Clinical Characterization of Patients With Autosomal Dominant Short Stature due to Aggrecan Mutations

Alexandra Gkourogianni,[1] Melissa Andrew,[2] Leah Tyzinski,[2] Melissa Crocker,[3] Jessica Douglas,[4] Nancy Dunbar,[5] Jan Fairchild,[6] Mariana F. A. Funari,[7] Karen E. Heath,[8] Alexander A. L. Jorge,[7] Tracey Kurtzman,[9] Stephen LaFranchi,[10] Seema Lalani,[11] Jan Lebl,[13] Yuezhen Lin,[12] Evan Los,[10] Dorothee Newbern,[14] Catherine Nowak,[4] Micah Olson,[14] Jadranka Popovic,[15] Štěpánka Průhová,[13] Lenka Elblova,[13] Jose Bernardo Quintos,[16] Emma Segerlund,[1,17] Lucia Sentchordi,[8,18] Marwan Shinawi,[19] Eva-Lena Stattin,[20] Jonathan Swartz,[3] Ariadna González del Angel,[21] Sinhué Diaz Cuéllar,[21] Hidekazu Hosono,[22] Pedro A. Sanchez-Lara,[23] Vivian Hwa,[2] Jeffrey Baron,[24] Ola Nilsson,[1,24,25]* and Andrew Dauber[2]*

[1]Division of Pediatric Endocrinology, Department of Women's and Children's Health, Karolinska Institutet and Karolinska University Hospital, Stockholm SE-171 76, Sweden; [2]Cincinnati Center for Growth Disorders, Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 70941; Divisions of [3]Endocrinology and [4]Genetics, Boston Children's Hospital, Boston, Massachusetts 02115; [5]Division of Pediatric Endocrinology, Connecticut Children's Medical Center, Hartford, Connecticut 06106; [6]Department of Endocrinology and Diabetes, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia; [7]Unidade de Endocrinologia do Desenvolvimento (LIM/42), Disciplina de Endocrinologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo 05508-020, Brazil; [8]Institute of Medical and Molecular Genetics (INGEMM) and Skeletal Dysplasia Multidisciplinary Unit, Hospital Universitario La Paz, Universidad Autónoma de Madrid, IdiPAZ, and CIBERER, ISCIII, Madrid 20849, Spain; [9]El Rio Community Health Center, Tucson, Arizona 85745; [10]Department of Pediatrics, Oregon Health and Science University, Portland, Oregon 97239; Departments of [11]Molecular and Human Genetics and [12]Pediatric Endocrinology and Metabolism, Baylor College of Medicine, Houston, Texas 77030; [13]Department of Pediatrics, Second Faculty of Medicine, Charles University in Prague and University Hospital in Motol, Prague 11636, Czech Republic; [14]Division of Endocrinology, Phoenix Children's Hospital, Phoenix, Arizona 85016; [15]Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15237; [16]Hasbro Children's Hospital, Providence, Rhode Island 02903; [17]Sunderby Hospital, Sunderby 95442, Sweden; [18]Department of Pediatrics, Hospital Universitario Infanta Sofia, Madrid 28703, Spain; [19]Division of Genetics, Washington University, St. Louis, Missouri 63130; [20]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala 75236, Sweden; [21]Laboratorio de Biología Molecular, Departamento de Genética Humana, Instituto Nacional de Pediatría, Insurgentes-Cuicuilco, Coyoacán 04530, México; [22]Cottage Children's Medical Center, Santa Barbara, California 93111; [23]Center for Personalized Medicine, Children's Hospital of Los Angeles, Los Angeles, California 90027; [24]Section on Growth and Development, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892; and [25]Department of Medical Sciences, Örebro University and University Hospital, Örebro 70185, Sweden

Context: Heterozygous mutations in the aggrecan gene (*ACAN*) cause autosomal dominant short stature with accelerated skeletal maturation.

Objective: We sought to characterize the phenotypic spectrum and response to growth-promoting therapies.

Patients and Methods: One hundred three individuals (57 females, 46 males) from 20 families with autosomal dominant short stature and heterozygous *ACAN* mutations were identified and

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*These authors contributed equally to this study.

Abbreviations: ACAN, aggrecan; BA, bone age; CLD, C-type lectin-binding domain; FOD, familial osteochondritis dissecans; GH, growth hormone; OA, osteoarthritis; OD, osteochondritis dissecans; SDS, standard deviation score; SHI, sitting height index.

confirmed using whole-exome sequencing, targeted next-generation sequencing, and/or Sanger sequencing. Clinical information was collected from the medical records.

Results: Identified *ACAN* variants showed perfect cosegregation with phenotype. Adult individuals had mildly disproportionate short stature [median height, −2.8 standard deviation score (SDS); range, −5.9 to −0.9] and a history of early growth cessation. The condition was frequently associated with early-onset osteoarthritis (12 families) and intervertebral disc disease (9 families). No apparent genotype–phenotype correlation was found between the type of *ACAN* mutation and the presence of joint complaints. Childhood height was less affected (median height, −2.0 SDS; range, −4.2 to −0.6). Most children with *ACAN* mutations had advanced bone age (bone age − chronologic age; median, +1.3 years; range, +0.0 to +3.7 years). Nineteen individuals had received growth hormone therapy with some evidence of increased growth velocity.

