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Clinical Characteristics of Pathogenic *ACAN* Variants and 3-Year Response to Growth Hormone Treatment: Real-World Data

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Keywords
ACAN · Aggrecan · Growth hormone · Treatment response

Abstract
Introduction: Heterozygous variants in the *ACAN* gene may underlie disproportionate short stature with characteristically accelerated bone age (BA) maturation and/or early-onset osteoarthritis (OA). ***Methods:*** The objective of this study was to describe phenotype, analyze genotype-phenotype correlations, and assess the response of growth hormone (GH) treatment in children with a heterozygous *ACAN* variant. Thirty-six subjects (23 boys, 13 girls) with *ACAN* deficiency and treated for ≥1 year with GH were identified in the Dutch National Registry of GH treatment in children. ***Results:*** We identified 25 different heterozygous *ACAN* variants in 36 subjects. Median (interquartile range) height SDS at start of GH was −2.6 SDS (−3.2 to −2.2). Characteristic features such as disproportion, advanced BA, early-onset OA, and dysmorphic features like midface hypoplasia and brachydactyly were present in the majority of children, but in ~20%, no specific features were reported. Subjects with a truncating *ACAN* variant had a shorter height SDS compared to subjects with a non-truncating variant (−2.8 SDS and −2.1 SDS, respectively, $p = 0.002$). After 3 years of GH, height gain SDS in prepubertal children was 1.0 SDS (0.9–1.4). In pubertal children, height SDS remained relatively stable. ***Conclusion:*** The phenotype of subjects with pathogenic heterozygous *ACAN* variants is highly variable, and genetic testing for *ACAN* deficiency should be considered in any child with significant short stature, even in the absence of disproportion, specific dysmorphic features, or BA advancement. Furthermore, children with *ACAN* deficiency may benefit from GH with a modest but significant response, which is sustained during 3 years of treatment.

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Introduction

Normal growth depends not only on the growth hormone (GH)-insulin-like growth factor (IGF) I axis but also on a normal functioning growth plate [1, 2]. Long bones grow by a process of endochondral ossification. This complex process is regulated by various endocrine and paracrine signals as well as interactions between intracellular proteins and extracellular matrix [3]. Pathogenic variants in genes involved in any of these pathways can result in a diverse group of skeletal dysplasias. One group of skeletal dysplasias results from variants in the gene coding for aggrecan (*ACAN*) [4].

Aggrecan is the primary proteoglycan of the cartilage growth plate [3]. Homozygous pathogenic *ACAN* variants lead to a severe form of skeletal dysplasia (spondyloepimetaphyseal dysplasia, aggrecan type; MIM 612813), whereas heterozygous *ACAN* variants can lead to much milder phenotypes, such as spondyloepiphyseal dysplasia, Kimberley type (MIM 608361), familial osteochondritis dissecans (OD) (MIM 165800), or idiopathic short stature [5–18]. Typically, the short stature is disproportionate and associated with accelerated bone age (BA) maturation, resulting in early cessation of growth and an unexpectedly short adult stature [7, 12, 15]. Other clinical characteristics that have been described are midface hypoplasia, a flat nasal bridge, brachydactyly, broad great toes, wide feet, and an increased arm span to height ratio [7, 14, 15]. However, as more individuals are being diagnosed with *ACAN* deficiency, the clinical phenotype has expanded in recent years.

Until now, only limited data on the effects of GH treatment or other growth-promoting interventions in children and adolescents with *ACAN* deficiency have been reported [14, 16, 19–22]. Muthuvel et al. [19] recently described improved linear growth in 10 patients with *ACAN* deficiency during 1 year of GH treatment. Prior to that, van der Steen et al. [14] had shown that GH treatment increased height SDS in combination with 2 years of gonadotrophin-releasing hormone analog (GnRHa) treatment in a number of *ACAN*-deficient patients. Based on these early data, a standardized, national GH treatment guideline for *ACAN* deficiency was introduced in The Netherlands with collection of long-term follow-up data.

For this study, we selected patients with pathogenic *ACAN* variants from the Dutch National Registry of GH Treatment in Children, described the clinical characteristics, and evaluated possible genotype-phenotype correlations. In addition, the longer-term response to GH treatment with or without GnRHa or aromatase inhibitor (AI) was evaluated.

Patients and Methods

Subjects

The Dutch National Population-Based Registry of GH Treatment in Children was searched for children with a heterozygous *ACAN* gene variant. Children were included if: (1) the *ACAN* gene variant was classified as likely pathogenic (class IV) or pathogenic (class V) according to the ACMG guidelines [23] (throughout the manuscript, we will use the term pathogenic variant for class IV and V); and (2) at least 1 year of GH treatment was given. Children were excluded if the *ACAN* gene variant was classified as a variant of unknown significance (class III), additional chromosomal defects or syndromes were present, and growth failure could have been influenced by other conditions (e.g., abnormal endocrine evaluation or severe chronic illness).

The *ACAN* variants were identified by either next-generation sequencing or Sanger sequencing (NM_001369268.1) (see online suppl. Table 1 for comparison with the previous reference sequence NM_013227.3; for all online suppl. material, see https://doi.org/10.1159/000535651). The variants found were defined as a heterozygous deletion of the entire gene or ≥1 complete exon, a truncating variant (frameshift or nonsense) or splice-site variant located less than 2 base pairs from the exon/intron boundary, a missense variant (not found in public databases, predicted to be damaging using prediction software, de novo or segregates with short stature in the family), or an in-frame insertion, duplication, or deletion of ≥1 amino acid meeting the same criteria as missense variants [23].

Of 52 eligible children, 36 were included in the analysis. Sixteen children were not eligible and therefore not included for the following reasons: $n = 12 < 1$ year of GH treatment, and $n = 4$ had additional chromosomal defects or syndromes ($n = 1$ Turner syndrome, $n = 1$ Wiedemann-Steiner syndrome, $n = 1$ SHOX haploinsufficiency, and $n = 1$ 22q11 duplication). Four children included in this study were previously reported by van der Steen et al. [14].

The Dutch National Registry of GH Treatment was initiated in 1997; the nationwide coverage of the registry is 100%. Data in this registry are provided by members of the Dutch Society for Pediatric Endocrinology. In the registry, all data are pseudonymized to comply with rigorous privacy guidelines. For data collection in this registry, informed consent and ethical approval were not required according to Dutch law [24–26].

