2. Family 2

The proband is a boy (12 years and 9 months of age) with short stature. He was born at 38 weeks of gestational age, with a birth weight of 2.95 kg (0.0 SDS) and birth length of 45 cm (−1.36 SDS). Short stature developed in early childhood, with appropriate weight for height [Fig. 1(c)]. He had attention deficit hyperactivity disorder managed with dextroamphetamine/amphetamine and guanfacine, which were the only medications he was receiving. He had no history of arthralgia or other joint problems.

He presented to the pediatric endocrinology clinic at Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center for evaluation of short stature at the age of



**Figure 1.** (a) Growth chart of proband in family 1. Height is shown as black circles, and height for bone age is shown as the tip of the arrows. The black line corresponds to the period of GH treatment. There is a discrepancy of 2 months between the height measurement at chronological age 5 years 11.5 months and the bone age x-ray performed at chronological age 6 years and 2 months (\*). (b) Bone age radiograph of proband in family 1 obtained at chronological age 6 years and 2 months. The bone age was interpreted as 9 years and 9 months using the Greulich and Pyle atlas. (c) Growth chart of the proband in family 2. Height and weight are indicated with black circles and height for bone age as the tips of the black arrows. The black line corresponds to the period of GH treatment. (d) Bone age radiograph of proband in family 2 obtained at chronological age 12 years and 7 months. The bone age was interpreted as 11 years and 9 months using the Greulich and Pyle atlas and as 11 years and 9 months using the Tanner Whitehouse method. (e) Pedigree of proband in family 1. The affected proband (II:1) is shown with an arrow as a filled square. The height in SDS is shown below each symbol. (f) Pedigree of proband in family 2. The affected proband (IV:10) is indicated with an arrow. The family members with short stature are indicated with filled symbols. The height in SDS and the genotype for the members who underwent genetic testing are reported below each symbol. The three paternal aunts and one of their offspring were short as reported by the proband's family, but no documentation of their height was available (\*).

11 years and 7 months. At that visit, he was short (height SDS,  $-4.0$ ) with low weight (weight SDS,  $-3.3$ ). The laboratory evaluation revealed normal complete blood count, chemistry panel, urinalysis, inflammatory markers, celiac panel, thyroid function tests, IGF-1, and IGFBP3. Two unprimed GH stimulation tests with arginine and insulin administration were performed, which produced a peak GH value of  $9.3$  ng/mL (Supplemental Table 1). The patient was started on GH treatment at the age of 11 years and 10 months with a dose of  $0.29$  mg/kg/wk, which appeared to result in modest growth acceleration.

He was referred to the National Institutes of Health at the age of 12 years and 9 months. At that visit, his height SDS was  $-3.8$ , and his sitting height index was  $+1.3$  SDS. His arm span was longer than his height ( $+7.6$  cm). He had mild dysmorphic facial features, including retrognathia and midface hypoplasia, and he was prepubertal.

The bone age of the proband was younger than his chronological age for two consecutive years. At a chronological age of 11 years and 7 months, his bone age was 11 years and 3 months based on the Greulich and Pyle atlas. At chronological age of 12 years and 7 months, his bone age was 11 years and 6 months [Fig. 1(d)]. The bone age assessments were similar when assessed with the Tanner Whitehouse (TW3) method (Table 1).

**Table 1. Bone Age Assessment for the Probands of the Two Families According to the Greulich and Pyle and the Tanner Whitehouse 3 Methods**

Subject Characteristics	Proband in Family 1	Proband in Family 2	
Age	6 y, 2 mo	11 y, 7 mo	12 y, 7 mo
Height, cm	116.2 <sup>a</sup>	119.2	124.9
Height, SDS	0.32 <sup>a</sup>	-4.0	-3.8
Bone age (Greulich/Pyle)	9 y, 9 mo	11 y, 3 mo	11 y, 6 mo
Carpal bone age (TW3)	9 y, 10 mo	10 y, 8 mo	11 y, 9 mo
RUS bone age (TW3)	9 y, 5 mo	11 y, 6 mo	11 y, 9 mo
Predicted adult height, cm	156.8	147.8	150.1

Abbreviations: RUS, radius, ulna and short bones; TW3, Tanner Whitehouse.