Conclusions: Heterozygous *ACAN* mutations result in a phenotypic spectrum ranging from mild and proportionate short stature to a mild skeletal dysplasia with disproportionate short stature and brachydactyly. Many affected individuals developed early-onset osteoarthritis and degenerative disc disease, suggesting dysfunction of the articular cartilage and intervertebral disc cartilage. Additional studies are needed to determine the optimal treatment strategy for these patients. (*J Clin Endocrinol Metab* 102: 460–469, 2017)

Longitudinal bone growth occurs at the growth plate and is the result of proliferation, hypertrophy, and matrix production by chondrocytes. This process, chondrogenesis, requires that growth plate chondrocytes integrate a complex network of signals from endocrine and paracrine systems and inputs from cell–cell and cell–matrix interactions. Consequently, growth failure can be caused by mutations in any 1 of the many genes that directly or indirectly affect the growth plate chondrocytes and the process of growth plate chondrogenesis (1). It is thus not surprising that the molecular cause of short stature remains undiagnosed in a large fraction of affected children. Recent implementation of exome sequence analysis, however, has started to identify genetic diagnoses in subgroups of patients with idiopathic short stature, including heterozygous mutations in the aggrecan gene (*ACAN*) causing autosomal dominant short stature with advanced bone age (BA) (2, 3). *ACAN* mutations are sometimes associated with early-onset osteoarthritis (OA) but no, or only minor, skeletal findings suggestive of skeletal dysplasia (2–4).

Aggrecan is a proteoglycan and important component of extracellular matrix, critical to the structure and function of growth plate cartilage and other cartilaginous tissues (5). The aggrecan core protein consists of a large, centrally located glycosaminoglycan attachment region flanked by 2 N-terminal and 1 C-terminal globular domains (G1 to G3; Fig. 1). Aggrecan forms large proteoglycan aggregates by interaction of the G1 domain with hyaluronan and cartilage link protein. The G2 domain is highly conserved during evolution, but its biological function is so far unknown. In contrast, the G3 domain contains a C-type lectin-binding domain (CLD) critical to the interaction with other extracellular proteoglycans, including tenascins and fibulins (4, 6) (Fig. 1).

Since our 2014 report of heterozygous *ACAN* mutations in patients with short stature and advanced BA (2), multiple new families with this condition have been identified. To better characterize the phenotypic spectrum, associated conditions, and response to growth-promoting therapies in individuals with heterozygous *ACAN* mutations, an international cohort of 103 individuals from 20 different families with heterozygous *ACAN* mutations has been assembled and are reported in the present study.

Patients and Methods

Subjects

The institutional review board of Cincinnati Children's Medical Center (Cincinnati, OH), the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (Bethesda, MD), Charles University in Prague and University Hospital in Motol (Prague, Czech Republic), Hospital Universitario La Paz (Madrid, Spain), and regional ethical review board of Umeå University (Umeå, Sweden) and Karolinska Institutet (Stockholm, Sweden) approved the present study. All the participants or their legal guardians provided written informed consent. All the subjects' height and weight data were plotted on the Centers for Disease Control and Prevention growth charts (7), and z-scores for height, weight, and sitting height index (SHI) were calculated using the Third National Health and Nutrition Examination Survey growth data (8). The clinical information was collected on a standardized data form (Supplemental Table 1) for all subjects. Birth length, birth weight, and head circumference were plotted according to the subject's week of gestation or age (7, 9). The evaluation of puberty was assessed according to the methods described by Marshall and Tanner (10, 11), and BA was assessed according to the method of Greulich and Pyle (12) by a single reader of the films, who was kept unaware of the age and identification of the individuals. Five of the families with heterozygous *ACAN* mutations causing either autosomal dominant short stature and advanced BA (families 1 to 4) (2, 3) or familial osteochondritis dissecans (FOD)

Family	c - DNA	Exon	Variant
18	c.61G>T	2	p.Glu21*
12	c.223T>C	3	p.Trp75Arg
1	c.272delA	3	p.Arg93Alafs*
20	c.492C>G	4	p.Tyr164*
10	c.532A>T	4	p.Asn178Tyr
16	c.903G>C	6	p.Trp301Cys
15	c.916A>T	6	p.Ser306Cys
13	c.1047_1048 delinsAC	6	p.Tyr349*
8	c.1443G>T	7	p.Glu415*
14	c.1425delA	8	p.Val478Serfs*
11	c.1526C>A	8	p.Ser509*
2	c.2026+1G>A	10	c.2026+1G>A
9	c.4657G>T	12	p.Glu1553*
4	c.5391delG	12	p.Gly1797Glyfs*
3	c.7064T>C	14	p.Leu2355Pro
7	c.7153G>A	15	p.Glu2385Lys
19	c.7203G>A	16	p.Trp2401*
5,6	c.7429G>A	16	p.Val2417Met
17	c.7276G>T	16	p.Glu2426*