Measurements

Height, weight, and Tanner stage were extracted from the Registry at start and subsequently yearly. Body mass index was calculated as weight divided by height squared (kg/m^2). Target height (TH) was calculated as TH = 44.5 + 0.376 × paternal height (cm) + 0.411 × maternal height (cm) for boys and TH = 47.1 + 0.334 × paternal height (cm) + 0.364 × maternal height (cm) for girls [27]. Height, weight, body mass index, and TH were expressed in SDS, adjusting for sex and age according to Dutch reference data [28]. Adult height SDS was calculated based on Dutch reference data at age 21 years [28].

The onset of puberty was defined as breast development stage 2 for girls and a testicular volume ≥4 mL for boys according to Tanner [29]. BA was determined either manually according to

Greulich and Pyle by the treating physician or automated using BoneXpert® [30, 31]. An advanced BA was defined as a BA minus calendar age (CA) of at least 0.5 years [14].

Treatment

As described in the Dutch national *ACAN* guideline, children with a class IV or V *ACAN* variant were treated with biosynthetic GH with a dose of 1.4 mg/m^2/day (~0.046 mg/kg/day) subcutaneously [19, 32]. GH treatment was started if height SDS was < −2.5 SDS and/or predicted adult height was expected to be < −2.5 SDS and/or an affected parent had a height < −2.5 SDS. Patients visited the outpatient clinic every 4 months. After each visit, the dose was titrated according to the calculated body surface area, growth velocity, and/or serum IGF-I levels. IGF-I levels were determined at least yearly.

Eight children were treated with a GH dose of 1 mg/m^2/day (~0.033 mg/kg/day) because of short stature after small for gestational age birth, while at that moment, the *ACAN* deficiency had not yet been diagnosed. Two children were treated with a GH dose of 2 mg/m^2/day (~0.067 mg/kg/day) according to the GH study design in which they were included [33].

Some patients came to medical attention when they were already in early puberty. In boys and girls, with a relatively short height at onset of puberty defined as a height <140 cm, postponement of puberty for 2 years using a GnRH analog was added to GH treatment (n = 9). Additionally, in 3 boys with an advanced BA and therefore an expected adult height <−2.5 SDS, adjunctive treatment with an AI was started to delay further advancement of BA. GnRHa and/or AI treatment was discussed and started at the discretion of the treating physician.

Statistical Analyses

Clinical characteristics and growth results are presented as median (interquartile range [IQR]), unless stated otherwise. Normal distribution of variables was tested using Kolmogorov-Smirnov test. Differences in characteristics between subgroups were evaluated using independent sample t test for normal distributed variables and Mann-Whitney test otherwise.

Changes in height SDS and BA over time were analyzed with repeated measure regression analysis to correct for multiple testing and missing data. For this analysis, the group was divided into children who were prepubertal at start of GH treatment and adolescents who were pubertal at start of GH. Two children in the prepubertal group started puberty during the first years of GH treatment; therefore, only their prepubertal measurements were included. The analysis for gain in height SDS was adjusted for GH dose and in the pubertal group also for GnRHa treatment. Analyses were performed using the statistical package SPSS for Windows (version 26; SPSS Inc., Chicago, IL, USA) and R, R Core Team (2022; Posit Team; RStudio: Integrated Development Environment for R. Posit Software, Boston, MA, USA). A p value <0.05 was considered significant.

Results

In total, 36 individuals (23 boys and 13 girls) from 25 different pedigrees were identified and included in the present study (Table 1).

ACAN *Gene Variants and Phenotypic Characteristics*

A total of 25 different *ACAN* variants were identified. There was 1 subject with a heterozygous deletion of >1 complete exon, 8 subjects with a frameshift variant, 17 subjects with a nonsense variant, 2 subjects with a splice-site variant, 7 subjects with a missense variant, and 1 subject with an in-frame insertion (online suppl. Table 1).

Most children were born full term, and 6 children were born preterm (<37 weeks gestational age). Eleven were born small for gestational age, while the other ones were born with normal birth weight and/or birth length. Median (IQR) birth length tended to be in the lower part of the normal range −1.7 SDS (−2.7 to −0.4) (Table 2). Median height SDS at start of GH treatment was −2.6 SDS (−3.2 to −2.2) for the total group. Height SDS was not correlated with age (r = 0.27, p = 0.11). Sitting height-to-height ratio was ≥2.0 SDS in only 10 subjects (28%) and ≥1.0 SDS in 20 subjects (56%). In 4/13 (30%) children with a known arm span, the arm span (cm) was at least 3 cm larger compared to the height (cm).

Dysmorphic features are described in Table 1. Midface hypoplasia was reported in 9 children. Brachydactyly was reported in 6 children, and wide feet in 5 children. Radiologically confirmed OD was present in 2 children, one had a variant in the glycosaminoglycan attachment region (subject 11a) and one in the G3 domain (subject 24). In subject 22, with a variant in the G3 domain, OD was diagnosed after start of GH treatment. OD was also described in parents with a pathogenic variant in other domains of the *ACAN* gene (Table 1). In ~20% (6/36) of the children, no specific clinical (advanced BA and/or increased sitting height and/or increased arm span to height ratio) or dysmorphic features were reported.

In 18 children (50%), before start of GH treatment or any other growth-promoting medication, BA was advanced (≥0.5 years), of whom only 6 had a markedly advanced BA (2 years or more). BA advancement was inversely correlated with age (r = −0.39, p = 0.02), also after correcting for height SDS and year of starting GH treatment.

Genotype-Phenotype Associations

The genetic variants were widely distributed over the *ACAN* gene (Fig. 1). The missense variants were located in the G1 and G3 domains. There were 26 subjects with a truncating variant, 8 subjects with a non-truncating variant, and 2 subjects with a splice-site variant (online suppl. Table 1).

