ACAN Gene Mutations in Short Children Born SGA and Response to Growth Hormone Treatment

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Background: Some children born small for gestational age (SGA) show advanced bone age (BA) maturation during growth hormone (GH) treatment. ACAN gene mutations have been described in children with short stature and advanced BA.

Objective: To determine the presence of ACAN gene mutations in short SGA children with advanced BA and assess the response to GH treatment.

Methods: BA assessment in 290 GH-treated SGA children. ACAN sequencing in 29 children with advanced BA ≥0.5 years compared with calendar age.

Results: Four of 29 SGA children with advanced BA had an ACAN gene mutation (13.8%). Mutations were related to additional characteristics: midface hypoplasia ($P = 0.003$), joint problems ($P = 0.010$), and broad great toes ($P = 0.003$). Children with one or fewer additional characteristic had no mutation. Of children with two additional characteristics, 50% had a mutation. Of children with three additional characteristics, 100% had a mutation. All GH-treated children with a mutation received gonadotropin-releasing hormone analog (GnRHa) treatment for 2 years from onset of puberty. At adult height, one girl was 5 cm taller than her mother and one boy was 8 cm taller than his father with the same ACAN gene mutation.

Conclusion: This study expands the differential diagnosis of genetic variants in children born SGA and proposes a clinical scoring system for identifying subjects most likely to have an ACAN gene mutation. ACAN sequencing should be considered in children born SGA with persistent short stature, advanced BA, and midface hypoplasia, joint problems, or broad great toes. Our findings suggest that children with an ACAN gene mutation benefit from GH treatment with 2 years of GnRHa. **(*J Clin Endocrinol Metab* 102: 1458–1467, 2017)**

Children born small for gestational age (SGA) comprise a heterogeneous group with a broad spectrum of clinical characteristics (1, 2). Although several factors have been identified in the etiology of children born SGA, the etiology remains unidentified in up to 40% of cases. Approximately 10% of children born SGA remain short and are therefore treated with growth hormone (GH) to increase adult height (AH) (3–7). AH is one of the most heritable human traits (8), but only a small number of genetic mutations (<1%) explaining short stature in children born SGA has been found (9–11). Uncovering the genetic etiology of short stature is important for health prognosis, genetic counseling, and treatment options.

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Abbreviations: AH, adult height; AI, aromatase inhibitor; BA, bone age; GH, growth hormone; GnRHa, gonadotropin-releasing hormone analog; SDS, standard deviation score; SGA, small for gestational age.

Some children born SGA have an accelerated bone age (BA) maturation during childhood, resulting in early closure of the epiphyseal growth plates and cessation of growth at a young age with a disappointing short AH. Heterozygous mutations in the ACAN gene have been identified in children with idiopathic short stature and advanced BA (12, 13). Various clinical characteristics have been described in affected individuals: advanced BA, early growth cessation, short stature [*i.e.*, height below –2.5 standard deviation score (SDS)], midface hypoplasia, flat nasal bridge, prognathism, posteriorly rotated ears, broad forehead, broad great toes, short thumbs, brachydactyly, joint problems, exaggerated lumbar lordosis, and genu valgum (12–14). Due to the variance in clinical characteristics, the identification of appropriate patients for genetic testing remains a challenge. We, therefore, assessed BA in 290 GH-treated children born SGA and performed genetic testing in 29 children suspected for mutations in the ACAN gene due to advanced BA. Phenotypic characteristics of children with advanced BA were used to develop a scoring system to identify children most likely to test positive for mutations in the ACAN gene and for distinguishing these children from those not likely to test positive. In addition, the response to GH treatment with additional gonadotropin-releasing hormone analog (GnRHa) for 2 years, and aromatase inhibitor (AI) treatment in boys, was evaluated in children with a confirmed mutation in the ACAN gene.

Subjects

The study population consisted of 290 children born SGA with persistent short stature, without known genetic abnormalities, who were participating in two prospective cohort trials evaluating the effects of GH treatment (15, 16). The inclusion criteria for these GH trials were: (1) birth length and/or birth weight SDS for gestational age less than –2.0 (17); (2) height SDS or predicted AH at start of GH treatment less than –2.5 SDS, based on Dutch references (18); (3) well-documented growth data from birth to start of GH treatment; and (4) an uncomplicated neonatal period, without signs of severe asphyxia (Apgar score >3 after 5 minutes) or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia.

Reported findings in patients with ACAN gene mutations showed that BA was persistently advanced during childhood. In our study population of 290 children born SGA, radiographs of the left hand and wrist were taken at start of GH treatment and yearly thereafter. BA was assessed according to Greulich and Pyle (19). We selected children for ACAN sequencing based on an advanced BA of at least 0.5 years at at least two radiographs prior to pubertal onset (Table 1; group 1). Some of the children in our study population came to medical attention in early puberty and started GH treatment with additional GnRHa for 2 years to postpone puberty and improve AH. Because GnRHa treatment influences bone maturation, and BA before start of combined GH/GnRHa treatment was not known in all children, only the first radiograph at start of treatment was reliable to assess BA in these children. Persistent advanced BA was therefore not a possible selection criteria, and selection was based on an advanced BA of at least 0.5 years at start of GH/GnRHa treatment (Table 1; group 2). Based on these criteria, 42 children were selected for ACAN sequencing. In 13 children, ACAN sequencing was not possible because they did not want to revisit the hospital after attainment of AH. In total, targeted ACAN sequencing was performed in 29 children after written informed consent.