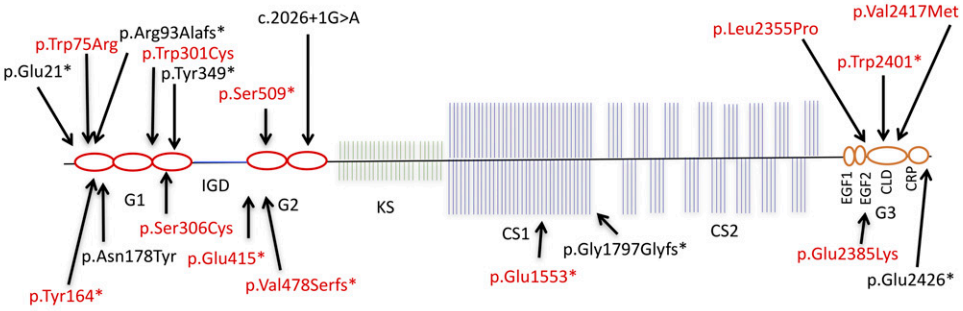

Figure 1. Structure of *ACAN* and the locations of pathogenic sequence variants. Aggrecan consists of a core protein, including 3 globular domains (G1 to G3), with the G3 domain including a C-type lectin-binding (CLD) multifunctional domain, plus 4 interglobular domains, a keratan-sulfate (KS), and 2 chondroitin-sulfate (CS1, CS2) attachment domains. The G1 region is encoded by exons 3 to 6, the interglobular domain region by exon 7, the G2 region by exons 8 to 10, the glycosaminoglycan (GAG; KS-CS1-CS2) attachment region by exons 11 to 12, and the G3 region by exons 13 to 19. The tabular data show the location of the sequence variant found in each family and the predicted change in amino acid sequence. The mutation in family 2 involved a splice site. The asterisk indicates a stop codon; and the red font, families with joint disease. fs, frame shift.

(family 5) with early-onset OA and disproportional short stature (4, 13) have been previously reported.

Sequencing

ACAN (MIM 155760, NM_013227.3) is located on chromosome 15q26 and encodes aggrecan, which contains 2530 amino acids with a calculated mass of 254 kDa (14) (Fig. 1). The *ACAN* mutations in families 1 to 5 were previously identified by either exome sequencing or linkage analysis (2–4). The proband of family 6 presented with short stature and early-onset OA and was found to be a carrier of the same mutation as the affected members in family 5. The DNA of additional members of family 6 was subsequently sequenced, confirming that the mutation cosegregated with the FOD phenotype (4, 13). Additional families were identified by Sanger sequencing, exome sequencing, or by targeted next-generation sequencing at various clinical and research laboratories around the world. All candidate variants were confirmed via Sanger sequencing and were shown to segregate with the skeletal phenotype in all available family members.

Variant analysis

None of the variants reported were present in the Exome Aggregation Consortium database. All 7 missense variants were predicted to be damaging by all 4 *in silico* prediction tools, CADD (complete annotation dependent depletion; available at http://cadd.gs.washington.edu/), SIFT (scale-invariant feature transform), PolyPhen, and MutationTaster (15). The level of conservation of the affected amino acids was determined using GerpN. Protein function was summarized using information found in the UniProt database (available at www.uniprot.org; NP_037359.3).

Results

Cohort characteristics

In total, 103 mutation-positive individuals (57 females, 46 males) from 20 different families with dominantly inherited short stature were identified and included in the present study. Their age ranged from 1.3 to 86 years (median, 15 years), including 33 children (age <18 years; 17 females, 16 males) and 70 adults (age ≥18 years; 40 females, 30 males; Fig. 2; Supplemental Fig. 1). Most of the probands (18 of 20) were identified in pediatric endocrine or genetics clinics and had been evaluated for short stature with normal endocrine evaluation results and no or only subtle findings on skeletal studies, except for advanced BA. Two of the probands (families 13 and 16) were evaluated for skeletal dysplasia soon after birth. The proband of family 13, owing to prenatally identified proportionate short stature and facial dysmorphism (frontal bossing, midface hypoplasia, anteverted ears, brachydactyly), and the proband of family 16 was identified because of short limbs at birth.