Table 1. Subjects with a heterozygous pathogenic *ACAN* variant: clinical and dysmorphic features

Subject	Sex	ACAN variant	Height SDS father	Height SDS mother	Age at start GH	Height SDS at start GH	BA-CA at start GH	SH/H ratio SDS	Dysmorphic and clinical features at start of GH treatment	Pubertal at start GH	GnRHa/AI treatment
1	M	c.1_9del p.Met1?	−0.2[§]	−0.6[§]	14.8	−3.3	−0.8	0.9	No	Yes	AI
2a	M	c.130G>A p.(Gly44Arg)	−1.7	**−3.8**	6.1	−2.5	0.8	1.3	No	No	No
2b	M	c.130G>A p.(Gly44Arg)	−1.7	**−3.8**	10.0	−2.3	0.6	2.8	No	No	No
3a	F	c.454+1G>A p.?	−0.5	**−2.7**	4.2	−3.0	1.9	2.5	No	No	No
3b	M	c.454+1G>A p.?	−1.7	**−1.7**	4.3	−3.8	0.0	1.1	Frontal bossing	No	No
4	M	c.547G>A p.(Ala183Thr)	0.7[§]	1.8[§]	7.1	−1.6	1.7	−0.6	Arm span>height	No	No
5a	F	c.706C>T p.(Arg236*)	1.0	**−4.2**	7.8	−2.2	1.6	0.9	Mild midface hypoplasia, wide feet	No	No
5b	M	c.706C>T p.(Arg236*)	NA	**−4.1**	8.7	−2.3	0.0	1.7	Stocky build, mild midface hypoplasia, arm span>height, wide feet, brachydactyly	No	No
5c	M	c.706C>T p.(Arg236*)	1.0	**−4.2**	9.2	−3.1	0.0	1.8	Mild midface hypoplasia, wide feet	No	No
6	M	c.775_784delins17 p.(Thr259 fs)	**−3.9**[#]	−2.5	14.9	−5.0	−2.8	1.8	No	Yes	GnRHa
7	F	c.1526C>A p.(Ser509*)	−0.5[§]	−1.1[§]	6.4	−3.3	2.0	0.8	No	No	No
8a	M	c.1608C>A p.(Tyr536*)	−1.0	**−3.4**	5.0	−3.2	0.9	2.7	No	No	No
8b	F	c.1608C>A p.(Tyr536*)	−1.4	**−4.9**[#]	5.0	−3.7	0.0	2.2	Midface hypoplasia, broad great toes, and short thumbs	No	No
8c	M	c.1608C>A p.(Tyr536*)	−1.4	**−4.9**[#]	11.9	−2.4	0.6	1.4	Midface hypoplasia, mild posteriorly rotated ears, broad great toes, absent left kidney	Yes	GnRHa
9	F	c.1631dup p.(Cys545Metfs*4)	0.9	0.4	11.2	−3.1	−1.2	0.5	Mild midface hypoplasia, Osgood Schlatter, pes plano valgus, patella dislocation, mild scoliosis	Yes	GnRHa

Table 1 (continued)

Subject	Sex	ACAN variant	Height SDS father	Height SDS mother	Age at start GH	Height SDS at start GH	BA-CA at start GH	SH/H ratio SDS	Dysmorphic and clinical features at start of GH treatment	Pubertal at start GH	GnRHa/AI treatment
10	M	c.2423C>G p.(Ser808*)	−1.5[§]	1.3[§]	7.7	−2.7	−0.3	0.7	No	No	No
11a	F	c.2735_2736delins31 p.(Leu912fs)	**−4.1**[#]	−0.5	4.2	−4.8	−2.1	<u>2.7</u>	OD knees	No	No
11b	F	c.2735_2736delins31 p.(Leu912fs)	1.3	**−2.9**[#]	11.9	−2.2	<u>1.4</u>	0.3	Brachydactyly dig I hand, sandal gap	Yes	GnRHa
12	M	c.2770G>T p.(Glu924*)	1.2[§]	−0.7[§]	3.3	−2.6	<u>0.8</u>	<u>2.0</u>	Frontal bossing, brachydactyly dig 5 hand, Baker's cyst both sides	No	No
13	F	c.4573del p.(Leu1525Serfs*11)	0.5[§]	−1.1[§]	11.2	−2.9	<u>1.0</u>	0.0	Arm span>height	Yes	GnRHa
14	F	c.4700del p.(Ser1567Metfs*10)	**−2.3**	−1.4	6.0	−3.7	0.0	<u>2.0</u>	Brachydactyly dig 5 hand	No	No
15	M	c.4762_4765del p.(Gly1588Cysfs*26)	**−3.7**[#]	0.6	12.5	−2.8	<u>1.0</u>	<u>1.6</u>	Midface hypoplasia, broad great toes, posteriorly rotated ears	Yes	GnRHa
16	M	c.5219C>A p.(Ser1740*)	−1.2	−4.9	13.4	−1.9	<u>1.0</u>	<u>2.1</u>	Lumbar lordosis, genu vara, arm span>height	Yes	No
17	M	c.5344dup p.(Ile1782Asnfs*3)	0.1	0.0	9.3	−2.1	0.0	0.9	Almond shaped eyes, downslant, offset ears, cafe-au-lait spots	No	No
18a	M	c.6673C>T p.(Gln2225*)	**−2.7**	−1.6	10.9	−2.2	0.3	<u>2.4</u>	Stocky build	No	No
18b	M	c.6673C>T p.(Gln2225*)	**−2.7**	−1.6	12.6	−2.0	0.2	0.9	No	Yes	No
19	M	c.7072T>C p.(Cys2358Arg)	0.9[§]	0.9[§]	10.1	−1.4	<u>2.9</u>	<u>1.4</u>	Wide feet, coarse bone structure hands and feet, stocky build	No	No
20	M	c.7096C>T p.(Gln2366*)	0.1	**−3.6**	4.3	−3.5	<u>1.0</u>	<u>2.4</u>	Hypertelorism, cafe-au-lait spots	No	No
21	F	c.7156_7161dup p.(Cys2386_Arg2387dup)	**−0.7**[#]	−0.4	6.1	−2.4	<u>2.5</u>	<u>1.7</u>	No	No	No

Table 1 (continued)

Subject	Sex	ACAN variant	Height SDS father	Height SDS mother	Age at start GH	Height SDS at start GH	BA-CA at start GH	SH/H ratio SDS	Dysmorphic and clinical features at start of GH treatment	Pubertal at start GH	GnRHa/AI treatment
22	M	c.7204C>T p.(Gln2402*)	−2.0	**−5.6#**	12.0	−2.8	0.5	1.4	Midface hypoplasia, mild prognathism, broad great toes, exaggerated lumbar lordosis	Yes	GnRHa
23a	F	c.7255G>C p.(Asp2419His)	**−3.0#**	−1.6	11.6	−1.6	0.5	−0.6	Cafe-au-lait spots, arm span>height	Yes	GnRHa
23b	M	c.7255G>C p.(Asp2419His)	**−3.0#**	−1.6	14.3	−2.1	−1.0	−0.6	Hypertension, arm span>height	Yes	No
24	F	c.7408T>G p.(Cys2470Gly)	0.2	**−1.1**	12.4	−2.0	−0.8	0.2	Mild midface hypoplasia, OD knees	Yes	No
25a	F	c.7602G>A p.(Trp2534*)	−1.2	**−3.8#**	5.9	−2.5	0.0	0.5	Frontal bossing, hypertelorism, wide feet, brachydactyly	No	No
25b	M	c.7602G>A p.(Trp2534*)	1.9	**−2.2#**	14.3	−2.4	−0.5	−0.6	Offset ears	No	No
25c	M	c.7602G>A p.(Trp2534*)	−0.3	**−2.2#**	15.7	−3.1	−2.5	−0.7	Brachydactyly, arm span>height	Yes	No