The following physical characteristics were assessed by one physician (M. v. d. S.) after instruction by a clinical geneticist and recorded as present or absent; midface hypoplasia, prognathism, flat nasal bridge, broad forehead, microcephaly defined as head circumference below –2.0 SDS, posteriorly rotated ears, joint problems, broad great toes, brachydactyly, exaggerated lumbar lordosis, and genu valgum.

Sequencing

Amplicon sequencing of ACAN was performed in all 29 participants essentially as previously described (20, 21). The sequences of the primers that were used are available in Supplemental Table 1.

Statistical analyses

Statistical analyses were performed using SPSS version 23 (IBM). Birth length and birth weight were transformed into SDS for sex and gestational age (17). Height was transformed into SDS for sex and chronological age according to Dutch references (18), using Growth Analyzer Research Calculation Tools (https://growthanalyser.org). AH SDS was calculated using references for Dutch adults (age 21 years) (18). Mann-Whitney *U* test and Fisher's exact test were used to assess the difference in clinical characteristics between children with and without mutations in the ACAN gene. *P* values <0.05 were considered statistically significant.

Results

ACAN sequencing was performed in 29 children (13 males, 16 females) with advanced BA, originating from 26 nonrelated Dutch families. Clinical characteristics are summarized in Table 1. Four children, three boys,

Table 1. Clinical Characteristics of Selected Individuals for ACAN Sequencing

Patient	Sex	Age at Start GH	Height SDS at Start GH	Height SDS Father	Height SDS Mother	BA-Calendar Age (Years) at Start of GH	BA-Calendar Age (Years) During GH	Midface Hypoplasia	Joint Problems	Broad Great Toes	ACAN Mutation
Group 1											
1 (A.IV:1)[a, b]	Female	5.0	−3.7	Unknown	−4.7	Conform	+2.0	Yes	Yes	Yes	Yes
2	Female	6.4	−2.6	−1.6	−3.2	−0.4	+1.0	No	No	Yes	No
3	Female	4.2	−2.8	−2.5	−2.4	−1.2	+1.2	Yes	No	No	No
4	Male	9.5	−2.9	−2.7	−1.3	+1.2	+2.0	No	No	No	No
5	Male	9.0	−4.2	−2.0	−1.3	Conform	+1.5	Yes	No	Yes	No
6	Male	12.8	−2.9	−2.0	−2.0	+0.5	+1.1	Yes	No	No	No
7	Female	4.1	−3.5	−0.8	−0.4	−0.6	+1.3	No	No	No	No
8	Male	3.0	−3.6	−0.8	−1.5	Conform	+1.9	No	No	No	No
9[c]	Female	5.2	−3.2	−1.4	−0.6	Conform	+2.8	No	No	Yes	No
10[c]	Male	4.5	−2.7	−1.4	−0.6	+0.5	+1.5	No	No	No	No
11	Female	5.3	−2.2	0.2	−1.0	+0.7	+1.4	No	No	No	No
12	Female	6.0	−2.9	−0.8	−0.1	+1.1	+2.0	No	No	No	No
13	Female	4.3	−3.0	0.6	−0.3	+0.7	+1.1	No	No	No	No
14[d]	Male	3.7	−3.4	−0.8	−2.0	−0.7	+1.2	No	No	No	No
15[d]	Male	3.7	−3.1	−0.8	−2.0	−1.2	+1.2	No	No	No	No
16	Female	4.7	−2.6	−0.6	−0.4	−0.2	+1.2	No	No	No	No
17	Male	4.5	−3.3	−1.3	0.5	−0.5	+1.4	No	No	No	No
18	Male	4.4	−2.5	−1.3	−1.0	−2.4	+1.4	No	No	No	No
19	Male	5.2	−2.5	0.4	−0.1	−0.2	+1.1	No	No	No	No
Group 2											
20 (A.IV:2)[b]	Male	11.9	−2.4	Unknown	−4.7	+0.6	Influenced by GnRHa treatment	Yes	Yes	Yes	Yes
21 (B.IV:1)[e]	Male	11.7	−2.7	−2.0	−4.9	+0.8	Influenced by GnRHa treatment	Yes	Yes	Yes	Yes
22 (C.III:6)[f]	Male	12.3	−2.7	−3.8	0.6	+1.0	Influenced by GnRHa treatment	Yes	No	Yes	Yes
23	Female	12.0	−3.1	−3.8	−0.8	+0.5	Influenced by GnRHa treatment	No	No	No	No
24	Female	11.0	−3.0	2.5	−3.2	+2.0	Influenced by GnRHa treatment	No	Yes	No	No
25	Female	9.5	−2.2	0.2	−3.1	+0.7	Influenced by GnRHa treatment	No	No	No	No
26	Female	11.3	−2.4	−2.3	−1.4	+0.7	Influenced by GnRHa treatment	No	No	Yes	No
27	Female	12.2	−2.4	0.7	−2.3	+0.5	Influenced by GnRHa treatment	Yes	No	No	No
28	Female	9.8	−2.8	−0.2	−2.1	+1.3	Influenced by GnRHa treatment	No	Yes	No	No
29	Female	11.0	−2.5	0.9	2.0	+0.7	Influenced by GnRHa treatment	No	No	No	No

Group 1: Persistently advanced BA of at least 0.5 years at more than two radiographs prior to pubertal onset.