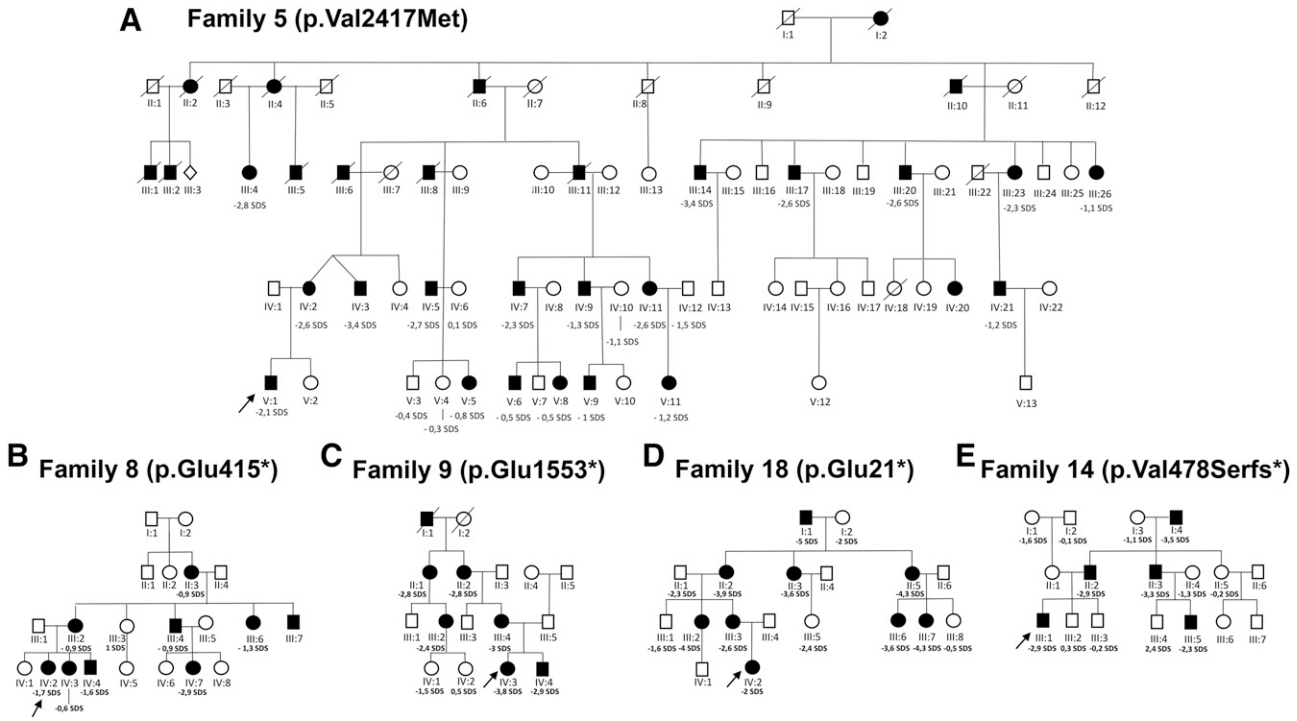

Figure 2. Pedigrees of the studied families: (A) Family 5. (B) Family 8. (C) Family 9. (D) Family 18. (E) Family 14. The arrows indicate the proband. Individuals carrying heterozygous *ACAN* mutations are indicated by solid symbols, and unaffected individuals by open symbols. The height SDS of each individual is indicated.

ACAN variants

We identified 19 different *ACAN* mutations (11 truncating, 7 missense, 1 splice site) in the 20 different pedigrees (Fig. 1; Supplemental Fig. 1). Five of the pathogenic sequence variants have been reported previously (2–4, 13). *In silico* analysis predicted that all 7 missense variants were deleterious (Supplemental Table 2). A second family with FOD due to the *p.Val2417Met* mutation (4) (family 6) was identified and likely represented a branch of the original FOD family (4) (family 5), because they originated from the same region in northern Sweden. The identified mutations were widely scattered over the gene (Fig. 1).

Heterozygous ACAN mutations affect linear growth and cause adult short stature

Of 33 children, 32 were born at full term. At birth, the affected children tended to have a birth weight SDS and birth length SDS in the lower part of the normal range [median birth weight (n = 29), −0.7 SDS; range, −4.1 to 0.5 SDS; median birth length (n = 29), −1.5 SDS; range, −3.8 to 1.1 SDS; Fig. 3].

During childhood [age <10 years (n = 26), 14 females, 12 males], short stature was more pronounced, with heights ranging from low-normal to substantially short [median height (n = 25), −2.0 SDS; range, −4.0 to −0.6 SDS; Figs. 3 and 4; Supplemental Fig. 2]. Children were also mostly proportionate, with a SHI SDS in the upper part of the normal range [6 of 9 children SHI within ± 2 SDS; median SHI (n = 9), 1.6 SDS; range, −0.3 to 2.7 SDS]. During childhood, the arm span was similar to the height [median arm span − height (n = 9), −0.6 cm; range, −5.1 to 5.8 cm].

Of 31 children with heterozygous *ACAN* mutations, 20 presented with a BA that was >12 months advanced, and no child had delayed BA [BA − chronological age; median (n = 31), +1.3 years; range, +0.0 to +3.7 years; Fig. 5].

Children in 6 families (families 1, 4, 12, 15, 18, and 20) had markedly advanced skeletal maturation (BA, 3 to 4 years advanced); however, the children in the other 14 families had normal or modestly advanced BA (BA, 0 to 2 years advanced; Fig. 5). This relative advancement of BA appears to reflect decreased remaining growth potential as evidenced by the history of early growth cessation in subjects [median age of growth cessation (n = 23), 12 years; range, 9 to 16 years] and/or decreased pubertal growth spurts (Fig. 4). In this cohort, no child presented with delayed BA. This is very different from the cohorts of patients with idiopathic short stature in which average BA delays of ≥2 years have been reported (16, 17).

Consequently, most adult individuals had a more obvious phenotype with short stature [median height (n = 62), −2.9 SDS; range, −5.9 to −0.9 SDS; Figs. 3 and 4] and often with some disproportion. Males (n = 24) and females (n = 38) had a similar degree of growth failure