If one of the parents has the same *ACAN* gene variant as the patient, height SDS of the affected parent is in bold. If the mutation is de novo, this is indicated by a §. Persistent joint problems of the knees and hips and/or knee or hip replacement at a young age due to osteoarthritis/osteochondritis dissecans in parents are indicated by a #. If BA is advanced (≥0.5 years), this is underlined. If the SH/H ratio is increased (≥1.0), this is underlined. GnRHa/AI treatment during the first 3 years of GH treatment. AI, aromatase inhibitor; BA, bone age; CA, calender age; F, female; GH, growth hormone; GnRHa, gonadotrophin-releasing hormone analog; H, height; M, male; NA, not available; SDS, standard deviation score; SH, sitting height.

Height SDS was not correlated with the location of the variant on the gene ($r = 0.23$, $p = 0.19$). However, median height was −2.8 SDS (−3.4 to −2.3) in subjects with a truncating variant, compared to a median height of −2.1 SDS (−2.4 to −1.6) in subjects with a non-truncating variant ($p = 0.002$).

BA advancement (BA-CA) was not correlated with the location of the variant on the gene ($r = -0.12$, $p = 0.50$). Median BA advancement was 0.1 years (−0.4 to 1.0) in the group with a truncating variant and 0.7 years (−0.5 to 2.3) in the non-truncating group ($p = 0.21$).

Response to GH Treatment

At start of GH treatment, median age was 9.7 years (6.0–12.4) (Table 2). Twenty-two children were prepubertal, and 14 were pubertal at start of GH treatment.

Prepubertal Children

Children who were prepubertal at start of GH treatment showed a significant median (IQR) increase in height SDS (ΔHSDS) of 0.6 SDS (0.5–0.8, $p < 0.01$) after 1 year and 1.0 SDS (0.9–1.4, $p < 0.01$) after 3 years of GH (Fig. 2).

Table 2. Baseline characteristics

	Total group (*n* = 36)	Prepubertal (*n* = 22)	Pubertal (*n* = 14)	*p* value
Boys/girls	23/13	14/8	9/5	0.98
Gestational age, weeks	40.0 (38.7–41.2)	39.7 (38.2–41.3)	40.1 (38.5–41.2)	0.86
Birth weight SDS	−0.3 (−1.2 to 0.1)	−0.4 (−1.4 to 0.0)	0.1 (−1.1 to 0.2)	0.35
Birth length SDS	−1.7 (−2.7 to −0.4)	−2.0 (−4.1 to −0.2)	−1.3 (−2.4 to −0.5)	0.26
TH SDS	−1.2 (−1.7 to −0.3)	−1.2 (−1.7 to −0.1)	−1.4 (−2.3 to −0.3)	0.35
At start of GH treatment				
Age, years	9.7 (6.0–12.4)	6.2 (4.8–9.3)	12.5 (11.8–14.4)	**<0.01**
Height SDS	−2.6 (−3.2 to −2.2)	−2.6 (−3.5 to −2.3)	−2.6 (−3.1 to −2.0)	0.52
TH – height SDS	2.0 (0.6–2.5)	2.1 (1.2–2.4)	1.6 (0.1 to 2.6)	0.20
BMI SDS	0.4 (−0.2 to 1.4)	0.8 (−0.1 to 1.5)	0.1 (−0.7 to 1.0)	0.25
Sitting height/height ratio SDS	1.4 (0.5–2.0)	1.7 (0.9–2.4)	0.7 (−0.2 to 1.5)	**0.01**
BA – CA, years	0.4 (−0.2 to 1.0)	0.5 (0.0–1.6)	0.4 (−1.1 to 1.0)	0.06

Values expressed as median (IQR). BMI, body mass index; GH, growth hormone; SDS, standard deviation score; TH, target height.