Group 2: Advanced BA of at least 0.5 years at start of GH/GnRHa treatment in early puberty.

[a]Proband of family A.

[b]Twin of family A.

[c]Siblings.

[d]Twin.

[e]Proband of family B.

[f]Proband of family C.

including one dizygotic twin, had a mutation in the ACAN gene.

Clinical presentation

Family A

Figure 1(a) shows the pedigree. The proband was a girl (A.IV:1) born SGA at 32 6/7 weeks of gestation. She had persistent short stature and was referred to our clinic at the age of 5 years with a BA equal to her calendar age (Table 2). She had midface hypoplasia, broad great toes, and short thumbs. A skeletal survey showed underdeveloped facial bones and no vertebral body deformities. Her twin brother (A.IV:2) was born SGA and referred to our clinic at the age of 11.9 years because of persistent short stature with a BA of 0.6 years advanced relative to his calendar age (Table 2). He had midface hypoplasia, mild posteriorly rotated ears, and broad great toes. Both children were referred to orthopedics

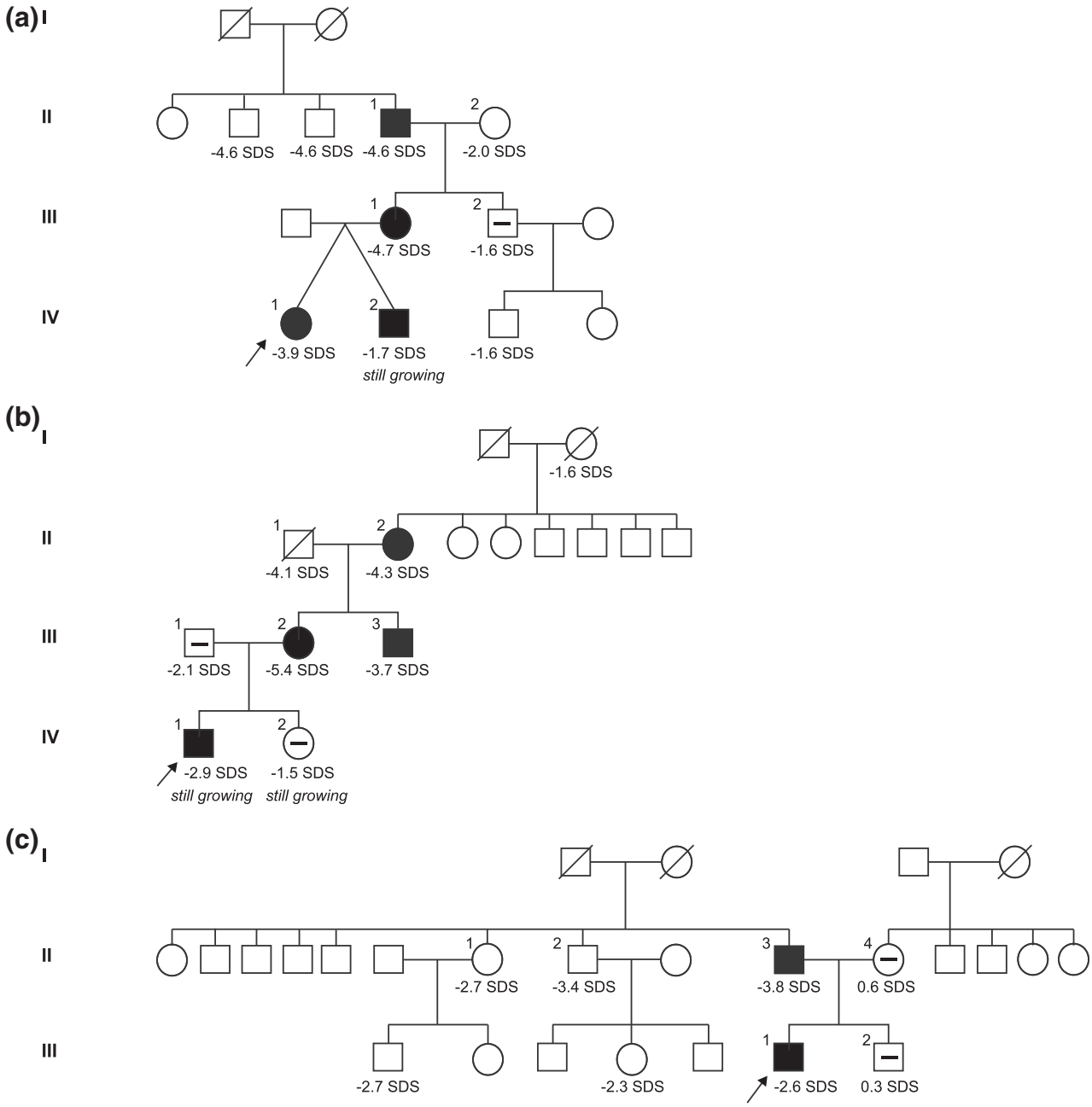

Figure 1. Pedigrees of families with ACAN gene mutations. (a) Family A. (b) Family B. (c) Family C. Black symbols represent patients with confirmed ACAN gene mutations through ACAN sequencing. White symbols with a horizontal line represent patients without ACAN gene mutations. White symbols without lines represent those individuals who did not undergo ACAN sequencing. White symbols with a slash represent the deceased. Reported height SDS is AH SDS; in children still growing, it represents the current height SDS. When height SDS is not reported, subjects had a height of >–1.5 SDS.

Table 2. Clinical Characteristics of the Four Children With a Mutation in the ACAN Gene

	A.IV:1	A.IV:2	B.IV:1	C.III:1
Birth characteristics				
Gestational age (wk)	32 6/7	32 6/7	38 6/7	41
Birth weight (SDS)	−2.1	−0.9	−0.1	−1.8
Birth length (SDS)	−4.4	−2.1	−3.0	−3.0
At referral to our clinic				
Age (y)	5.0	11.9	11.7	12.3
BA (y)	5.0	12.5	12.5	13.3
Height (cm)	94.5	135.6	131.7	135.4
Height (SDS)	−3.7	−2.4	−2.7	−2.7
Weight (SDS; for height)	−1.1	2.0	0.7	−0.4
Sitting-height-to-height ratio (SDS)	2.2	1.4	1.4	1.6
Head circumference (SDS)	−3.0	−1.3	−0.2	−1.6

because of persistent pain and dysfunction of their knees and elbows, and osteoarthritis was diagnosed. Because of recurrent urinary tract infections, the boy was referred to a pediatric urologist, and abdominal ultrasound showed an absent left kidney.

