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ORIGINAL ARTICLE

Genotype and phenotype in patients with *ACAN* gene variants: Three cases and literature review

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Abstract

Objective: To characterize the phenotype spectrum, diagnosis, and response to growth-promoting therapy in patients with *ACAN* variants causing familial short stature.

Methods: Three families with *ACAN* variants causing short stature were reported. Similar cases in the literature were summarized, and the genotype and phenotype were analyzed.

Results: Three novel heterozygous variants, c.757+1G>A, (splicing), c.6229delG, p.(Asp2078Tfs*1), and c.6679C>T, p.(Gln2227*) in the *ACAN* gene were identified. A total of 314 individuals with heterozygous variants from 105 families and 8 individuals with homozygous variants from 4 families were confirmed to have *ACAN* variants from literature and our 3 cases. Including our 3 cases, the variants reported comprised 33 frameshift, 39 missense, 23 nonsense, 5 splicing, 4 deletion, and 1 translocation variants. Variation points are scattered throughout the gene, while exons 12, 15, and 10 were most common (25/105, 11/105, and 10/105, respectively). Some identical variants existing in different families could be hot variants, c.532A>T, p.(Asn178Tyr), c.1411C>T, p.(Gln471*), c.1608C>A, p.(Tyr536*), c.2026+1G>A, (splicing), and c.7276G>T, p.(Glu2426*). Short stature, early-onset osteoarthritis, brachydactyly, midfacial hypoplasia, and early growth cessation were the common phenotypic features. The 48 children who received rhGH (and GnRHa) treatment had a significant height improvement compared with before (-2.18 ± 1.06 SD vs. -2.69 ± 0.95 SD, $p < 0.001$). The heights of children who received rhGH (and GnRHa) treatment were significantly improved compared with those of untreated adults (-2.20 ± 1.10 SD vs. -3.24 ± 1.14 SD, $p < 0.001$).

Conclusion: Our study achieves a new understanding of the phenotypic spectrum, diagnosis, and management of individuals with *ACAN* variants. No clear genotype–phenotype relationship of patients with *ACAN* variants was found. Gene sequencing is necessary to diagnose *ACAN* variants that cause short stature. In general, appropriate rhGH and/or GnRHa therapy can improve the adult height of affected pediatric patients caused by *ACAN* variants.

KEYWORDS
ACAN, aggrecan, bone age, rhGH, short stature

1 | INTRODUCTION

Short stature is defined as a height below 2 standard deviations (SD) or the third percentile of people of the corresponding age, sex and race. Most children are diagnosed with idiopathic short stature (ISS) for unknown reasons. The normal growth of children is regulated by many systems, including multiple hormones (growth hormone, thyroid hormone, and sex hormones), paracrine factors, extracellular matrix molecules, and intracellular proteins that regulate the activity of growth plate chondrocytes (Bai et al., 2023; Baron et al., 2015). Short stature can potentially be caused by variants in any of the genes that directly or indirectly affect growth plate chondrocytes and the process of growth plate chondrogenesis (Baron et al., 2015). Hundreds of genes have been confirmed to be associated with growth plate dysfunction and disorders of linear growth, benefiting from high-throughput sequencing. The two most common single-pathogenic gene variants, *SHOX* and *NPR2*, have been reported in 2%–4.2% and 2% of ISS patients, respectively (Amano et al., 2014; Binder et al., 2003; Rappold et al., 2007, 2002). Heterozygous *ACAN* (MIM 155760) variants account for approximately 1.1%–1.4% of familial short stature patients (Hauer et al., 2017; Hu et al., 2017; Lin et al., 2021; Yang et al., 2018).

Aggrecan, encoded by *ACAN*, located on chromosome 15q26.1 with 19 exons, is a major component of the extracellular matrix of the growth plate, articular cartilage, and intervertebral disc cartilage. The aggrecan core protein is encoded by exons 2–19 and is composed of 2454 amino acids (Valhmu et al., 1995). Aggrecan is mainly composed of N-terminal G1 and G2 domains and a C-terminal G3 domain, with an interglobular domain (IGD) located between the G1 and G2 domains and keratan sulfate (KS) and chondroitin sulfate (CS) binding to the glycosaminoglycan attachment region between the G2 and G3 domains (Valhmu et al., 1995). Heterozygous variants in *ACAN* could lead to spondyloepiphyseal dysplasia, Kimberley type (SEDK, MIM 608361) or early-onset osteoarthritis (OA), osteochondritis dissecans (OCD, MIM 165800), while homozygous variants can cause spondyloepimetaphyseal dysplasia, aggrecan type (SEMDAG, MIM 612813). Over 100 variants of *ACAN* have been identified in patients with highly variable clinical manifestations from nearly normal to severe short stature with multiple facial and skeletal deformities since the first SEDK patient was reported in 2005 (Table 1; Gleghorn et al., 2005).

We reported 3 families with short stature caused by *ACAN* variants. This study aims to establish the phenotypic spectrum and the genotype–phenotype relationship of patients with heterozygous variants in *ACAN* and to summarize all published *ACAN* variants that cause clinical symptoms confirmed by genetic testing, clinical characteristics, and the efficacy of treatment with recombinant human growth hormone (rhGH) and/or gonadotropin-releasing hormone analog (GnRHa) in some children with short stature.

1.1 | Case presentation

Proband 1 is a 4-year and 2-month-old boy who was the second child of nonconsanguineous parents. He was born at full term with a birth weight of 3.15 kg (−0.41 SD) and a birth length of 50 cm (0.06 SD). He was referred to our hospital due to growth retardation for over 3 years. His height was 94.1 cm (−2.49 SD), with a sitting height of 51 cm and a weight of 15 kg (−0.87 SD). His bone age was 5 years 6 months by x-ray imaging of the left hand. He had a proportional short stature, and no other anomalies were observed. No skeletal deformity was detected. Laboratory investigations revealed normal insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-1 binding protein-3 (IGFBP3) and thyroid function. The proband's follow-up is ongoing.

Peripheral blood samples were drawn from proband 1 and possibly affected families (Figure 1a: III2, III3, III4 and IV2). Whole-exome sequencing (WES) was performed for the proband, and a candidate *ACAN* variant was selected. A novel heterozygous variant, c.757+1G>A (splicing) in exon 5 (hg19 reference sequence) was identified in the proband's mother (III3) and uncle (III4) by Sanger sequencing. Proband 1 inherited the splicing variant from his mother. The variant was considered a possible pathogenic variant (PVS1 + PM2) according to the American College of Medical Genetics and Genomic (ACMG) guidelines (Richards et al., 2015). His father was 175 cm (−0.11 SD), his mother was 130 cm (−5.16 SD), his sister was 145 cm (0.97 SD) at the age of 10 years, his maternal grandmother was 138 cm (−3.95 SD), and his uncle was 140 cm (−5.11 SD). The proband's grandmother might be a carrier, but no blood sample was obtained to confirm the variant.