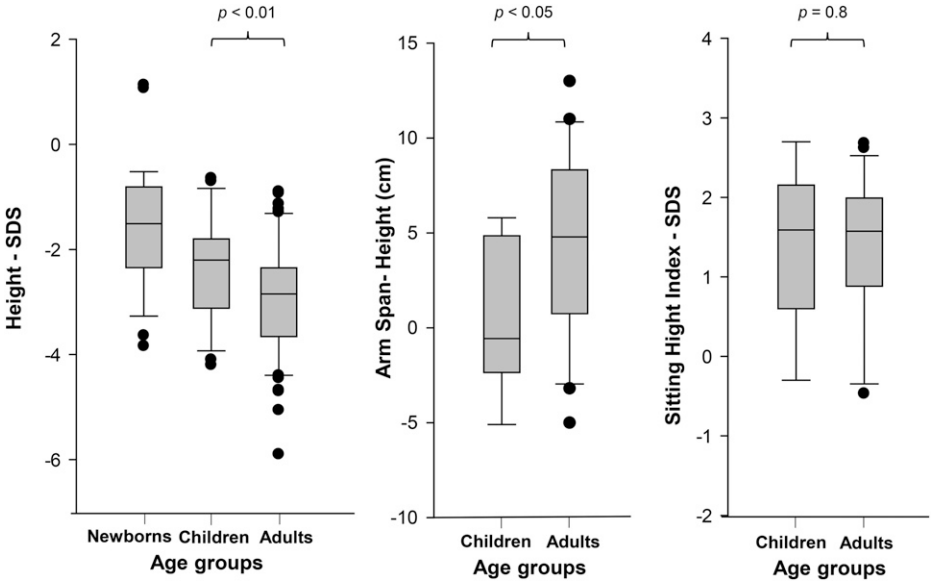

Figure 3. Height SDS, SHI SDS, and arm span minus height in individuals with heterozygous *ACAN* mutations at different ages. (A) Height SDS at birth, during childhood, and in adulthood. (B) SHI SDS during childhood and in adults. (C) Arm span minus height during childhood and adulthood.

(average height of females, 143.9 cm; −3.0 SDS; average height of males, 156 cm; −2.9 SDS). The average adult height was similar in families with missense and truncating variants (average height, −3.0 SDS; range, −2.4 to −4.5; vs −3.2 SDS; range, −1.0 to −4.3; $P = 0.66$, respectively). In addition, the variation of adult height in our cohort (adult height SDS, female, 7.0; male, 6.9) was not larger than that of the general population (18). Taken together, these findings suggest that different mutations have a similar effect on final height. In adult individuals, the SHI was commonly at the upper part of the normal range or slightly elevated [median SHI ratio (n = 22), +1.6 SDS; range, −0.5 to 2.7 SDS]. The growth of the upper extremities appeared to be less affected, with arm spans commonly greater than the height [median arm span − height (n = 22), 5.0 cm; range, −5.0 to 13.3 cm]. Most patients had a normal head circumference [median for females (n = 11), 54.5 cm; +0.2 SDS; range, 51.3 to 58.5 cm; median for males (n = 12), 56 cm; 1.0 SDS; range, 53.3 to 58.5 cm]. Two male patients and 1 female patient had a head circumference >+2 SDS.

Other skeletal features

In some families, the mutations were associated with mild midface hypoplasia and flat nasal bridge (8 of 20 families; Supplemental Fig. 3), and young children in 2 families were noted to have frontal bossing (families 16 and 18).

The growth of the hands varied. Brachydactyly was reported in 5 of 20 families. In 4 of these families, the affected individuals also had notably short thumbs (Supplemental Fig. 3). Complete skeletal surveys were performed on 8 individuals with no or only subtle signs of skeletal dysplasia noted (Supplemental Fig. 4).

Early-onset OA and osteochondritis dissecans

Heterozygous *ACAN* mutations can also affect the articular cartilage (4, 19). In our cohort, early-onset OA was reported in 12 (families 3, 5 to 9, 11, 12, 14, 16, 19, 20) of 20 families, with knee pain the most commonly reported complaint (7 children and 34 adults), except for short stature and commonly started in late adolescence. In these families, severe OA was established during the second and fourth decade of life. Penetrance appeared to be complete but with a variable degree of severity among the families. The knees were the most commonly affected joints, but other joints were also affected (4, 13). The joint disease was associated with missense, truncating, and nonsense mutations located in various regions of the gene (Fig. 1). Thus, in this cohort of 20 families, no correlation was no evident between the presence or absence of joint disease and either the domain in which the mutation occurred or the type of mutation. In some families (families 3, 5, and 6), the *ACAN* mutations were associated with osteochondritis dissecans (OD) (4, 13, 20). In these families, the 2 *ACAN* mutations occurred in the CLD region.

Intervertebral disc disease

Aggrecan is a major proteoglycan of the cartilaginous intervertebral discs, and its degradation has been suggested to be an important pathogenic mechanism in intervertebral disc degenerative disease (21). Back pain was also a common complaint in affected adult individuals of 11 families (families 1, 5 to 9, 12, 15, 16, 19, and 20), who