Height of the mother (A.III:1) was 140.3 cm (–4.7 SDS), and she had midface hypoplasia, broad great toes, short thumbs, exaggerated lumbar lordosis, osteoarthritis, and partial adrenal insufficiency. Height of the children's maternal grandfather (A.II:1) was 151.6 cm (–4.6 SDS) and maternal grandmother (A.II:2) was 158.0 cm (–2.0 SDS). The grandfather had midface hypoplasia and knee and hip replacement surgery at a young age due to osteoarthritis. ACAN sequencing identified a pathogenic heterozygous nonsense mutation (c1608C>A) in the proband, her twin brother, her mother, and her maternal grandfather. The maternal uncle (A.III:2) had a height of 173 cm (–1.6 SDS) and no ACAN gene mutation.

Family B

Figure 1(b) shows the pedigree. The proband was a boy (B.IV:1) born SGA at 38 6/7 weeks of gestation. He had persistent short stature and was referred to our clinic at the age of 11.7 years with a BA of 0.8 years advanced relative to his calendar age (Table 2). He had midface hypoplasia, mild prognathism, broad great toes, and exaggerated lumbar lordosis. At the age of 14 years, he was referred to orthopedics because of persistent pain in both knees, and osteochondritis dissecans was diagnosed based on knee x-rays and magnetic resonance imaging.

Height of his father (B.III:1) was 169.5 cm (–2.1 SDS) and mother (B.III:2) was 135.9 cm (–5.4 SDS). His mother had midface hypoplasia, short thumbs, broad great toes, exaggerated lumbar lordosis, and osteoarthritis of her knees, for which she had replacement surgery several times. The mother had no nails on most of her digits (hands and feet). The proband had one sister with a height of 156.3 cm (–1.5 SDS) at the age of 14.5 years (B.IV:2). The proband's maternal grandfather (B.II:1) was deceased, his height was 155.0 cm (–4.1 SDS), and he had osteoarthritis of neck and vertebrae. Height of the proband's maternal grandmother (B.II:2) was 143.0 cm (–4.3 SDS), and she had midface hypoplasia, short thumbs, and problems with both knees and neck. Height of the proband's maternal uncle (B.III:3) was 158.0 cm (–3.7 SDS). ACAN sequencing identified a pathogenic heterozygous nonsense mutation (c.7090C>T) in the proband, his mother, and his maternal grandmother and uncle. In addition, ACAN sequencing identified two additional heterozygous missense mutations in the proband's mother and his maternal uncle, c.1973A>G [p.(Asn658Ser)] and c.5419G>A [p.(Gly1807Arg)]. The pathogenicity of the second mutation was uncertain. These two missense mutations could not be tested in the proband's maternal grandfather because he was deceased. The unaffected father and sister of the proband had no ACAN gene mutation.