Proband 2 came to our hospital when he was 4 years and 10 months old with a height of 94 cm (−3.3 4 SD), a sitting height of 52.6 cm and a weight of 14.4 kg (−1.75 SD). He had a proportional short stature and no facial deformity. He was born at 39 weeks through cesarean delivery

TABLE 1 Genotypes and phenotypes information.

No	Ref	Variant	Exon/ intron	Aggrecan domain	Protein	Mutation type	Phenotype	ACMG criteria	Inheritance pattern
1	Sentchordi-Montane et al. (2018)	c.-7-2A>C	Intron 1		Splicing	Splicing	SEDK	Pathogenic	AD
2	Hu et al. (2017)	c.6_13delCACTTTAC	Exon 2	Signal peptide	p.Thr3Leufs*21	Frameshift	ISS, OA, thoracic deformities	Pathogenic	AD
3	Wang et al. (2021)	c.23delT	Exon 2	Signal peptide	p.Phe8Serfs*2	Frameshift	ISS, delayed BA	Pathogenic	AD
4	Gkourogianni et al. (2017) and Sentchordi-Montane et al. (2018)	c.61G>T	Exon 2	Signal peptide	p.Glu21*	Nonsense	ISS, advanced BA, macrocephaly, brachydactyly, FB, MFH, lordosis	Pathogenic	AD
5	Stavber et al. (2020)	c.71_1051del	Intragenic deletion	G1	p.His25_Thr350del	Deletion	ISS, OA	Pathogenic	AD
6	Wei et al. (2021)	c.116dupT	3	G1	p.Arg40Glufs*51	Frameshift	ISS, delayed BA, FNB, macrocephaly, short neck, low-set ears, short thumbs, thoracic deformities	Probably pathogenic	AD
7	Hauer et al. (2017)	c.151T>G	3	G1	p.Cys51Gly	Missense	ISS, SGA, thoracic deformities, advanced BA, Limited supination	–	AD
8	Gkourogianni et al. (2017)	c.223T>C	3	G1	p.Trp75Arg	Missense	ISS, OA, IDD	Pathogenic	AD
9	Nilsson et al. (2014) and Gkourogianni et al. (2017)	c.272delA	3	G1	p.Arg93fs*41	Frameshift	ISS, IDD, MFH, brachydactyly, FNB, FB	Pathogenic	AD
10	Stavber et al. (2020)	c.301C>T	3	G1	p.Gln101Ter	Missense	ISS, OA, IDD	Pathogenic	AD
11	Sentchordi-Montane et al. (2018)	c.371G>A	3	G1	p.Arg124His	Missense	ISS, delayed BA, brachydactyly, lordosis, coxa valga, high arched palate, thin lips, broad nose and philtrum	VUS	AD
12	Stavber et al. (2020)	c.410_418delinsTGGA		G1	p.His137Leu fs*30	Frameshift	ISS	Pathogenic	AD
13	Gkourogianni et al. (2017)	c.492C>G	4	G1	p.Tyr164*	Nonsense	ISS, OA, IDD	Pathogenic	AD
14	Kim et al. (2020)	c.512C>T	4	G1	p.Ala171Val	Missense	ISS, IDD, advanced BA, MFH, FB, short neck, ptosis, brachydactyly, low posterior hairline	Probably pathogenic	AD
15	Hauer et al. (2017)	c.515del A	4	G1	p.Gln172Argfs*59	Frameshift	ISS, delayed BA, FB, SN, brachydactyly, thoracic deformities, limited supination	–	AD
16	Freire et al. (2019); Gkourogianni et al. (2017)	c.532A>T	4	G1	p.Asn178Tyr	Missense	ISS, advanced BA, SGA	Probably pathogenic; Pathogenic	AD
17	Ma, (2021)	c.534C>G	4	G1	p.N178K	Missense	ISS, advanced BA	Probably pathogenic	AD
18	Lin et al. (2021)	c.560dupA	4	G1	p.Leu188fs*13	Frameshift	ISS, equal BA	Probably pathogenic	AD
19	Wang et al. (2021)	c.630-1G>A		G1	Splicing	Splicing	ISS, advanced BA	Probably pathogenic	AD
20	Lin et al. (2021)	c.631_632insA	5	G1	p.Tyr211fs	Frameshift	ISS, equal BA	Probably pathogenic	AD
21	Hu et al. (2017) and Lin et al. (2021)	c.661delT	5	G1	p.Tyr221Metfs*10	Frameshift	ISS, equal BA, acanthosis nigricans	Pathogenic	AD
22	Sentchordi-Montane et al. (2018)	c.742G>A	5	G1	p.Ala248Thr	Missense	ISS, OA, IDD, equal BA, brachydactyly, coxa valga, joint anomalies	VUS	AD
23	Case1*	c.757+1G>A	5	G1	Splicing	Splicing	ISS, OA, advanced BA		AD
24	Sentchordi-Montane et al. (2018) and Gkourogianni et al. (2017)	c.903G>C	6	G1	p.Trp301Cys	Missense	ISS, OA, OCD, IDD, equal BA,FB, MFH, FNB, brachydactyly, short limbs, coxa valga, joint anomalies	VUS; Pathogenic	AD

(Continues)

TABLE 1 (Continued)