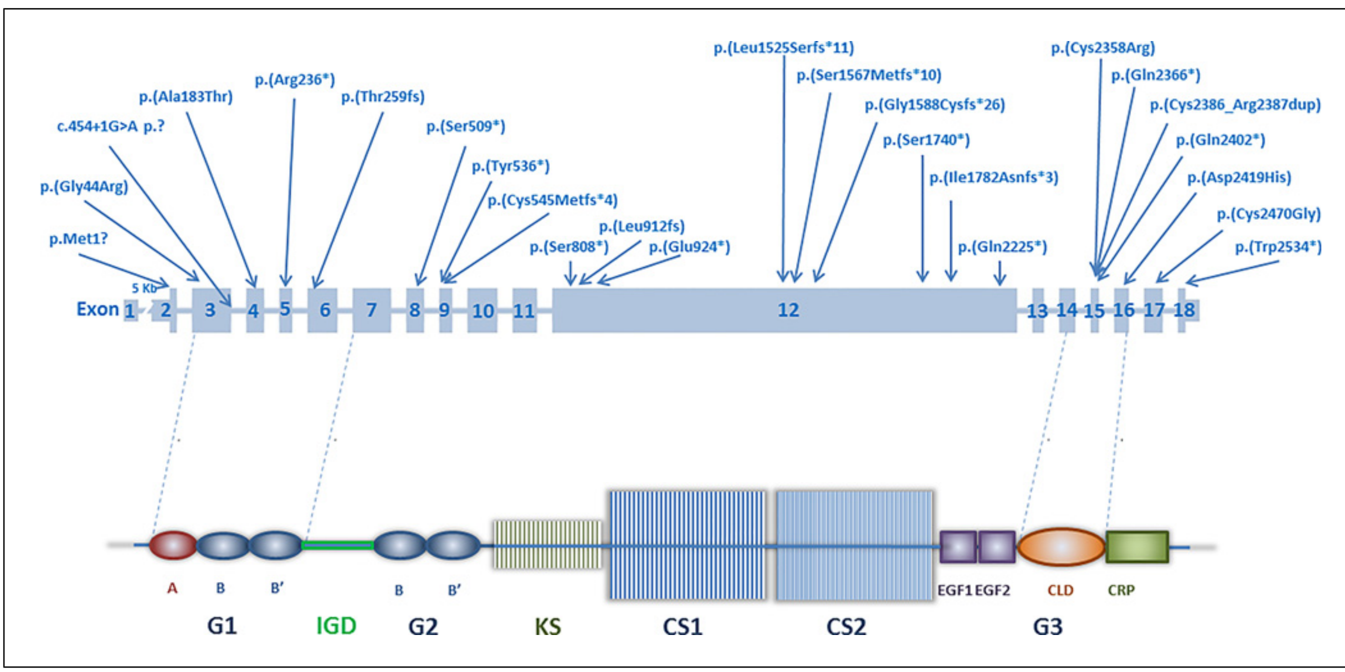

Fig. 1. Distribution of variants in exonic location of *ACAN* (NM_001369268.1) and domain structure of the aggrecan proteoglycan. The structure of the *ACAN* gene is shown in the upper row with exon numbers. The structure of the aggrecan protein is shown in the lower row with crucial domains drawn approximately to scale. Two structures (G1 and G3) are linked by dashed line to indicate the exonic locations of respective domains. Variants above are reported in this study. Figure adapted from Hu et al. [18]. G, globular domain; IGD, interglobular domain; KS, keratan sulfate; CS, chondroitin sulfate; CLD, C-type lectin domain; CRP, complement regulatory-like domain; EGF, epidermal growth factor-like domain.

During these 3 years, the GH dose was increased in 1 subject because of a poor response to GH treatment and reduced in 5 subjects because of serum IGF-I levels >2 SDS. Adjusting ΔHSDS for GH dose did not significantly change the results (β: 0.23, p = 0.34). One subject (No. 11a) showed a suboptimal response to GH with a ΔHSDS of 0.4 SDS after 1 year and 0.3 SDS after 3 years, due to noncompliance.

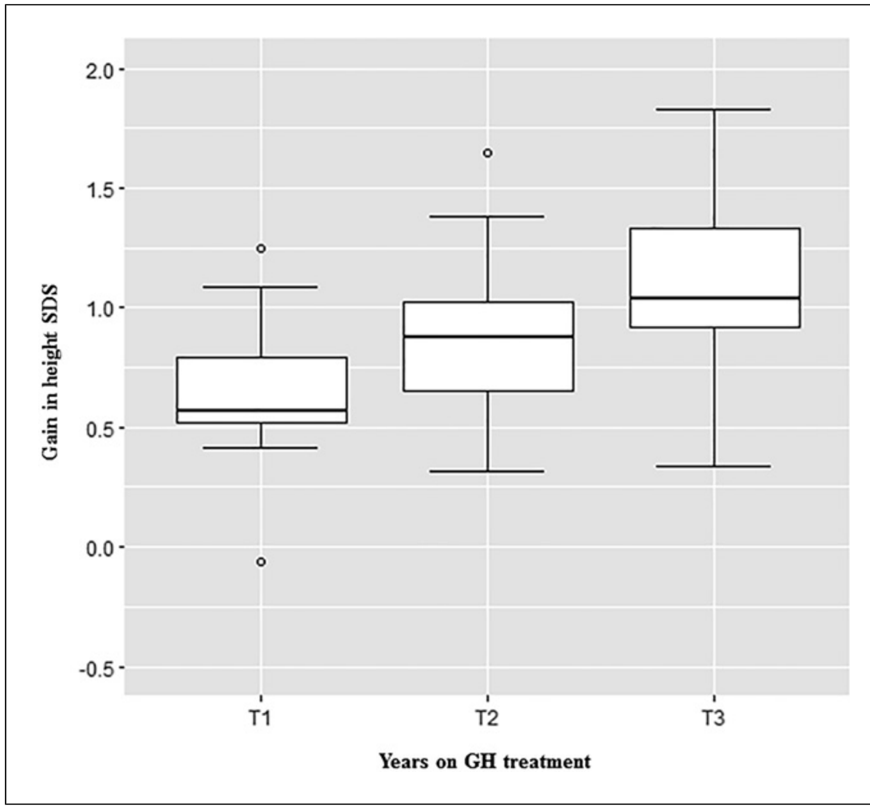

Fig. 2. Gain in height SDS during 3 years of GH treatment in prepubertal children (≥1 year of GH in 22 subjects, ≥2 years n = 17, and ≥3 years n = 10). GH, growth hormone; SDS, standard deviation score.

During GH treatment, BA did not accelerate. At start of GH treatment, BA/CA was 1.02 (1.0–1.2), after 1 year of GH treatment, 1.05 (1.0–1.2, p = 0.88), and after 3 years, 1.06 (1.0–1.1, p = 0.83).

Pubertal Children

Pubertal children showed an increase in HSDS (ΔHSDS) compared to baseline of 0.3 SDS (0.2–0.3, $p < 0.01$) after 1 year of GH treatment and 0.5 SDS (0.2–0.7, p = 0.03) after 3 years (Fig. 3, 4).

In this group, 8 patients were also treated with GnRHa. In 3 patients, the GH dose during these 3 years could not be increased or was even reduced because of serum IGF-I levels >2 SDS. Adjusting the ΔHSDS for GnRHa use and GH dose did not significantly change the results (β: 0.07, p = 0.83).

One subject (No. 6) showed a suboptimal response to GH treatment with a decrease in height SDS. He was 14.9 years old at start of GH and was treated with GH 1.4 mg/m^2/day and GnRHa simultaneously.

Adult Height

Ten patients reached adult height, namely, subjects 8b, 8c, 9, 10, 15, 18b, 22, 23a, 23b, and 25c (Fig. 5). Four subjects reached an adult height ≥ −2 SDS, and in 5 subjects, adult height SDS was greater than the affected parent. In subject 10, the variant was de novo, and his final height was −2.4 SDS. Subjects 10, 18b, 23b, and 25c were treated with GH only, and they reached a height of −2.4 SDS, −1.6 SDS, −2.3 SDS, and −1.9 SDS, respectively.

Subjects 8b, 9, 15, and 23a were treated with GH and GnRHa, and they reached an adult height of −4.0 SDS, −2.0 SDS, −2.6 SDS, and −2.4 SDS, respectively. Subjects 8c and 22 were both treated with GH 2 mg/m^2/day, GnRHa, and AI, and they reached an adult height of −1.3 SDS and −3.1 SDS, respectively.

Safety

GH treatment was overall well tolerated. Two pubertal subjects developed a thoracic lumbar scoliosis during treatment: in one subject, this was mild and no intervention was needed, and in the other subject, a brace was prescribed. Serum IGF-I values varied during GH treatment. Values >2 SDS were observed in 9 individuals (6 prepubertal and 3 pubertal). The IGF-I levels normalized without intervention or after reduction of the GH dose.