Family C

Figure 1(c) shows the pedigree. The proband (C.III:1) was a boy born SGA at 41 weeks of gestation. He had persistent short stature and was referred to our clinic at the age of 12.3 years with a BA of 1 year advanced relative to his calendar age (Table 2). He had midface hypoplasia, broad great toes, and posteriorly rotated ears, but no joint problems.

Height of his father (C.II:3) was 157.4 cm (–3.8 SDS) and mother (C.II:4) was 174.6 cm (0.6 SDS). The proband's father had midface hypoplasia and joint problems with his hips. The proband had one brother (C.III:2) with an AH of 185.0 cm (0.2 SDS). One paternal aunt (C.II:1) had severe knee problems and a height of 153 cm (–2.7 SDS), and one paternal uncle (C.II:2) had a height of 160 cm (–3.4 SDS). ACAN sequencing identified a pathogenic heterozygous frameshift mutation, c.4762_4765del [p.(Gly1588fs)], which leads to an early stopcodon, in the proband and his

father. The proband's mother and brother had no ACAN gene mutation. Siblings of the proband's father were not tested for ACAN gene mutations.

Presence of mutations in the ACAN gene and phenotypic characteristics

Overall, 13.8% of the children with advanced BA had an ACAN gene mutation (4 of 29). Supplemental Table 2 provides additional phenotypic information of all 29 children. Children with an ACAN gene mutation were clinically different from children without mutations (Table 3; $P = 0.001$). Children with an ACAN gene mutation differed significantly for presence of midface hypoplasia, joint problems, and broad great toes from children without ACAN gene mutations. Figure 2 shows a flowchart on the probability of having an ACAN gene mutation. Children with advanced BA and none or only one of the additional characteristics (midface hypoplasia, joint problems, or broad great toes) had no ACAN gene mutation. Of children with two additional characteristics, 50% had an ACAN gene mutation. When two additional characteristics were present and at least one parent had a height below –3.5 SDS, the occurrence rate of ACAN gene mutations increased to 100%. All children with advanced BA ≥0.5 years and all three additional characteristics had a mutation in the ACAN gene (100%).

In the families with confirmed mutations in the ACAN gene, all affected individuals had midface hypoplasia and broad great toes. Joint problems were present in all affected individuals, except in the proband of family C (C.III:2). All affected individuals without GH treatment had a height below –3.5 SDS.

Table 3. Statistical Comparison of the Clinical Characteristics of Patients With and Without ACAN Gene Mutations

	ACAN Gene Mutation		No ACAN Gene Mutation		*P* Value
n	4		25		
Mean (standard deviation) number of factors recorded "yes"	5.25 (1.0)		1.92 (1.5)		0.001
Characteristics	n	%	n	%	
Midface hypoplasia	4	100	4	16	0.003
Broad great toes	4	100	4	16	0.003
Joint problems	3	75	2	8	0.010
Posteriorly rotated ears	2	50	4	16	NS
Brachydactyly	1	25	4	16	NS
Prognathism	1	25	5	20	NS
Microcephaly	1	25	2	8	NS
Exaggerated lumbar lordosis	1	25	2	8	NS
Flat nasal bridge	0	0	6	24	NS
Broad forehead	0	0	5	20	NS
Genu valgum	0	0	4	16	NS

Abbreviation: NS, not significant.

Growth response and bone maturation during GH treatment

The four children with an ACAN gene mutation were treated with GH based on persistent short stature after SGA birth. Timing of pubertal onset was normal in all four children, but relatively early for their short stature. Because of an AH expectation below –2.5 SDS at pubertal onset, they were all treated with 2 years of additional GnRHa treatment from onset of puberty to postpone puberty (leuprolide acetate depots, 3.75 mg subcutaneous every 4 weeks). Figure 3 shows the growth charts of the children with an ACAN gene mutation.

The girl of family A started GH treatment at the age of 5 years with a dose of 1 mg/m^2/d (0.033 mg/kg/d) [Fig. 3(a)]. From the age of 7 years, her BA was 1.5 to 2 years advanced. She started puberty at the age of 10 years with a height of 132.3 cm (–1.8 SDS) and was additionally treated with GnRHa. During GnRHa treatment, her bone maturation slowed compared with the years before, but her growth rate decreased, and GnRHa treatment was therefore discontinued after 1.5 years, while GH treatment was continued. At the age of 13.3 years, the GH dose was increased to 2 mg/m^2/d because of a disappointing pubertal growth spurt. Her growth ceased at the age of 14 years, when BA was 17 years, with a height of 145.5 cm (AH, –3.9 SDS), which was 5 cm taller than her mother (AH, –4.7 SDS).