No	Ref	Variant	Exon/ intron	Aggrecan domain	Protein	Mutation type	Phenotype	ACMG criteria	Inheritance pattern
25	Kim et al. (2018)	c.910G>A	6	G1	p.Asp304Asn	Missense	ISS, equal BA, MFH, FNB, brachydactyly, FB, brittle teeth, blue sclera, triangular face	–	AD
26	Gkourogianni et al. (2017)	c.916A>T	6	G1	p.Ser306Cys	Missense	ISS, IDD	Pathogenic	AD
27	Hattori et al. (2017)	c.1046A>G	6	G1	p.Tyr349Cys	Missense	ISS, equal BA	–	AD
28	Gkourogianni et al. (2017)	c.1047_1048delinsAC	6	G1	p.Tyr349*	Nonsense	ISS, FB, MFH, brachydactyly, anteverted ears	Pathogenic	AD
29	Riera et al. (2021)	c.1061_1062 del			p.Phe354Cys fs*13	Frameshift	ISS	–	AD
30	Riera et al. (2021)	c.1097dup			p.Glu367*	Nonsense	ISS, retrognathia, ears set low	–	AD
31	Hu et al. (2017); Lin et al. (2021)	c.1117_1120delCAGA	7	IGD	p.Thr374*	Nonsense	ISS, equal BA	Pathogenic	AD
32	Hauer et al. (2017)	c.1180 C>T	7	IGD	p.Arg394*	Nonsense	ISS, OCD, FB, SN, brachydactyly, delayed BA, thoracic deformities, limited supination, broad great toes	–	AD
33	Liang et al. (2020); Lin et al. (2021)	c.1411C>T	7	IGD	p.Gln471*	Nonsense	ISS, advanced/equal BA, FB, FNB, short neck, scoliosis, long philtrum	Probably pathogenic	AD
34	Gkourogianni et al. (2017)	c.1425delA	7	IGD	p.Val478fs*14	Frameshift	ISS, OA	Pathogenic	AD
35	Gkourogianni et al. (2017)	c.1443G>T	8	G2	p.Glu415*	Nonsense	ISS, OA, IDD	Pathogenic	AD
36	Lin et al. (2021)	c.1467C>G	8	G2	p.Tyr489*	Nonsense	ISS, equal BA	Probably pathogenic	AD
37	Zhao et al. (2021)	c. 1504C>T	8	G2	p.R502C	Missense	ISS, delayed BA	–	AD
38	Gkourogianni et al. (2017)	c.1526C>A	8	G2	p.Ser509*	Nonsense	ISS, OA	Pathogenic	AD
39	Sentchordi-Montane et al. (2018)	c.1598C>T	9	G2	p.Thr533Ile	Missense	SEDK	VUS	AD
40	Sentchordi-Montane et al. (2018), Gkourogianni et al. (2017) and van der Steen et al. (2017)	c.1608C>A	9	G2	p.Tyr536*	Nonsense	ISS, OA, MFH, SGA, broad great toes, equal BA, lordosis, short thumbs, brachydactyly, low-set posteriorly rotated ears	Pathogenic	AD
41	Hauer et al. (2017)	c.1702G>A	9	G2	p.Asp568Asn	Missense	ISS, OCD, delayed BA, thoracic deformities, Limited supination	–	AD
42	Liang et al. (2020)	c.1733-1G>A	Intron 9	G2	Splicing	Splicing	ISS, EGC, short neck, long philtrum	–	AD
43	Dateki et al. (2017)	c.1744delT	10	G2	p.Phe582fs*69	Frameshift	ISS, IDD, MFH, brachydactyly, advanced/ equal BA, lordosis	–	AD
44	Liang et al. (2020)	c.1762C>T	10	G2	p.Gln588*	Nonsense	ISS, delayed BA, FNB, long philtrum, rib valgus, short phalanges	–	AD
45	Hauer et al. (2017)	c.1774 C>T	10	G2	p.Gln592*	Nonsense	ISS, SN, thoracic deformities	–	AD
46	Liang et al. (2020)	c.1817C>A	10	G2	p.Ala606Asp	Missense	ISS, FNB, brachydactyly, delayed BA, short metacarpal, long philtrum, rib valgus	–	AD
47	Lin et al. (2021)	c.1861A>T	10	G2	p.Lys621*	Nonsense	ISS, advanced BA	Probably pathogenic	AD
48	Lin et al. (2021)	c.1880_1883dupTGGC	10	G2	p.Asp629fs	Frameshift	ISS, advanced BA	Probably pathogenic	AD
49	Sentchordi-Montane et al. (2018)	c.1930G>A	10	G2	p.Gly644Ser	Missense	ISS, equal BA, brachydactyly, joint anomalies	VUS	AD
50	Sentchordi-Montane et al. (2018)	c.1948G>A	10	G2	p.Val650Met	Missense	ISS, FNB, delayed BA, brachydactyly, thin lips, epicanthus	VUS	AD

TABLE 1 (Continued)

No	Ref	Variant	Exon/intron	Aggrecan domain	Protein	Mutation type	Phenotype	ACMG criteria	Inheritance pattern
51	Kim et al. (2021)	c.1968C>G	10	G2	p.Tyr656*	Nonsense	ISS, SGA, equal BA, genu valgum, cubitus valgus	Pathogenic	AD
52	Hattori et al. (2017)	c.1979C>T	10	G2	p.Thr660Met	Missense	ISS, equal BA	–	AD
53	Nilsson et al. (2014), Gkourogianni et al. (2017) and Wang et al. (2021)	c.2026+1G>A	Intron10	G2	exon skipping	Splicing	ISS, advanced /delayed BA, MFH, EGC, brachydactyly, low-set posteriorly rotated ears, exaggerated lumbar lordosis, broad great toes	Pathogenic	AD
54	Stavber et al. (2020)	c.2099G>A	11	KS	p.Trp700Ter	Missense	ISS	Pathogenic	AD
55	Wang et al. (2020)	C.2164C>G	11	KS	p.P722A	Missense	ISS, equal BA, SGA, precocious puberty	–	AD
56	Lin et al. (2021)	c.2173delG	11	KS	p.Glu725fs	Frameshift	ISS, advanced BA	Probably pathogenic	AD
57	Sentchordi-Montane et al. (2018)	c.2218A>T	11	KS	p.Thr740Ser	Missense	ISS, FB, MFH, equal BA, brachydactyly, high arched palate, triangular face	VUS	AD
58	Liang et al. (2020)	c.2266G>C	11	KS	p.Gly756Arg	Missense	ISS, equal BA, FB, FNB, long philtrum, short neck, scoliosis, rib valgus	–	AD
59	Wei et al. (2021)	c.2367delC	12	KS	p.Ser790Glnfs*20	Frameshift	ISS, advanced BA, precocious puberty	Probably pathogenic	AD
60	Sentchordi-Montane et al. (2018)	c.2369C>G	12	KS	p.Ser790*	Nonsense	ISS, FB, advanced BA, brachydactyly, SGA, lordosis, hypertelorism, broad nose and philtrum	Pathogenic	AD
61	Hattori et al. (2017)	c.2535_2536insTTCA	12	KS	p.Pro846Phefs*9	Frameshift	ISS, equal BA	–	AD
62	Toscano et al. (2021)	c.2677delG	12	CS1	p.Gly893AspfsTer52	Frameshift	ISS	Pathogenic	AD
63	Ristolainen et al. (2015)	c.2836_2892del	12	CS1	p.Gly46_Glu64del	Deletion	SEMD, Hodgkin lymphoma	–	AR
64	Gleghorn et al. (2005)	c.3986dupC	12	CS1	p.Gly1330fs*221	Frameshift	SEDK	–	AD
65	Fukuhara et al. (2019)	c.4138G>T	12	CS1	p.V1380F	Missense	SEMD	–	AR
66	Mancioppi et al. (2021)	c.4390delG	12	CS1	p.Val1464Ter	Missense	ISS, OCD, OA, IDD, brachydactyly, macrocephaly, short neck, FB, FNB, low-set rotated ears, broad thumbs, thoracic deformities, muscle hypertrophy	Pathogenic	AD
67	Uchida et al. (2020)	c.4634delT	12	CS1	p.Leu1545Profs*11	Frameshift	ISS, IDD, advanced BA, MFH, broad great toes	–	AD
68	Gkourogianni et al. (2017)	c.4657G>T	12	CS1	p.Glu1553*	Nonsense	ISS, OA, IDD	Pathogenic	AD
69	van der Steen et al. (2017)	c.4762_4765del	12	CS1	p.Gly1588fs	Frameshift	ISS, OA, SGA, MFH, equal BA, broad great toes, joint anomalies, posteriorly rotated ears	Pathogenic	AD
70	Wang et al. (2021)	c.4790-4792delTGG	12		p.Val1597del	Deletion	ISS, equal BA	Probably pathogenic	AD
71	Tatsi et al. (2017)	c.4852C>T	12	CS2	p.Gln1618*	Nonsense	ISS, OA, IDD, MFH, retrognathia	–	AD
72	Zhao et al. (2021)	c.5026_5027del	12	CS2	p.Ser1676Ter	Missense	ISS, advanced BA	–	AD
73	Fukuhara et al. (2019)	c.5061T>A	12	CS2	p.S1687R	Missense	SEMD	–	AR
74	Gkourogianni et al. (2017) and Quintos et al., 2015	c.5391delG	12	CS2	p.Gly1797Glyfs*52	Frameshift	ISS, MFH, FNB, EGC, advanced BA	Pathogenic	AD