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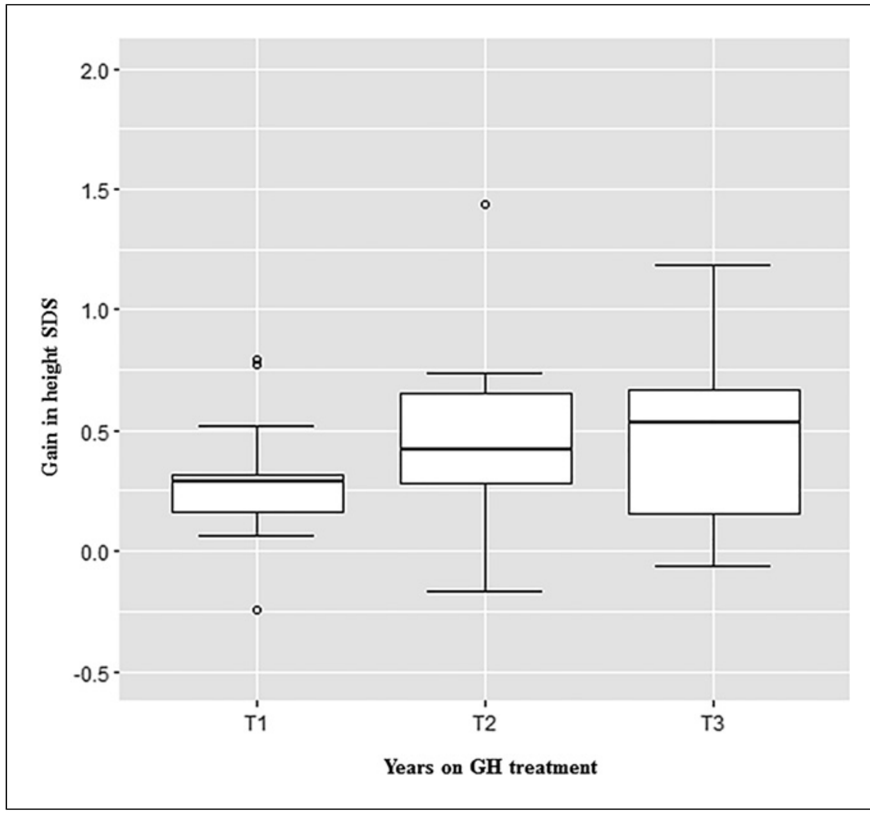

Fig. 3. Gain in height SDS during 3 years of GH treatment and additional GnRHa/AI treatment in pubertal children (≥1 year of GH in 14 subjects, ≥2 years $n = 11$, and ≥3 years $n = 7$). AI, aromatase inhibitor; GH, growth hormone; GnRHa, gonadotrophin-releasing hormone analog; SDS, standard deviation score.

Discussion

In this study, we present detailed clinical characteristics of a large group of patients with pathogenic heterozygous *ACAN* variants who were followed longitudinally, thereby expanding our knowledge of this clinical phenotype. Twenty-two of the 25 variants were not previously reported [14]. Although the majority of children displayed mild dysmorphic features, 20% of children did not have any clinical signs suggestive of a skeletal dysplasia and presented with isolated short stature. To our knowledge, this is the first cohort of *ACAN*-deficient children who were treated according to a standardized, national GH treatment protocol with 3-year follow-up. We found that GH treatment had a modest but significant growth-promoting effect in the majority of children, which was maintained during 3 years of GH treatment.

The *ACAN* gene is located on the long arm of chromosome 15 and consists of 19 exons (NM_001369268.1), with the first exon being noncoding [34, 35]. Only patients with de novo or segregating (likely) pathogenic *ACAN* variants were included in our study. The 25 variants found were spread throughout the gene and included mostly truncating or splice-site variants ($n = 19$) and less often a missense or in-frame variant ($n = 6$) (Fig. 1). We found that subjects with a truncating *ACAN* variant had a significantly shorter stature compared to those without a truncating variant. These findings are in line with the results of Wu et al. [16]. In contrast to Wu et al. [16], we did not observe that truncating variants led to a more advanced BA.

Interpretation of variants in the *ACAN* gene, especially non-truncating variants, can be difficult. Missense variants are often classified as a variant of unknown significance. In line with previous reports, the missense variants found in our cohort were located in the folded, globular domains of the aggrecan core protein (G1 and G3) [36]. This may reflect that a missense variant in any of the glycosaminoglycan attachment sites of the non-folded parts of aggrecan only has a marginal effect on aggrecan function and would not result in a clear phenotype [36]. On the other hand, the globular domains mediate important interactions for cartilage extracellular matrix assembly and organization [36, 37]. Indeed, Stattin et al. [36] found a link between missense variants affecting the G3 domain of *ACAN* and OD. We also found that subjects and parents with a pathogenic *ACAN* variant in the G3 domain presented with OD/early-onset