The three boys all started GH treatment combined with two years of GnRHa in early puberty, aged 11.5 to 12 years [Fig. 3(b–d)]. Due to the GH trial design, they were randomized to receive either GH 1 mg/m^2/d (boy from family C) or GH 2 mg/m^2/d (boys from families A and B). Bone maturation was advanced at start of GH treatment but stopped during GnRHa treatment. Because of the rapid development of Tanner stage and bone maturation after discontinuation of GnRHa, the boys of families A and B were treated with an AI from the age of 14 and 15 years, respectively (letrozole, 2.5 mg daily). Due to abnormalities in the knee joint, AI treatment was discontinued for 4 months in the boy of family B, during which time BA rapidly advanced. AI treatment was restarted after the diagnosis of osteochondritis dissecans. Currently, BA is delayed in both boys (1 to 1.5 years) while being treated with AI. They have not yet reached AH and have a current height of 164.7 and 161.3 cm, respectively. In the boy of family C, growth ceased at the age of 18.6 years, with a height of 165.3 cm (AH, –2.6 SDS) [Fig. 3(d)], which is 8 cm taller than his father (–3.8 SDS).

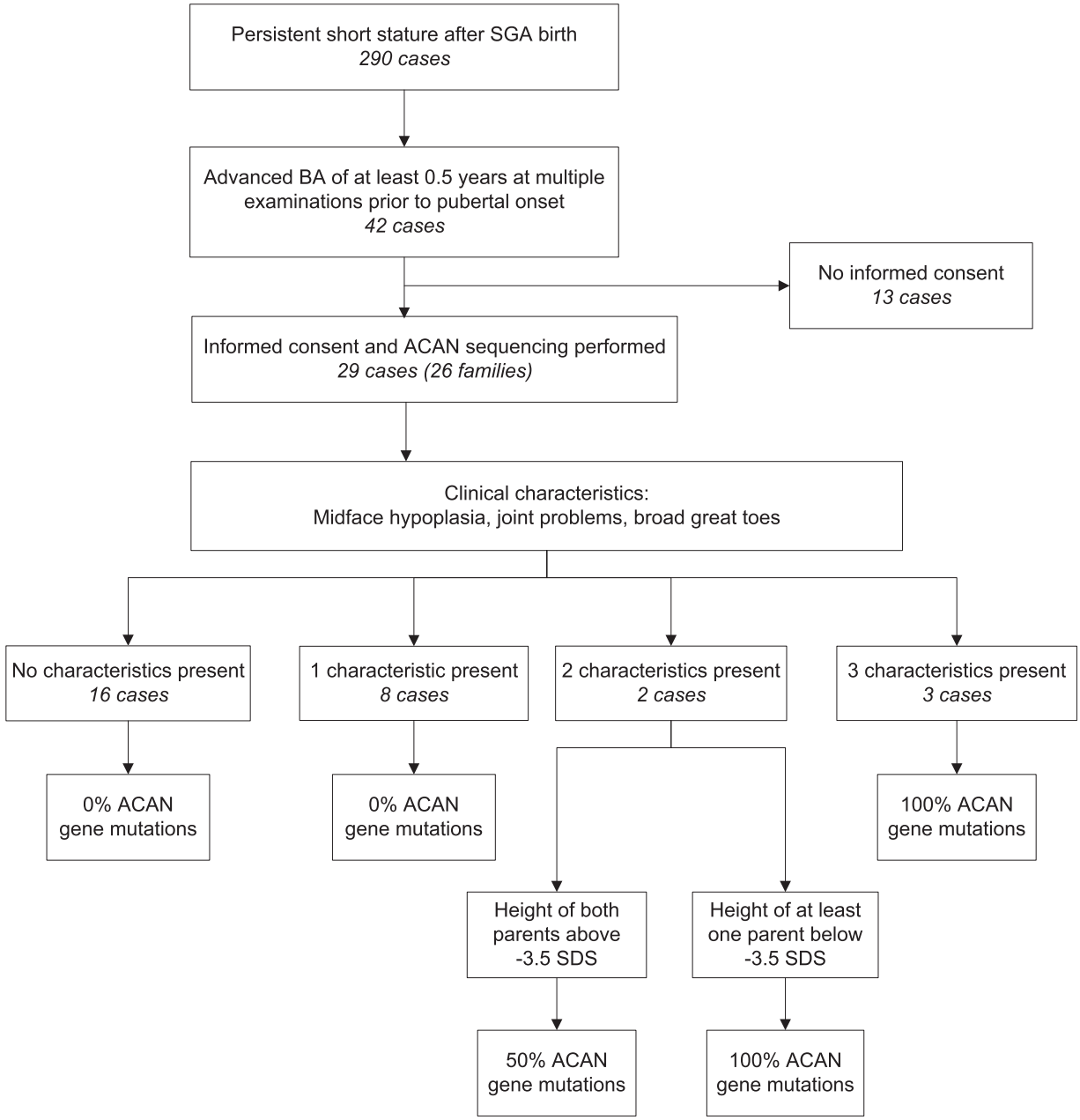

Figure 2. Flowchart on the probability of having an ACAN gene mutation.

Treatment had no adverse effects, and dual-energy X-ray absorptiometry scans of the total body and lower lumbar spine showed normal bone mineral density in all children.