(Continues)

TABLE 1 (Continued)

No	Ref	Variant	Exon/ intron	Aggrecan domain	Protein	Mutation type	Phenotype	ACMG criteria	Inheritance pattern
75	Lin et al. (2021)	c.5443delC	12	CS2	p.Leu1815fs	Frameshift	ISS, equal BA	Probably pathogenic	AD
76	Lin et al. (2021)	c.5579delC	12	CS2	p.Gly1861fs	Frameshift	ISS, equal BA	Probably pathogenic	AD
77	Hauer et al. (2017)	c.5597C>A	12	CS2	p.Ser1866*	Nonsense	ISS, advanced BA, brachydactyly, thoracic deformities, FB, SN	–	AD
78	Wang et al. (2021)	c.6122dupT	12	CS2	p.Val2042Argfs*6	Frameshift	ISS, equal BA	Pathogenic	AD
79	Sentchordi-Montane et al., 2018	c.6142C>G	12	CS2	p.Pro2048Ala	Missense	ISS, brachydactyly, equal BA, SGA	VUS	AD
80	Case3' father*	c.6229delG	12	CS2	p.D2078Tfs*14	Frameshift	ISS		AD
81	Zeng et al. (2018)	c. 6193delC	12	CS2	p.Gln2065Serfs*27	Frameshift	ISS, equal BA	Pathogenic	AD
82	Tatsi et al. (2017)	c.6404delC	12	CS2	p.Ala2135Aspfs	Frameshift	ISS, MFH, advanced BA	–	AD
83	Case2*	c.6679C> T	12	CS2	P.Q2227*	Nonsense	ISS		AD
84	Tompson et al. (2009)	c.6799G> A	15	G3(CLD)	P.D2267N	Missense	SEMD	–	AR
85	Lin et al. (2021)	c.6861delC	15	G3	p.Cys2288fs*28	Frameshift	ISS, delayed BA	Probably pathogenic	AD
86	Stattin et al., 2008, 2010 and Gkourogianni et al. (2017)	c.6907G>A	15	G3	p.Val2303Met	Missense	ISS, OCD, OA, IDD	Pathogenic	AD
87	Florio et al. (2019)	c.6970T>C	15	G3	p.Trp2324Arg	Missense	ISS, OCD, delayed BA, brachydactyly, dolichocephaly, hypotelorism, arched palate	–	AD
88	Ye et al. (2020)	c.7007dupC	15	G3	p.Asp2337Glyfs*9	Frameshift	ISS, EGC, equal BA, short neck, dentinogenesis imperfecta, blue sclera	Pathogenic	AD
89	Stavber et al. (2020)	c.7041delG	15	G3	p.Cys2348Val fs*8	Frameshift	ISS, OA, OCD	Pathogenic	AD
90	Nilsson et al. (2014); Gkourogianni et al. (2017)	c.7064T>C	15	G3	p.Leu2355Pro	Missense	ISS, OCD, OA, EGC, short thumbs, macrocephaly, broad thumbs	Pathogenic	AD
91	Stavber et al. (2020)	c.7069A>T	15	G3	p.Ser2357Cys	Missense	ISS	Probably pathogenic	AD
92	van der Steen et al. (2017)	c.7090C>T	15	G3	p.Gln2364*	Nonsense	ISS, OA, OCD, SGA, MFH, equal BA, broad great toes, lordosis, FB, short thumbs	Pathogenic	AD
93	Hattori et al. (2017)	c.7093_7095delGAG	15	G3	p.Glu2365del	Deletion	ISS, delayed BA	–	AD
94	Gkourogianni et al. (2017)	c.7153G>A	15	G3	p.Glu2385Lys	Missense	ISS, OA, IDD	Pathogenic	AD
95	Gkourogianni et al. (2017)	c.7203G>A	16	G3	p.Trp2401*	Nonsense	ISS, OA, IDD	Pathogenic	AD
96	Yang et al. (2018)	c.7222dupA	16	G3	p.Asp2407fs	Frameshift	ISS, equal BA	Pathogenic	AD
97	Sentchordi-Montane et al. (2018)	c.7269delG	16	G3	p.Glu2424 fs*5	Frameshift	ISS, FNB, advanced BA, brachydactyly	Pathogenic	AD
98	Gkourogianni et al. (2017), Riera et al. (2021) and Sentchordi-Montane et al. (2018)	c.7276G>T	16	G3	p.Glu2426*	Nonsense	ISS, OA, macrocephaly, equal BA, SGA, brachydactyly, joint anomalies	Pathogenic	AD
99	Sentchordi-Montane et al. (2018)	c.7276G>A	16	G3	p.Glu2426Lys	Missense	ISS, SGA, equal BA, FB, FNB, brachydactyly, joint anomalies	VUS	AD
100	Deng, 2018	c.7339_7349delTGCGGAGAGCC		G3	p.C2447fs*17	Frameshift	SEMD	Pathogenic	AR
101	Sentchordi-Montane et al. (2018)	c.7342G>A	17	G3	p.Gly2448Arg	Missense	ISS, FB, MFH, brachydactyly	VUS	AD
102	Gkourogianni et al. (2017)	c.7429G>A		G3	p.Val2417Met	Missense	ISS, OA, IDD, OCD	Pathogenic	AD

TABLE 1 (Continued)