<sup>a</sup>There is a discrepancy of 2 months between the height measurement performed at chronological age 5 years and 11.5 months and the bone age x-ray performed at chronological age 6 years and 2 months.

The proband's immediate family was also clinically evaluated at the outpatient clinic of the National Institutes of Health Clinical Center. His father was noted to be short (height SDS, -3.1) with a normal sitting height index (+1 SDS) and arm span. The proband's siblings and his mother were of average height with normal body proportions. Extended family history revealed multiple members with short stature in a pattern consistent with an autosomal dominant inheritance [Fig. 1(f)]. The father reported substantial knee and shoulder pains starting in early adulthood. Similarly, the affected 58-year-old paternal grandmother has a history of two previous hip replacement arthroplasties at 45 and 56 years of age and severe hand arthritis and scoliosis. Her 68-year-old sister [Fig. 1(f), II:5] has also undergone bilateral hip replacement arthroplasties, and she has persistent hand osteoarthritis and degenerative disc disease. Blood samples were collected from affected [Fig. 1(f), II:2, II:5, III:9, III:12] and unaffected family members [Fig. 1(f), III:10, III:17, IV:9, IV:11].

Exome sequencing identified a heterozygous nonsense mutation in *ACAN* (c.4852C>T: p.Q1618X) that cosegregated with the short stature phenotype in all studied family members [Fig. 1(f)]. The mutation leads to the predicted loss of the C-terminus of the protein, including part of the chondroitin sulfate 1 (CS1) domain and all of the CS2 and Globular 3 (G3) domains. It was not found in a large database (ExAC). Sanger sequencing confirmed the mutation in all affected family members studied.

### 3. Discussion

We studied two boys with short stature (height SDS, -2.0 and -4.0). The first patient had accelerated bone age but no family history of short stature. The second patient had an extended family pedigree consistent with an autosomal dominant inheritance of short stature, which included some affected family members who also had joint disease. His bone age was approximately 1 year less than his chronological age. Exome sequencing, confirmed by Sanger sequencing, revealed in both families two novel mutations in *ACAN*, the gene encoding aggrecan. In the first family the mutation was a *de novo* event, whereas in the second patient it cosegregated with the short stature.

The proband in family 1 represents the first reported child with nonfamilial short stature due to a mutation in *ACAN*. All previously reported cases have shown an autosomal dominant inheritance. In family 1, the *ACAN* mutation was not present in either parent (with paternity confirmed by single nucleotide polymorphism array analysis), and therefore the mutation arose *de novo* in the proband, which explains the sporadic presentation.

Several lines of evidence strongly indicate that the *ACAN* mutation is responsible for the short stature in this child. First, the phenotype of short stature with marked bone age advancement closely resembles that of individuals reported to have heterozygous *ACAN* mutations. Second, the mutation is not present in a large database (ExAC) and thus is rare in the general population. Third, the identified frameshift mutation leads to a predicted premature

termination of the protein, affecting the G3 domain of the protein. As previously described in an animal model, the G3 domain is important for the glycosylation of the protein, and its absence results in defective protein secretion and processing [11]. Additionally, pathogenic mutations in this domain have been previously reported in patients with familial short stature due to *ACAN* mutations. Fourth, as noted previously, the *de novo* occurrence of the mutation matches the sporadic occurrence of the phenotype.

The proband in family 2, unlike previously reported subjects, had a bone age less than chronological age. Indeed, one of the main characteristic features of short stature associated with aggrecan mutations is the presence of advanced bone age. Gkourogianni *et al.* [10] report that the bone age in their cohort was advanced by +0 to +4.0 years, with 6 out of the 20 families (30%) presenting with a markedly advanced bone age of +3.0 to +4.0 years. Similarly, adult patients may report a history of linear growth cessation at an early age. To our knowledge, the only description of delayed bone age associated with an *ACAN* mutation was made by Anderson *et al.* [12] in a family with spondyloepiphyseal dysplasia syndrome, Kimberley type. However, there was no mention of the actual bone age of the patient or the method used for the assessment. Thus, our patient is the first described to have isolated familial short stature and bone age less than chronological age.