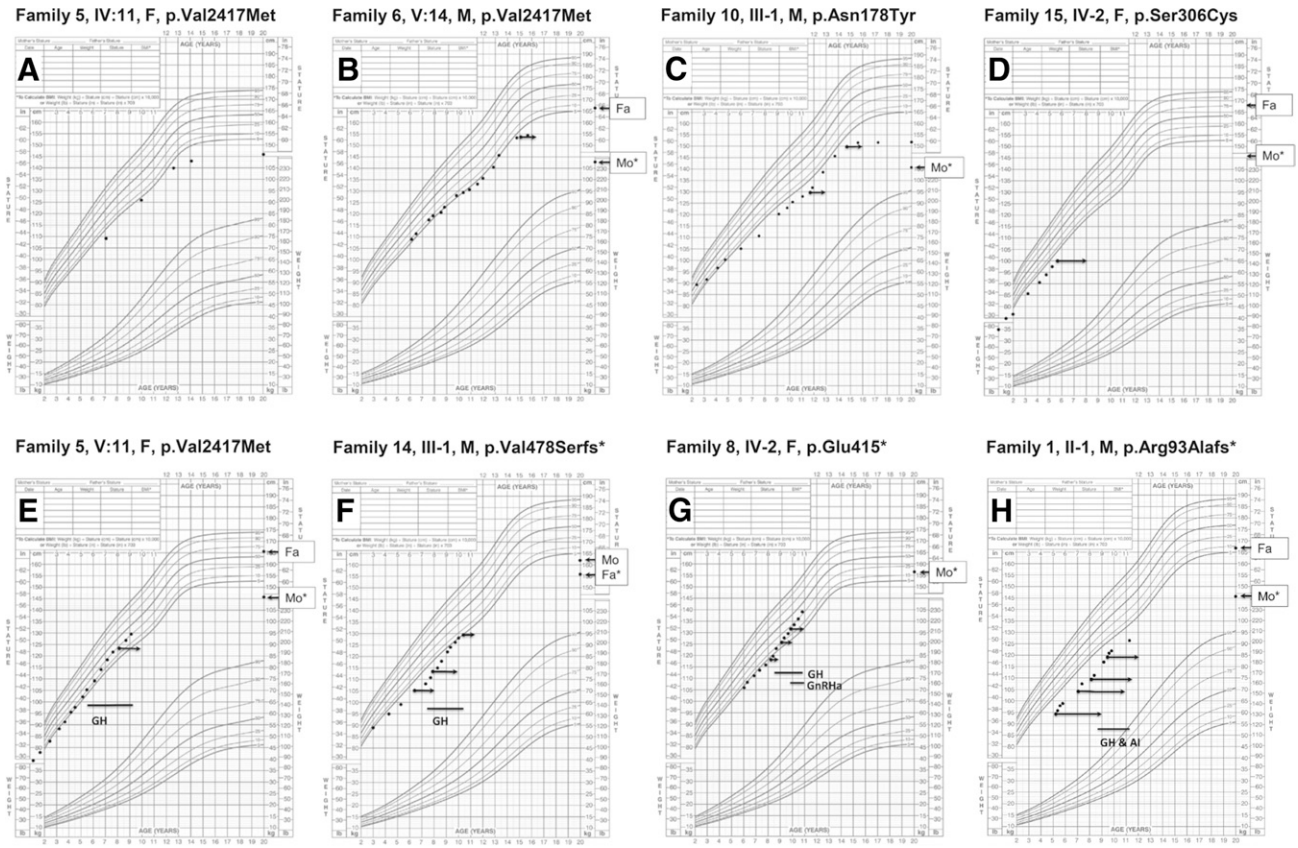

Figure 4. Growth charts of individuals with heterozygous *ACAN* mutations. (A) Mother of proband in family 5 (IV:11). (B) *ACAN* mutation-positive child of family 6 (V:14). (C) Proband of family 10 (III:1). (D) Proband of family 15 (IV:2). (E) *ACAN* mutation-positive child of family 5 (V:11). (F) Proband of family 14 (III:1). (G) Proband of family 8 (IV:2). (H) Proband of family 1 (II:1). The left end of each arrow represents the subject's chronological age (CA) and height and the right end, the BA. Lines indicate periods with GH or gonadotropin-releasing hormone analog (GnRHa) or GH and GnRHa or GH and aromatase inhibitor (AI) letrozole treatment. Asterisk indicates parent carrying the mutation. Fa, father's height; Mo, mother's height.

reported back problems with confirmed disc disease or symptoms suspicious for intervertebral disc disease (3 children and 14 adults). Symptoms of back problems typically started later than the joint disease, with symptoms beginning in the fourth and fifth decade of life and commonly progressing to severe disease with neurologic symptoms often requiring surgical intervention. However, 2 sisters from family 20 presented with symptoms in the first and second decade of life.

Treatment of short stature

In total, 14 children from 8 families had received growth hormone (GH) treatment of short stature (8 females, 6 males) at 1 of 3 different doses: 30 μg/kg/d (4 individuals), 43 μg/kg/d (9 individuals), and 50 μg/kg/d (1 individual). In addition, 5 adult individuals had previously received GH treatment of short stature. The average height SDS of these 5 GH-treated adult individuals was −2.5 and that of untreated adult individuals (n = 65) was −3.0 SDS. Longitudinal growth data were available for the 14 children treated with GH for ≥12 months, including growth data after 2 (n = 3) and 3 (n = 3) years in some individuals. During GH treatment, the height SDS increased on average by +0.4, +0.7, and +1.0 SDS during the first year, the first 2 years, and the first 3 years of treatment, respectively (Fig. 6). In addition to GH treatment, 5 subjects had received treatment with gonadotropin-releasing hormone analogs (4 females, 1 male; families 3, 5, 8, and 20; Fig. 6) to delay BA progression and thereby increase adult height. BA radiographs were available before and after the beginning of treatment in 2 individuals. During treatment, BA progression was halted (0 years of progression/chronological year) during 9 and 22 months of gonadotropin-releasing hormone analog treatment, respectively. Thus, treatment appeared to halt BA progression. In addition, 1 male patient had received treatment with GH and an aromatase inhibitor (letrozole; family 1) for 11 months. During letrozole treatment, BA progression was halted (Fig. 6). Moreover, no adverse events or serious side effects from the treatments, including no new or worsened joint pain, were recorded for any of the patients.