No	Ref	Variant	Exon/intron	Aggrecan domain	Protein	Mutation type	Phenotype	ACMG criteria	Inheritance pattern
103	Xu et al., 2018	c.7465T>C		G3	p.Gln2364Pro	Missense	SEDK	Pathogenic	AD
104	Liang et al. (2020)	c.7469G>A	18	G3	p.Cys2490Tyr	Missense	ISS, delayed BA, brachydactyly, short neck, thoracic deformities, mild scoliosis, lordosis	–	AD
105	Crippa et al., 2018	t(10;15)(q22.3;q26.1)	Intron1		–	Translocation	ISS, equal BA, scoliosis, flat feet, broad great toes/thumbs	–	AD

Abbreviations: *, GRCh37/hg19; AD, autosomal dominant; AR, autosomal recessive; BA, bone age; EGC, early growth cessation; FB, front bossing; FNB, flat nasal bridge; IDD, intervertebral disc diseases; ISS, idiopathic short stature; MFH, midfacial hypoplasia; OA, early-onset osteoarthritis; OCD, osteochondritis dissecans; SEDK, spondyloepiphyseal dysplasia, Kimberley type; SEMD, spondyloepimetaphyseal dysplasia; SGA, small for gestational age; SN, short neck; VUS, variant of uncertain significance.

with a birth weight of 2.5 kg (−1.90 SD) and a birth length of 50 cm (0.06 SD).

WES was performed for proband 2, his brother and his parents and identified a novel heterozygous variant in *ACAN* (Figure 1b). A nonsense variant occurring in exon 12 (c.6679C>T, hg19 reference sequence) is pathogenic according to ACMG guidelines (PVS1), which is predicted to cause early truncation of the aggrecan protein (p.(Gln2227*)). His father was 165 cm (−1.67 SD), and his mother was 130 cm (−6.48 SD). His brother was 11 years and 3 months old with a height of 130 cm (−2.19 SD) and a weight of 37 kg (1.26 SD). His mother and his brother both denied arthritis and joint and back pain.

Patient 3 came to our hospital due to growth delay for a half year at the age of 3 years old. He was born at 36 weeks through cesarean delivery with a birth weight of 4 kg (1.26 SD) and birth height of 52 cm (1.12 SD). He showed no abnormal facial or body features. The growth hormone peak was 19 ng/mL at 30 minutes. The chemical tests were all normal, including thyroid function, liver function, renal function, glucose level, sex hormones, IGF-1, and IGFBP3. RhGH therapy had begun at the age of 6 at a dose of 66 μg/kg/day. He had a delayed bone age of 1 month. His height was 131.8 cm (−0.78 SD) at the last visit at the age of 9 years and 9 months. The WES test was performed for the patient and his parents and identified a novel heterozygous variant in *ACAN* of his father only (Figure 1c). A nonsense variant occurring in exon 12 (c.6229delG) is possibly pathogenic (PVS1 + PM4) and may cause early truncation of the aggrecan protein (p.(Asp2078Thrfs*14), hg19 reference sequence). His father was 150 cm (−3.74 SD) and denied arthritis and back pain.

2 | GENETIC ANALYSIS

Genomic DNA was extracted from the peripheral blood of the patients and possibly affected relatives. The entire exome was captured using SureSelectXT Human A11 Exon v5 (Agilent, USA), followed by sequencing using NextSeq (Illumina, USA). The DNA sequences obtained by sequencing were aligned with the human genome (GRCh37/hg19) provided by the University of California Santa Cruz (UCSC) database. The coverage of the target region and sequencing quality were also evaluated. The detected variants were confirmed by Sanger sequencing. The pathogenicity of variants was evaluated in accordance with the ACMG guidelines.

Identified variants were checked against multiple variant databases, such as the Clin var Database (https://www.ncbi.nlm.nih.gov/clinvar), the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/about), and the Leiden Open Variation Database (LOVD, https://databases.lovd.nl). The functional consequence of each variant

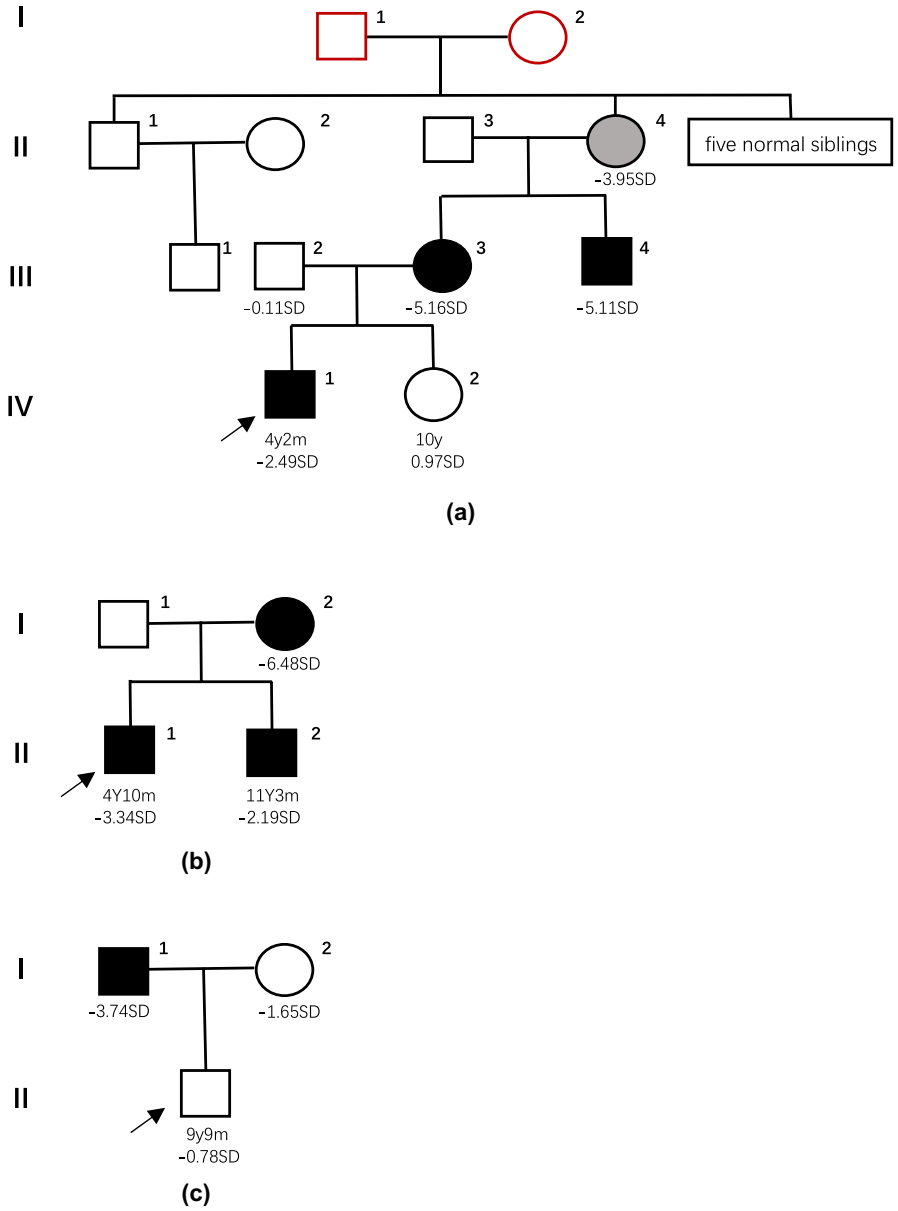

FIGURE 1 Four family pedigrees: (a) Case 1; (b) Case 2; (c) Case 3.