Because the proband has a feature not previously reported for this genetic condition, it is particularly important to establish that the observed mutation is pathogenic and not an incidental finding. Several lines of evidence strongly indicate that the *ACAN* mutation is responsible for the growth abnormality in this family. First, the overall phenotype (except for skeletal maturation) of affected family members closely resembles that of prior individuals reported with heterozygous *ACAN* mutations, including short stature, a tendency to have relatively short legs but long arms compared with the trunk, midface hypoplasia, and accompanying joint disease. Second, the mutation was rare (*i.e.*, not found in the ExAC database). Third, the sequence variant is a nonsense mutation, which results in predicted loss of the C-terminus of the protein, including part of the CS1 domain and all of the CS2 and G3 domains, which are considered to be important for aggrecan functions in water retention and structure of the cartilage [1]. Additionally, as mentioned previously, the G3 domain is important for the processing of the protein [11]. Fourth, the nonsense mutation cosegregated with the disorder.

The reason that the proband in family 2 had a more delayed bone age than previously reported subjects is unclear. It seems unlikely that his particular mutation has a unique effect because it is likely a simple null mutation, and other null mutations have been reported. A more likely possibility is that the variability in skeletal age in this disorder is analogous to the wide variability in the normal population. For example, for healthy 12-year-old boys, the standard deviation in bone age is approximately 10 months [13]. This variability likely involves multiple genetic and environmental factors. Because the father had not been evaluated for short stature during his childhood, his bone age was not available to evaluate whether the variability in bone age is consistent in the family.

Our findings have important clinical implications. Prior studies suggest that the diagnosis of an *ACAN* mutation should only be sought in patients with familial short stature and bone age equal to or greater than chronological age. The current findings suggest that this diagnosis should also be considered in children with nonfamilial short stature and in those with bone age less than chronological age. The relative frequency of these presentations may have been previously underestimated because targeted sequencing for *ACAN* has focused on patients with familial short stature and advanced bone ages. To determine the prevalence of *ACAN* mutations in children with nonfamilial short stature or delayed bone age will require targeted or exome sequencing studies in a sufficiently large set of subjects, including those with no family history and with delayed bone age.

In summary, we describe cases of heterozygous *ACAN* mutation presenting as nonfamilial short stature because the mutation arose *de novo* and as familial short stature with bone age younger than the chronological age. Thus, these findings expand the currently known spectrum of heterozygous *ACAN* mutations. This diagnosis should not be excluded based solely on the absence of a family history of short stature or of advanced skeletal maturation.

## Acknowledgments

Address all correspondence to: Jeffrey Baron, MD, NICHD, NIH, 10 Center Drive, Building 10, NIH-Clinical Research Center, Room 1-3330, MSC1103, Bethesda, Maryland 20892. E-mail: [baronj@cc1.nichd.nih.gov](mailto:baronj@cc1.nichd.nih.gov).

This work was supported by the Intramural Research Program, Eunice Kennedy Shriver National Institute of Child Health & Human Development, National Institutes of Health (NIH) and by funding from the Deputy Director for Intramural Research through the Clinical Center Genomics Opportunity, NIH.

T.M. is supported by the Intramural Research Program of the National Human Genome Research Institute and the Common Fund, Office of the Director, NIH.

The work by O.N., A.G., A.C.A., was supported by grants from the Swedish Research Council (project K2015-54X-22 736 -01 -4 & 2015-02227), the Swedish Governmental Agency for Innovation Systems (Vinnova) (2014-01438), Marianne and Marcus Wallenberg Foundation, the Stockholm County Council, Byggmästare Olle Engkvist Stiftelse, the Swedish Society of Medicine, Novo Nordisk Foundation (grant NNF16OC0021508), Erik och Edith Fernström Foundation for Medical Research, HKH Kronprinsessan Lovisas förening för barnsjukvård, Sällskapet Barnavård, Stiftelsen Frimurare Barnhuset i Stockholm, and Karolinska Institutet, Stockholm, Sweden, and Örebro University, Örebro, Sweden.