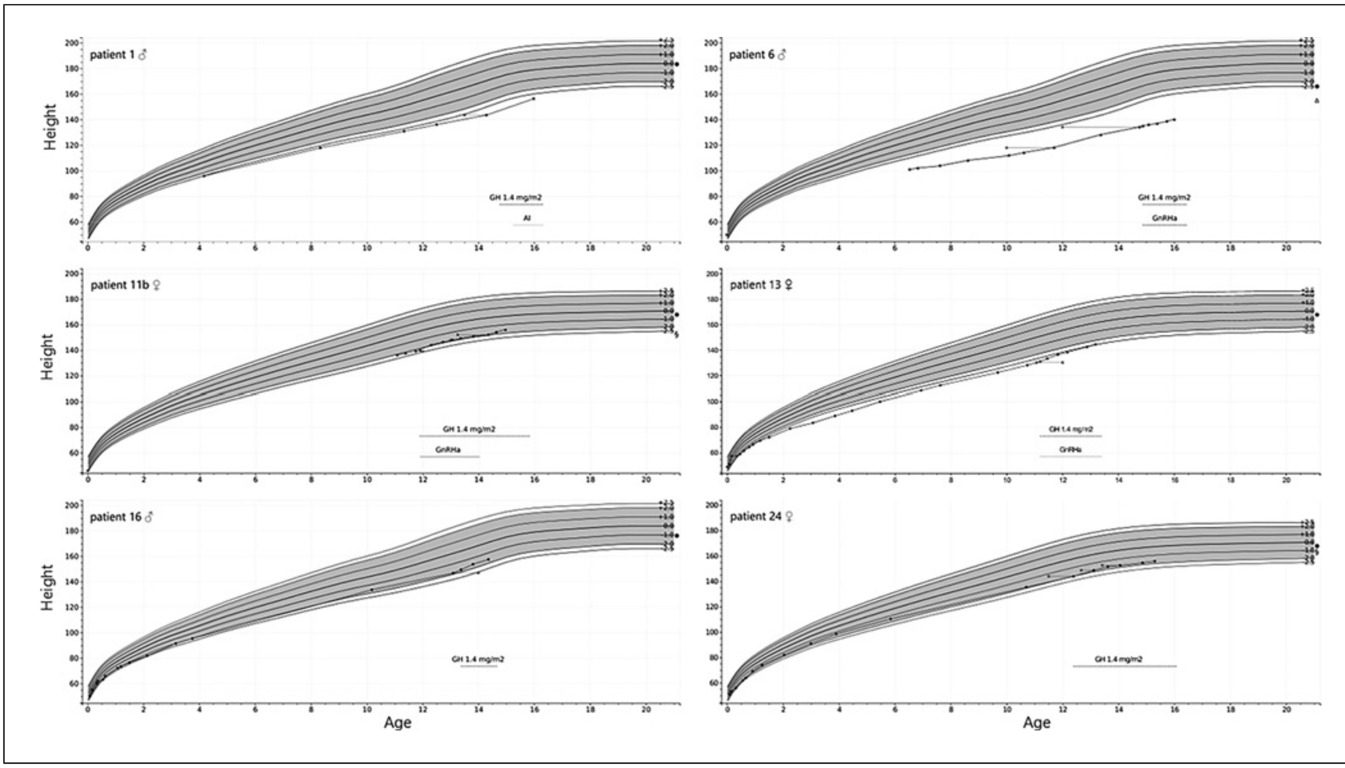

Fig. 4. Growth charts of pubertal subjects with a pathogenic *ACAN* gene variant. Individual growth charts of children with an *ACAN* gene variant who were pubertal at start of GH treatment and are still treated with GH and GnRHa/AI. Pubertal children who reached adult height are depicted in Figure 5. TH is indicated by a ●. Height of the affected parent is indicated by a Δ for father and by a § for mother. In patient 1, the variant was de novo, and in patients 13 and 16, parents were not tested (Growth charts according to Growth Analyser 4.0).

osteoarthritis; however, the same association was observed in families with a variant in other domains of the *ACAN* gene (Table 1).

In most of the previous papers describing children with an *ACAN* variant, BA was typically advanced compared to CA. In contrast, in our cohort, BA advancement was only present in 50% of the subjects. Remarkably, children who were older at start of GH treatment were more likely to show a delayed BA. A delayed BA in children with an *ACAN* variant has been described [13, 16, 38]. In our cohort, 1 subject with a delayed BA was diagnosed because of the presence of OD, 2 were diagnosed because of an affected family member, and in 5 older pubertal children with a delayed BA, the diagnosis was made because of severe short stature (<−3 SDS). It is possible that the latter group is overrepresented in our study because genetic testing was performed not on the basis of clinical features suggestive of *ACAN* deficiency but rather to quickly identify any underlying genetic condition that would qualify the patient for GH treatment under Dutch guidelines. Also, some of these children (4/8) had delayed pubertal development which could explain the delay in BA.

Approximately 20% of our subjects did not have any clinical or dysmorphic features known to be associated with *ACAN* deficiency. For example, disproportion, defined as a sitting height to height ratio ≥2 SDS, was only found in 28% of the subjects and was more prominent in prepubertal children. It might be that some symptoms become more or less apparent throughout childhood. Clearly, a wide phenotypic spectrum exists for *ACAN* deficiency [16, 19]. Based on our findings, we suggest to include *ACAN* as a candidate gene in genetic analyses in all patients with isolated short stature only (height <−2.5 SDS and/or below TH range), even in the absence of disproportion, specific dysmorphic features, or BA advancement.

Until now, short-term GH, either with or without GnRHa and/or AI, is the only available treatment for improving height in patients with *ACAN* deficiency, but data were limited. We found that after 1 year of GH,