was predicted by four in silico programs, such as SIFT (http://sift.jcvi.org), PP2 HVAR (http://genetics.bwh.harvard.edu/pph2), MutationTaster (http://www.mutationtaster.org), and CADD V1.3 (http://cadd.gs.washington.edu).

2.1 | Ethical compliance

This study protocol was reviewed and approved by the ethics committee of Children's Hospital, Zhejiang University, School of Medicine (2022-IRB-230).

2.2 | Literature review result and statistical analysis

Until November 1, 2021, we searched online databases (PubMed, Web of Science, and CNKI) for published papers with identified *ACAN* variants that cause clinical symptoms by "*ACAN*" or "aggrecan." Thirty-nine articles were collected to evaluate the affected individuals' genetic features, clinical manifestations, biochemical and radiologic characteristics, and response to therapy of rhGH and/or GnRHa (Crippa et al., 2018; Dateki et al., 2017; Deng, 2018; Florio et al., 2019; Freire et al., 2019; Fukuhara et al., 2019; Gkourogianni et al., 2017; Gleghorn et al., 2005; Hattori et al., 2017; Hauer et al., 2017; Hu et al., 2017; Kim et al., 2018, 2020, 2021; Liang et al., 2020; Lin et al., 2021; Ma, 2021; Mancioppi et al., 2021; Nilsson et al., 2014; Quintos et al., 2015; Riera et al., 2021; Ristolainen et al., 2015; Sentchordi-Montane et al., 2018; Stattin et al., 2008; Stattin et al., 2010; Stavber et al., 2020; Tatsi et al., 2017; Tompson et al., 2009; Toscano et al., 2021; Uchida et al., 2020; van der Steen et al., 2017; Wang et al., 2020, 2021; Wei et al., 2021; Xu et al., 2018; Yang et al., 2018; Ye et al., 2020; Zeng et al., 2018; Zhao et al., 2021).

Data were expressed as the mean ± standard deviation (SD) for continuous variables confirmed to be normally distributed with equal variance and as numbers (percentages) for categorical variables. Heights in different groups were compared using the *t*-test of two independent samples. The efficacy of different groups was compared using a paired-samples *t*-test. All statistical data were analyzed by SPSS 25.0. A p value <0.05 was considered to be statistically significant.

3 | RESULTS

3.1 | ACAN variants in humans

A total of 100 heterozygous *ACAN* variants, 3 homozygous variants and 1 compound heterozygous variant were identified from literature and our 3 cases (Table 1), including 33 frameshift, 39 missense, 23 nonsense, 5 splicing, 4 deletion, and 1 translocation variants. It is worth noting that duplicate cases were removed from the evaluation. Some identical variation existing in different families could be hot variants, c.532A>T, c.1411C>T, c.1608C>A, c.2026+1G>A, and c.7276G>T. De novo variants account for 6.7% (7/105) of all available data (Hauer et al., 2017; Kim et al., 2021; Lin et al., 2021; Ma, 2021; Sentchordi-Montane et al., 2018; Tatsi et al., 2017; Toscano et al., 2021). Variants are scattered throughout the gene, while exon 12 composes the main part (25/105), followed by exons 15 (11/105) and 10 (10/105). Interestingly, no variant was located in exons 13, 14, and 19. The 105 variants are detailed in Table 1.

3.2 | Clinical manifestations in patients with heterozygous ACAN variants

In total, 314 heterozygous mutation-positive individuals (148 males, 160 females, no sex information of 6 patients) from 105 different families were identified and included in our study. Over 20 possibly affected relatives without gene testing data were not recruited. A total of 132 variants were found in pediatric patients (age < 18 years), 129 of whom had assessed bone age (BA). We defined advanced BA as a BA greater than 1 year compared to the chronological age (CA), while delayed BA referred to a BA late over 1 year to the CA.

The most common clinical features are described in Table 2. Seven reported patients were all short in stature, and no facial deformity, body deformity, joint pain, or back pain was observed (Table 3). Proband 1 had an advanced BA.

Four heterozygous *ACAN* variants were associated with SEDK found in 30 patients (14 males and 16 females) from 4 different families, including 2 missense variants, 1 splicing variant, and 1 frameshift variant located in different aggrecan domains (Anderson et al., 1990; Gleghorn et al., 2005; Sentchordi-Montane et al., 2018; Xu et al., 2018). They showed moderate anomalies, including platyspondyly, short neck, short trunk, brachydactyly, waddling gait, bilateral irregular femoral epiphyses, severe premature degenerative arthropathy, cervical–vertebral clefts, and apophyses in the upper and lower thoracic vertebrae, but no obvious facial dysmorphism. The inheritance pattern was autosomal dominant.

Five SEMDAG-related *ACAN* variants were identified in 8 patients (5 males, 3 females) from 4 families; they were autosomal recessive inherited, and all demonstrated extremely severe short stature, craniofacial abnormalities, and radiological features, as detailed in Table 4 (Deng, 2018; Fukuhara et al., 2019; Ristolainen et al., 2015; Tompson et al., 2009).

Only 3 patients were associated with prenatal diagnosis. A pregnant woman of advanced maternal age underwent a second-trimester screening exam at 21 w + 3 d of gestation, skeletal dysplasia with prevalent involvement of the limb's rhizomelic tracts was suspected, and a

TABLE 2 Clinical features of patients with ACNA heterozygous mutation.