Discussion

We present the clinical characterization of a large international cohort of patients with 19 heterozygous

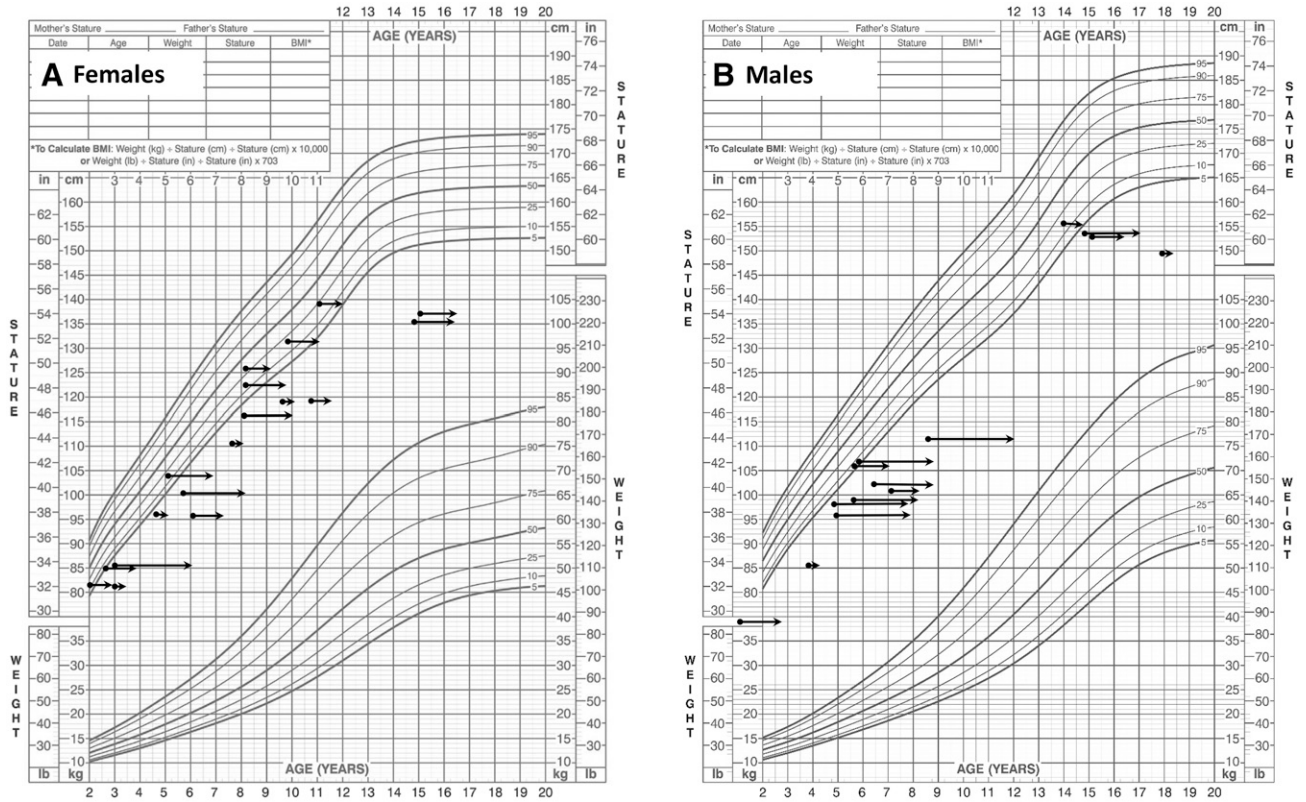

Figure 5. Height and BA in girls (A) and boys (B) with heterozygous *ACAN* mutations. BA was assessed according to the method of Greulich and Pyle (12) by a single, blinded, expert reader. The left end of each arrow represents the subject's chronological age (CA) and the right end represents the BA. Each subject is represented only once on the graph.

ACAN mutations, including 103 mutation-positive individuals from 20 families (Supplemental Fig. 1). Our study results greatly widen the genotypic and phenotypic spectrum of *ACAN* deficiency, demonstrating a lack of a simple genotype/phenotype correlation with regard to joint disease, and better defining the associated comorbidities and the natural history and response to growth-promoting therapies (2, 3).

The probands of all 20 families presented with autosomal dominant short stature and, in most cases, no or only subtle indications of chondrodysplasia. Affected individuals typically manifested mildly disproportionate short stature, often with advanced BA, a family history of early growth cessation, and/or a family history of early-onset OA (2, 3, 22–24).

In each family, a rare, nonsynonymous *ACAN* variant predicted to perturb protein function was identified by Sanger sequencing of *ACAN*, targeted next-generation sequencing, or exome sequencing with confirmation by Sanger sequencing. In each family, the rare variant cosegregated perfectly with the phenotype (Fig. 2; Supplemental Fig. 1; Supplemental Table 2). The variants were spread throughout the gene and included truncating, splice site, and missense variants, and included both missense and truncating variants in the G1, chondroitin sulfate attachment region, and G3 domains, and truncating variants in the G2 domain (Fig. 1) (6), thus greatly broadening the spectrum of the 7 previously published *ACAN* mutations (2, 3, 19, 25–27).