Disclosure Summary: The authors have nothing to disclose.

---

## References and Notes

- Kiani C, Chen L, Wu YJ, Yee AJ, Yang BB. Structure and function of aggrecan. *Cell Res.* 2002;**12**(1): 19–32.
- Myllyharju J. Extracellular matrix and developing growth plate. *Curr Osteoporos Rep.* 2014;**12**(4): 439–445.
- Nilsson O, Marino R, De Luca F, Phillip M, Baron J. Endocrine regulation of the growth plate. *Horm Res.* 2005;**64**(4):157–165.
- Domowicz MS, Cortes M, Henry JG, Schwartz NB. Aggrecan modulation of growth plate morphogenesis. *Dev Biol.* 2009;**329**(2):242–257.
- Lauing KL, Cortes M, Domowicz MS, Henry JG, Baria AT, Schwartz NB. Aggrecan is required for growth plate cytoarchitecture and differentiation. *Dev Biol.* 2014;**396**(2):224–236.
- Tompson SW, Merriman B, Funari VA, Fresquet M, Lachman RS, Rimoin DL, Nelson SF, Briggs MD, Cohn DH, Krakow D. A recessive skeletal dysplasia, SEMD aggrecan type, results from a missense mutation affecting the C-type lectin domain of aggrecan. *Am J Hum Genet.* 2009;**84**(1):72–79.
- Gleghorn L, Ramesar R, Beighton P, Wallis G. A mutation in the variable repeat region of the aggrecan gene (AGC1) causes a form of spondyloepiphyseal dysplasia associated with severe, premature osteoarthritis. *Am J Hum Genet.* 2005;**77**(3):484–490.
- Stattin EL, Wiklund F, Lindblom K, Onnerfjord P, Jonsson BA, Tegner Y, Sasaki T, Struglics A, Lohmander S, Dahl N, Heinegård D, Aspberg A. A missense mutation in the aggrecan C-type lectin domain disrupts extracellular matrix interactions and causes dominant familial osteochondritis dissecans. *Am J Hum Genet.* 2010;**86**(2):126–137.
- Nilsson O, Guo MH, Dunbar N, Popovic J, Flynn D, Jacobsen C, Lui JC, Hirschhorn JN, Baron J, Dauber A. Short stature, accelerated bone maturation, and early growth cessation due to heterozygous aggrecan mutations. *J Clin Endocrinol Metab.* 2014;**99**(8):E1510–E1518.
- Gkourogianni A, Andrew M, Tyzinski L, Crocker M, Douglas J, Dunbar N, Fairchild J, Funari MF, Heath KE, Jorge AA, Kurtzman T, LaFranchi S, Lalani S, Lebl J, Lin Y, Los E, Newbern D, Nowak C, Olson M, Popovic J, Pruhová Š, Elblova L, Quintos JB, Segerlund E, Sentschordy L, Shinawi M, Stattin EL, Swartz J, Angel AG, Cuéllar SD, Hosono H, Sanchez-Lara PA, Hwa V, Baron J, Nilsson O, Dauber A. Clinical characterization of patients with autosomal dominant short stature due to aggrecan mutations. *J Clin Endocrinol Metab.* 2017;**102**:460–469.
- Li H, Schwartz NB, Vertel BM. cDNA cloning of chick cartilage chondroitin sulfate (aggrecan) core protein and identification of a stop codon in the aggrecan gene associated with the chondrodystrophy, nanomelia. *J Biol Chem.* 1993;**268**(31):23504–23511.
- Anderson IJ, Tsipouras P, Scher C, Ramesar RS, Martell RW, Beighton P. Spondyloepiphyseal dysplasia, mild autosomal dominant type is not due to primary defects of type II collagen. *Am J Med Genet.* 1990;**37**(2):272–276.
- Greulich W, Pyle S. Radiographic atlas of skeletal development of the hand and wrist. doi:10.1097/00000441-195909000-00030.