Clinical manifestation	Prevalence ($n = 314$)
BA versus CA (advanced/equal/delayed)	54/57/18 ($n = 129$)
Short stature	289(92.0%)
Early-onset osteoarthritis	60(19.1%)
Brachydactyly	42(13.4%)
Midfacial hypoplasia	40(12.7%)
Early growth cessation	38(12.1%)
Flat nasal bridge	28(8.9%)
Intervertebral disc diseases	25(8.0%)
Osteochondritis dissecans	24(7.6%)
Joint anomalies	19(6.1%)
Front bossing	18(5.7%)
Short thumbs/metacarpals	17(5.4%)
Short neck	16(5.1%)
Thoracic deformities	16(5.1%)
Small for gestational age	14(4.5%)
Hyperlordosis	11(3.5%)
Broad great toes	9(2.9%)
Macrocephaly	9(2.9%)
Scoliosis	7(2.2%)
Recurrent patellar dislocations	6(1.9%)
Low-set posteriorly rotated ears	5(1.6%)

Abbreviations: BA, bone age; CA, chronological age.

TABLE 3 The clinical characteristics and variation bioinformation of each proband and the family members.

	Family1			Family2			Family3
	Proband1	III-3	III-4	Proband2	I-2	II-2	I-1
Birth weight (kg)	3.15	NA	NA	2.5	NA	NA	NA
Birth length (cm)	50.0	NA	NA	50.0	NA	NA	NA
Age (year)	4.2	34	36	4.8	33	11.2	32
Total height (cm)	94.1	130	140	94	130	130	150
Height (SD)	−2.49	−5.16	−5.11	−3.34	−6.48	−2.19	−3.74
Sitting height (cm)	51.0	71.7	74.3	52.6	NA	66.3	78.3
Ratio	0.54	0.55	0.57	0.56	–	0.51	0.52
cDNA	c.757+1G>A			c.6679C>T			c.6229delG
Protein change	Splicing			p.(Gln2227*)			p.(Asp2078Thrfs*14)
LOVD	NA			NA			NA
Clin Var	NA			NA			NA
ACMG	Likely pathogenic			Pathogenic			Possibly pathogenic
SIFT	NA			NA			NA
PP2	NA			NA			NA
Mutation taster	NA			Disease causing			Disease causing
CADD	26.7			36			NA

Note: Ratio, sitting height/total height; SIFT scores of <0.050, PolyPhen-2 scores of >0.800, MutationTaster scores of disease causing, CADD scores of >15.0 means deleterious effects.

Abbreviations: *, GRCh37/hg19; CADD V1.3, Combined Annotation Dependent Depletion V1.3; Clin Var, Clin var Database; LOVD, Leiden Open Variation Database; NA, data not available; PP2, Polymorphism Phenotyping v2; SIFT, sorting intolerant from tolerant.

slight prominence of the forehead with a low nasal bridge and mild nasal hypoplasia was observed. A pathogenic *ACAN* variant was confirmed after amniocentesis and next-generation sequencing (NGS) (Toscano et al., 2021). Another 2 patients were both evaluated for skeletal dysplasia soon after birth: one patient was prenatally found to have proportionate short stature and facial dysmorphism, and the other patient was suspected due to short limbs at birth (Gkourogianni et al., 2017). Therefore, an early diagnosis is feasible for neonatal suspicious patients.

3.3 | The height management of children

We recruited 48 affected individuals (23 females and 25 males) with detailed medical data to evaluate the efficacy of therapy with rhGH only (29 cases) or rhGH combined with GnRHa (19 cases); details are shown in Table 5. We also collected the adult heights (SD) of 44 untreated probands' parents who were confirmed by genetic testing. We defined GHD as a peak GH value < 10 ng/mL in GH stimulation tests.

The mean age of beginning rhGH therapy was 8.01 years, and the mean duration was 2.5 years, while the mean starting age for GnRHa therapy for females and males was 9.46 years old and 11.58 years old, respectively, and the mean durations for females and males were 1.78 years and 1.54 years, respectively. None of them began both therapies at the same time. The efficacy between all subjects, females, males, receiving rhGH therapy only, receiving rhGH combined with GnRHa therapy, among different variant groups, and whether combined with GHD are shown in Table 6.

4 | DISCUSSION

4.1 | Short stature

Approximately 92.0% of the patients with a heterozygous *ACAN* variant had a height below −2 SD. The children's heights ranged from −5.51 SD to +1.06 SD, while the adults' heights ranged from −6.48 SD to −0.9 SD. A significant difference in height in siblings with the same variant revealed that aggrecan was not the only factor that impacted chondrogenesis in the growth plate (Stavber et al., 2020). The mechanism of linear growth was associated with multiple endocrine hormones and regulating factors. An individual with a heterozygous frameshift variant exhibited a normal height (+1.06 SD), advanced BA and precocious puberty, making the mechanism more elusive (Wei et al., 2021). Two large cohort studies showed the same prevalence of *ACAN* variants in European and

TABLE 4 Clinical features of patients with ACNA homozygous mutation.

Patient/ref	1	2	3	4	5	6–8
Reference	Tompson et al. (2009)	Tompson et al. (2009)	Tompson et al. (2009)	Deng (2018)	Fukuhara et al. (2019)	Ristolainen et al. (2015)
Gender	Female	Male	Male	Female	Male	2 males, 1 female
Age (year)	26	19	13	13.5	45	NA
Protein	p.(Asp2267Asn)	p.(Asp2267Asn)	p.(Asp2267Asn)	p.(Cys2447fs*17)	p.(Val1380Phe); p.(Ser1687Arg)	p.(Gly46_Glu64del)
Type	Homozygous	Homozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous
Height	66 cm	68 cm	71 cm	88 cm	118.3 cm	NA
BA versus CA	NA	NA	NA	Delayed	NA	NA
Macrocephaly	√	√	√	√	√	
Low-set posteriorly rotated ears	√	√	√			
Midface hypoplasia	√	√	√	√		
Absent nasal bridge	√	√	√	√		
Prognathism	√	√	√			
Short neck	√	√	√			
Hoarse voice		√	√			
Bronchospasm		√				
Barrel chest	√	√	√			
Acromesomelic shortening of the limbs					√	
Pectus excavatum					√	
Pectus carinatum				√		
Genu valgum				√	√	
Short thumbs short metacarpals				√	√	
Lumbar lordosis	√	√	√		√	
Cervical lordosis					√	
Thoracolumbar kyphosis					√	
Widened metaphyses	√	√	√		√	
Irregular epiphyses	√	√	√		√	
Platyspondyly	√	√	√		√	
Multiple cervical-vertebral clefts		√				
Rhizomelia	√	√	√	√		
Mesomelia	√	√	√	√	√	
Brachydactyly	√	√	√		√	
Broad thumbs	√	√	√			
Horizontal nail beds	√	√	√			
Joint laxity	√	√	√		√	

Abbreviations: BA, bone age; CA, chronological age.

TABLE 5 The effects of rhGH therapy in patients with ACAN mutation.