All the current and previously reported heterozygous aggrecan mutations cause adult short stature of similar severity (2, 3, 13, 19, 26). In addition, the variation in adult height in our cohort was similar to the adult height variation in the general population. Taken together, these findings suggest that different disease-causing heterozygous *ACAN* variants impair growth plate chondrogenesis similarly, which, in turn, suggests a common mechanism. Therefore, it is likely that the impairment of growth plate chondrogenesis is due to functional haploinsufficiency of aggrecan, rather than different mutation-specific mechanisms (25).

Before the present report, early-onset OA due to heterozygous *ACAN* mutations, including 1 truncating mutation in the chondroitin-sulfate domain and 2 missense mutations in the G3 lectin-binding domain, had been reported in 3 of 7 families (2, 4, 13, 19). In the present study, we report 12 families with 11 mutations associated with early-onset OA. Two of the 11 mutations associated with early-onset OA had been reported previously and 9 of the mutations have not been previously

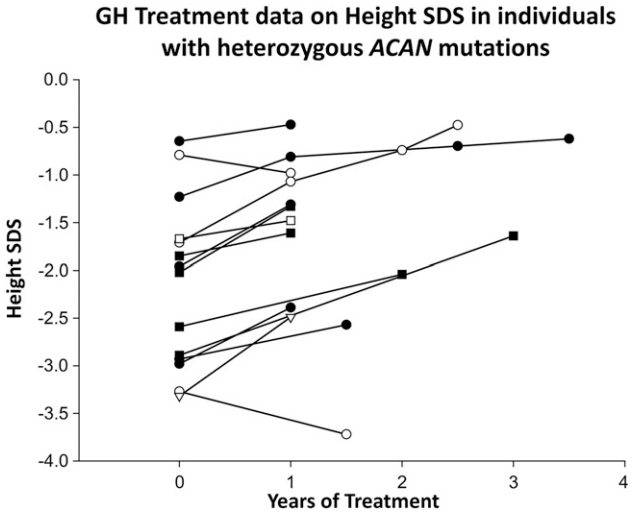

Figure 6. Change in height SDS of subjects during treatment of short stature. Longitudinal growth data were available for 14 individuals with *ACAN* mutations from different families who had received GH treatment of short stature (8 females and 6 males). Solid squares represent boys who had received GH only; solid circles, girls who had received GH only; open circles, girls who received GH and gonadotropin-releasing hormone agonist treatment; open triangles, 1 boy who had received GH and aromatase inhibitor (letrozole) therapy.

reported (2, 4). New mutations associated with early-onset OA include early truncating mutations in the G1 and G2 domains and missense mutations in the G1 and chondroitin-sulfate 1 domains (Fig. 1). Mutations not associated with early-onset OA also include early and late truncating mutations and missense mutations involving different regions of the protein. Therefore, no obvious genotype–phenotype correlation for early-onset OA could be detected in the present study, suggesting that additional genetic and environmental factors might ay modify the susceptibility to OA development caused by pathogenic gene variants in *ACAN*. Moreover, OA was common in adult individuals (53%) and children (21%) with heterozygous *ACAN* mutations and usually established well before 40 years of age. This is different from the general population, in which OA prevalence is very low, at approximately 0.04% in children (age <18 years), remains low until the mid-30s, and then increases rapidly, such that approximately 33% of the population aged >65 years are affected (28–31). Radiologic signs of OD have to date only been detected in individuals with missense mutations in the CLD. Whether OD also occurs in individuals with truncating mutations and missense mutations in other domains remains to be determined.

In our cohort, the affected individuals of several families also reported early-onset intervertebral disc disease that often caused neurologic symptoms and required surgical intervention. This finding is consistent with aggrecan being the major proteoglycan component of the intervertebral disc and loss of aggrecan being an important pathogenic mechanism for degenerative disc disease (21).

Several of the affected children were treated with GH for short stature. We also compared previously GH-treated and non–GH-treated adult individuals and calculated the change in height SDS during treatment. Both of these observations suggest a modest response to GH, with a magnitude similar to that seen with GH treatment of idiopathic short stature (32, 33). However, to rigorously assess the efficacy and safety of GHs for the treatment of children with short stature due to *ACAN* mutations, a randomized controlled trial would be required.

In summary, we report 20 families with heterozygous variants in *ACAN* associated with short stature, advanced BA, variable mild dysmorphic features, early-onset OA, and degenerative disc disease. The findings from the families indicate that heterozygous *ACAN* mutations sometimes present to the clinician as evident skeletal dysplasia or with early-onset OA but more often as autosomal dominant short stature with normal or advanced BA and early growth cessation despite normally timed puberty. Our findings greatly increase the number of reported families with *ACAN* mutations and suggest that heterozygous *ACAN* mutations often cause early-onset OA and intervertebral disc disease and that heterozygous *ACAN* mutations might be relatively common in families with autosomal dominant short stature with accelerated skeletal maturation and early growth cessation.

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Address all correspondence and requests for reprints to: Ola Nilsson, MD, PhD, Department of Women's and Children's Health, Karolinska University Hospital, Solna, Astrid Lindgren Children's Hospital, Q2:08, Stockholm SE-171 76, Sweden. E-mail: ola.nilsson@ki.se.

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