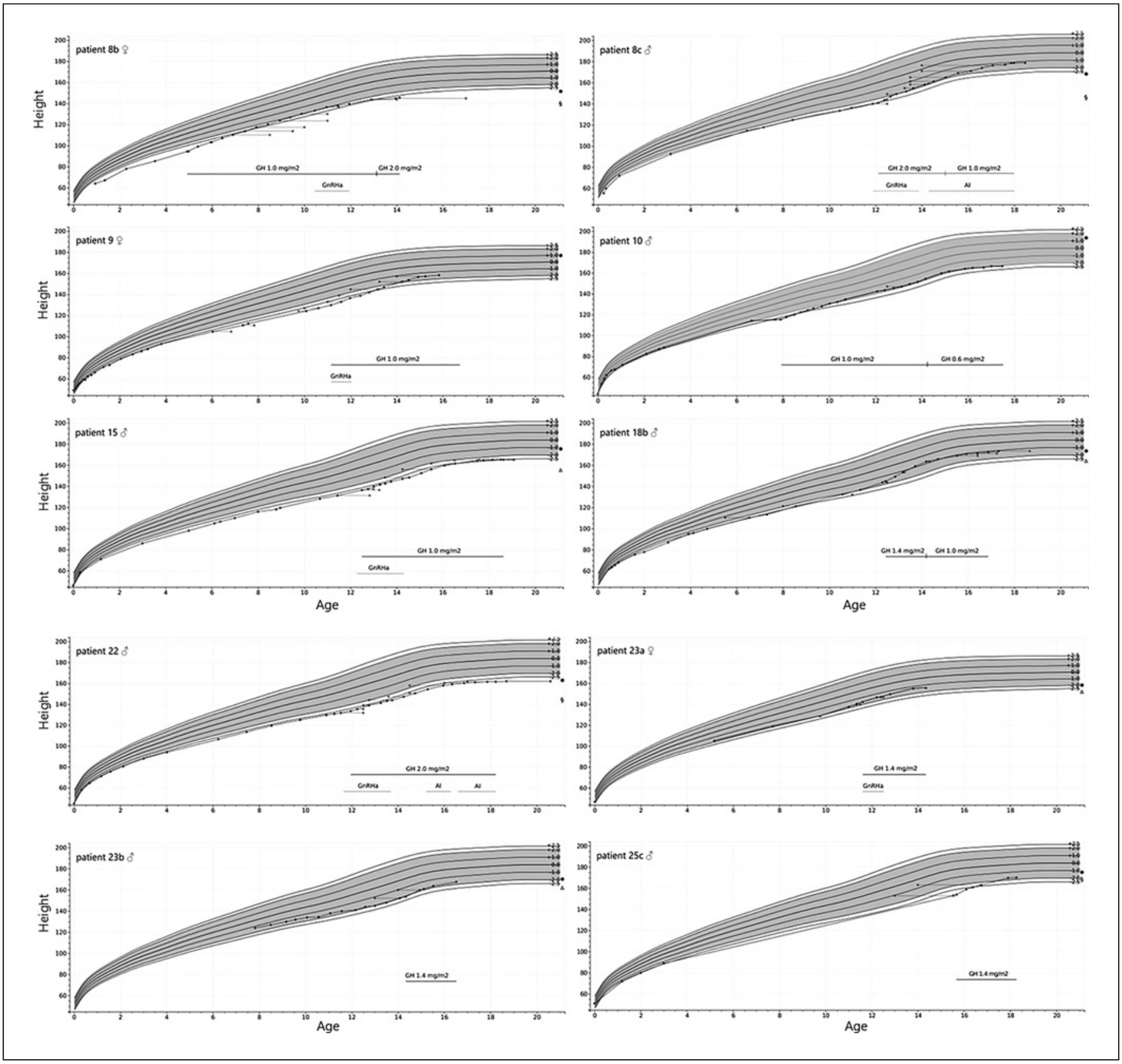

Fig. 5. Growth charts of subjects with a pathogenic *ACAN* gene variant who reached adult height. Individual growth charts of subjects with an *ACAN* gene variant who reached adult height and were either prepubertal or pubertal at start of GH. Target height is indicated by a ●. Height of the affected parent is indicated by a Δ for father and by a § for mother. In patient 10, the mutation was de novo, and in patients 9 and 25c, parents were not tested (Growth charts according to Growth Analyser 4.0).

median HSDS improved by 0.6 SDS in 22 prepubertal children. These results are in line with Muthuvel et al. [19] who described a ΔHSDS of 0.6 SDS after 1 year of GH with a similar dose in 10 prepubertal patients. We now show that continuing GH treatment further improves height SDS with a median height gain SDS of 1.0 SDS after 3 years of GH. As to be expected, further height gain appears to stabilize with ongoing GH treatment [21]. It will be crucial to evaluate whether the significant catch-up growth during the first 3 years of GH will persist throughout puberty and ultimately result in improved adult height.

We also describe growth of 14 pubertal adolescents. Untreated children with *ACAN* deficiency often show a severe decrease in height SDS during their pubertal years because of the rapidly advancing BA [5–7, 15]. This pattern of accelerated epiphyseal fusion under the influence of pubertal estrogen without an adequate compensatory growth spurt is typical of *ACAN* deficiency. We found that after 1 year of GH with or without the addition of GnRHa and/or AI, median height gain SDS was 0.3 SDS, and after 3 years of treatment, it was 0.5 SDS. This is comparable to previously described patients and suggests that even in pubertal children, GH treatment with or without GnRHa/AI may beneficially affect adult height [14, 15, 21]. Given the complexity of the multifactorial pubertal growth process in *ACAN* deficiency, further research is needed to optimize timing of adjunctive GnRHa and/or AI treatment.

In our study, we did not observe a GH dose effect on the change in height SDS. Children who started GH at a dose of 1 mg/m^2/day responded similar to children who started with 1.4 mg/m^2/day. Elevated IGF-I levels were reported in 9 children, and although this might not directly relate to IGF-I bioactivity, one may consider starting GH at a dose of 1 mg/m^2/day in young (<8 years) prepubertal children. If the response after 1 year is unsatisfactory (ΔHSDS <0.5 SDS) [39], the GH dose could be increased to 1.4 mg/m^2/day while maintaining IGF-I levels within the normal range. In pubertal children, however, we would advise to directly start GH treatment at 1.4 mg/m^2/day due to a limited treatment period until adult height attainment.

Our study design has some limitations which are important to consider. First of all, the subjects and data in this study are collected from the Dutch National Registry of GH Treatment. Theoretically, this could have caused a bias in the presented *ACAN* phenotypes with a subset of *ACAN* patients actively seeking GH treatment rather than *ACAN* patients preferring an expectant approach, and this may very well correlate with disease severity. Second, to rigorously evaluate the effect of GH in children with *ACAN* deficiency, a randomized controlled trial is needed. Although the number of individuals with known *ACAN* deficiency has increased in recent years, it is still a rare condition which makes it very difficult to perform a randomized controlled trial. Third, it is also important to emphasize that several subjects were not only treated with GH but also received additional GnRHa and/or AI treatment, which makes the interpretation of GH efficacy in this age range difficult. It will be important to follow these subjects to obtain longitudinal and adult height data before any definitive conclusions can be drawn about overall GH efficacy in this subset of patients.

In summary, we found that the phenotypic spectrum of *ACAN* deficiency is highly variable and ranges from symptoms suggestive of an underlying skeletal dysplasia to isolated short stature. Furthermore, our findings show that prepubertal children with *ACAN* deficiency show a modest but significant response to GH, which is sustained during 3 years of treatment. Finally, *ACAN*-deficient children who are pubertal at the time of diagnosis may still benefit from GH, but adult height data of this cohort are needed before any definitive conclusions can be drawn in this subgroup, given the effects of concurrent GnRHa and/or AI treatment.

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Statement of Ethics

Ethical approval is not required for this study in accordance with national guidelines. Patient consent was not required for this study in accordance with national guidelines.

Conflict of Interest Statement

The authors have nothing to disclose for this study.

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Author Contributions

Judith S. Renes made substantial contributions to the acquisition, analysis and interpretation of data, and drafted the work. Ardine M.J. Reedijk made substantial contributions to the acquisition, analysis and interpretation of data, and helped in drafting the work. Monique Losekoot made substantial contributions to the analysis and interpretation of data and critically reviewed the work. Sarina G. Kant, Manouk van der Steen, and Danielle C.M. van der Kaay made substantial contributions to the interpretation of data and critically reviewed the work. Anita C.S. Hokken-Koelega made substantial contributions to the design of the work, interpretation of data, and critically reviewed the work. Hermine A. van Duyvenvoorde made substantial contributions to analysis and interpretation of data, helped in drafting the work, and critically reviewed the work. Christiaan de Bruin made substantial contributions to the design of the work, analysis and interpretation of data, and critically reviewed the work. All authors approved the final version.

Data Availability Statement

All data generated and analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

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