Discussion

In this study, we performed ACAN sequencing in 29 short children born SGA with an advanced BA who participated in prospective GH trials, including 290 children born SGA. In four children (13.8%), including one dizygotic twin, we identified heterozygous genetic mutations in the ACAN gene. Our data show that ACAN gene mutations are related to the presence of midface hypoplasia, joint problems, and broad great toes. ACAN gene mutations were present in 50% to 100% of the children with advanced BA and at least two of these characteristics. We therefore suggest that ACAN sequencing could be considered in children with a combination of advanced BA and at least two of these clinical characteristics. When BA is advanced in the absence of these characteristics, a mutation in the ACAN gene is less likely, regardless of parental height. GH treatment with 2 years of additional GnRHa followed by AI treatment in boys, which successfully delayed bone maturation, was beneficial in all four patients with a confirmed mutation in the ACAN gene.

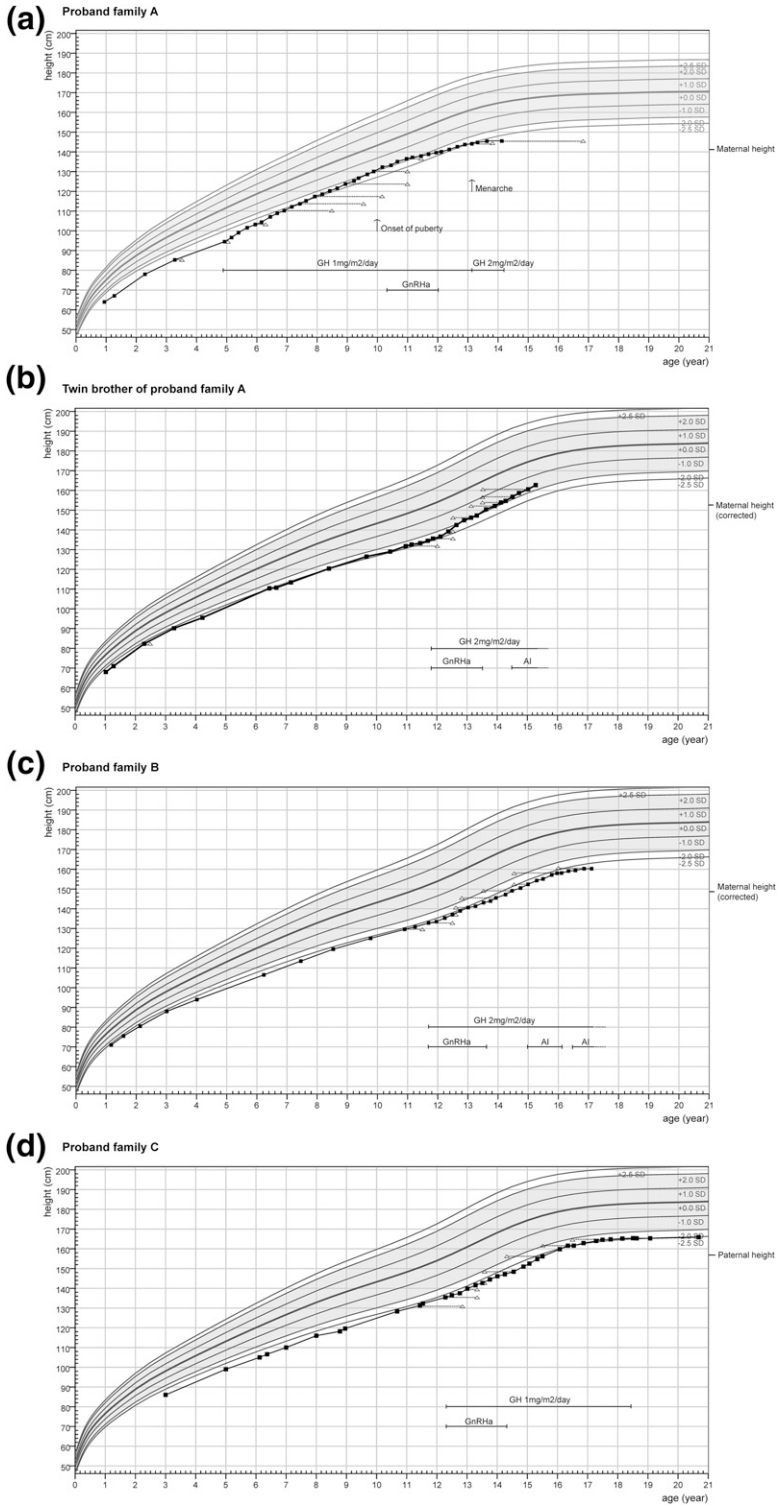

Figure 3. Growth charts of patients with an ACAN gene mutation, according to Growth Analyzer 4.0 (18). Triangles represent BA.

Longitudinal bone growth occurs at the growth plate by forming and remodeling cartilage into bone tissue. The ACAN gene is located on chromosome 15q26 and consists of 19 exons ranging in size from 77 to 4224 bp (22). It encodes for the aggrecan protein, which is a member of the lectican (chondroitin sulfate proteoglycan) family. The encoded protein is a proteoglycan and a critical component of the extracellular matrix in both articular and growth plate cartilaginous tissue, explaining the effects on joints (articular cartilage) and growth (growth plate cartilage) in case of a mutation. Mutations in the ACAN gene result in a broad phenotypic spectrum of nonlethal skeletal dysplasias, including spondyloepimetaphyseal dysplasia, spondyloepiphyseal dysplasia (Kimberley type), familial osteochondritis dissecans, and various idiopathic short-stature phenotypes (12–14, 20, 23–25). The exact mechanism of how each distinct mutation in the ACAN gene leads to a range of phenotypes is unknown, but in all published studies, affected individuals had short stature and early growth cessation. It is unknown if all previously reported individuals with ACAN gene mutations had an advanced BA, and because we selected on advanced BA, there is a small chance that ACAN gene mutations were present in the children not tested. However, an antiangiogenic function of the aggrecan matrix has been proposed (26), and mutations in the ACAN gene might, therefore, lead to premature or increased invasion of the growth plate by blood vessels and osteoblasts. This causes ossification of growth cartilage, resulting in advanced BA, early epiphyseal fusion, and premature growth cessation (27). Because all previously reported individuals with ACAN gene mutations had early growth cessation and short stature, advanced BA could be assumed in these subjects.

We show that having an advanced BA is insufficiently indicative for a mutation in the ACAN gene in children born SGA because only 13.8% of the children with advanced BA had an ACAN mutation. Patients should, therefore, have additional characteristics. Various different characteristics have been described in patients with ACAN gene mutations (12–14, 20, 24, 25), but we only found an association with midface hypoplasia, joint problems, and broad great toes. These

characteristics have also been described in other individuals with ACAN gene mutations (12–14). The occurrence of short thumbs was not indicative for ACAN gene mutations when analyzed in our 29 patients. However, all women with an ACAN gene mutation, and none of the men, had short thumbs. This might indicate that phenotypic characteristics of ACAN gene mutations differ in females and males, which is also true for short stature homeobox gene deficiency in which Madelung deformity is more common in females (28). It could, therefore, be that there are more phenotypic characteristics indicative for mutations in the ACAN gene that we did not find because of the small number of patients in our study. Nevertheless, this study proposes a clinical scoring system for ACAN sequencing (Fig. 2), showing that advanced BA combined with the presence of midface hypoplasia, joint problems, or broad great toes has a predictive value of 50% to 100% for identifying an ACAN gene mutation.

GH treatment with additional 2 years of GnRHa resulted in an AH of 145.5 cm (–3.9 SDS) in the girl from family A, and an AH of 165.3 cm (–2.6 SDS) in the boy from family C. This is, respectively, 5 and 8 cm taller than their parent with the same sex and ACAN gene mutation. Another study reported combined GH/GnRHa treatment in a girl aged 11.5 years with a heterozygous ACAN gene mutation in the C-type lectin domain (12). GnRHa treatment successfully blocked bone maturation, but both GH and GnRHa treatment were discontinued at the age of 13.5 years for unknown reasons, and growth ceased shortly thereafter at an AH of 135.8 cm (–4.2 SDS). A case report of GH treatment in a boy with a heterozygous missense mutation in the C-type lectin domain of the ACAN gene showed that the treated boy was 11.5 cm taller than the mean height of the untreated males with ACAN gene mutations in his family (20, 25). These findings together suggest that children born SGA who have a mutation in the ACAN gene benefit from GH treatment with 2 years of additional GnRHa treatment in early puberty. In our study, two boys received also AI treatment. Because these two boys have not yet reached their AH, effectiveness of AI treatment is still uncertain. However, because bone maturation was successfully stopped and bone mineral density remained normal during AI treatment, this could be considered in boys with an ACAN gene mutation.

Remarkably, accelerated bone maturation did not occur in early childhood, but from the age of 7 years in the affected girl and the age of 11 years in the affected boys. This highlights the importance of regularly reevaluating the original diagnosis in children born SGA who comprise a heterogeneous group with a broad spectrum of clinical characteristics, because SGA is a definition and not a diagnosis (1, 2). Being born SGA has been associated with an adverse health profile in young adulthood, and short stature during childhood and adolescence, resulting in a short AH, is associated with reduced quality of life (29). Therefore, identifying underlying causes of being born SGA remains important to alert physicians to potential comorbidities and to improve future treatment options. In most cases, however, no diagnosis is made because only a small number of genetic mutations (<1%) explaining short stature after SGA birth has been found (9–11).

In conclusion, our study expands the differential diagnosis of genetic variants in short children born SGA and proposes a clinical scoring system for identifying subjects most likely to have an ACAN gene mutation and for distinguishing these subjects from those not likely to test positive. Although the full phenotypic spectrum of this disorder is yet to be elucidated, our findings show that children born SGA with persistent short stature, advanced BA, and at least two of the additional clinical characteristics (midface hypoplasia, joint problems, or broad great toes) have a high likelihood of an ACAN gene mutation. Patients with advanced BA without additional characteristics are less likely to have a mutation in the ACAN gene. Our findings suggest that children with an ACAN gene mutation benefit from GH treatment with 2 years of additional GnRHa, and in boys, this could be followed by treatment with an AI, which might further increase AH. Further research on treatment options is warranted to improve height in children with ACAN gene mutations.

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