No	Gender	Height SDS before therapy	GHD	Mutation type	rhGH start age (y/m)	rhGH dose (μg/kg/day)	rhGH duration (y/m)	GnRH start age (y/m)	GnRH dose (mg/4wk)	GnRH duration (y/m)	Height SDS after therapy	ΔSD
1	Male	−1.35	Yes	Frameshift	4y	31.4	2y10m				0.62	1.97
2	Male	−4.0	Yes	Nonsense	11y10m	41.4	11m				−3.8	0.2
3	Male	−4.3	No	Frameshift	7y7m	NR	1m				−4.0	0.3
4	Male	−2.25	Yes	Frameshift	11y6m	25	3y6m	11y	NA	2y6m	−2.0	0.25
5	Female	−3.2	No	Missense	12y	NR	1y6m	11y7m	11.25	1y11m	−3.9	−0.7
6	Female	−3.7	No	Nonsense	5y	33–66	9y	10y	3.75	1y6m	−3.9	−0.2
7	Male	−2.4	No	Nonsense	11y10m	66	3y6m	NR	3.75	2y	−1.6	0.8
8	Male	−2.7	No	Nonsense	11y8m	66	5y7m	NR	3.75	2y	−2.9	−0.2
9	Male	−2.7	No	Frameshift	12y4m	33	6y2m	NR	3.75	2y	−2.6	0.1
10	Male	−3.3	No	Frameshift	8y8m	30–50	2y7m				−2.7	0.6
11	Male	−1.8	No	Frameshift	6y4m	30–50	2y5m				−1.6	0.2
12	Male	−2.6	No	Frameshift	6y2m	30–50	1y				−2.0	0.6
13	Female	−0.8	No	Missense	11y4m	30–50	1y1m	NA	NA	NA	−1.1	−0.3
14	Female	−1.2	No	Missense	5y6m	30–50	3y10m				−0.7	0.5
15	Female	−1.7	No	Nonsense	8y6m	30–50	2y1m	NA	NA	1y	−0.5	1.2
16	Male	−1.7	No	Nonsense	5y8m	30–50	6m				−1.5	0.2
17	Female	−0.7	No	Nonsense	8y5m	30–50	10m				−0.5	0.2
18	Female	−3.0	No	Nonsense	3y2m	30–50	10m				−2.3	0.7
19	Female	−2.9	No	Nonsense	8y8m	30–50	10m				−2.6	0.3
20	Male	−2.0	No	Missense	5y6m	30–50	1y				−1.3	0.7
21	Female	−1.9	No	Missense	8y4m	30–50	1y				−1.2	0.7
22	Male	−2.9	No	Frameshift	7y5m	30–50	3y				−1.7	1.2
23	Female	−3.0	No	Translocation	12y	25–37	3y5m				−2.82	0.18
24	Female	−2.16	No	Translocation	9y7m	35–50	3y8m	9y	1–2	3y3m	−2.26	−0.1
25	Male	−3.74	No	Missense	6y5m	60	7y8m				−3.75	−0.01
26	Female	−2.09	No	Missense	5y7m	40	3y11m	7y	NA	2y6m	−1.07	1.02
27	Male	−0.88	No	Missense	7y10m	50	5y	12y2m	NA	7m	−0.31	0.57
28	Male	−2.4	Yes	Missense	10Y	NA	4y6m				−3.5	−1.1
29	Male	−3.2	NA	Missense	NA	NA	NR				−1.7	1.5
30	Female	−2.5	No	Deletion	3y4m	34.8	1y2m				−1.9	0.6

TABLE 5 (Continued)

No	Gender	Height SDS before therapy	GHD	Mutation type	rhGH start age (y/m)	rhGH dose (μg/kg/day)	rhGH duration (y/m)	GnRH start age (y/m)	GnRH dose (mg/4wk)	GnRH duration (y/m)	Height SDS after therapy	ΔSD
31	Female	−4.3	No	Missense	7y11m	34.8	4y3m				−2.7	1.6
32	Female	−2.5	No	Frameshift	11y8m	34.8	2y3m	NR	3.75	NR	−2.9	−0.4
33	Female	−2.5	Yes	Missense	11y	34.8	3y2m	NR	3.75	NR	−2.8	−0.3
34	Male	−2.0	NA	Missense	10y7m	34.8	3m	NR	3.75	NR	−1.8	0.2
35	Male	−2.0	Yes	Frameshift	4y6m	34.8	6m				−1.6	0.4
36	Female	−3.13	Yes	Missense	5y4m	33–50	1y6m	5y10m	1.88	1y	−2.08	1.05
37	Male	−4.38	No	Frameshift	4y10m	48	2y6m				−3.08	1.3
38	Female	−2.91	Yes	Frameshift	9y6m	48	1y7m				−2.3	0.61
39	Male	−3.6	No	Missense	4y1m	50	1y6m				−1.7	1.9
40	Female	−3.4	No	Missense	5y6m	50	3m				−3.1	0.3
41	Male	−3.9	Yes	Missense	3y	33	1y10m				−2.86	1.04
42	Male	−2.88	Yes	Missense	9y5m	NA	2y6m				−1.91	0.97
43	Male	−2.92	Yes	Missense	3y10m	NA	10m				−2.16	0.76
44	Male	−4.53	Yes	Missense	15y1m	NA	2m	NA	NA	2m	−4.37	0.16
45	Female	−2.22	No	Missense	5y7m	50–60	1y8m				−1.28	0.94
46	Female	−3.93	Yes	Nonsense	9y6m	230–300	3y6m	10y6m	NR	2y6m	−2.91	1.02
47	Female	−2.5	No	Missense	11y	35.7	3y8m	10y9m	11.25	1y9m	−2.3	0.2
48	Female	−2.4	No	Missense	8y2m	38.6	3y5m	11y	11.25	7m	−2.1	0.3

TABLE 6 The efficacy of different groups.

	Before treatment (M ± SD)	After treatment (M ± SD)	*p* value
All patients	−2.69 ± 0.95	−2.18 ± 1.06	<0.001
All female	−2.55 ± 0.91*	−2.14 ± 0.98**	0.003
All male	−2.82 ± 0.98*	−2.23 ± 1.15**	<0.001
Children vs adults	−2.20 ± 1.10	−3.24 ± 1.14	<0.001
Only rhGH ($n = 29$)	−2.79 ± 0.96#	−2.13 ± 1.05##	<0.001
rhGH+GnRHa ($n = 19$)	−2.53 ± 0.92#	−2.28 ± 1.10##	0.071
Nonsense ($n = 10$)	−2.67 ± 1.08	−2.25 ± 1.21	0.022
Missense ($n = 23$)	−2.68 ± 1.00	−2.16 ± 1.07	0.002
Frameshift ($n = 12$)	−2.75 ± 0.91	−2.16 ± 1.12	0.008
Without GHD ($n = 33$)	−2.58 ± 0.97^	−2.12 ± 1.02^^	<0.001
Combined GHD ($n = 13$)	−2.98 ± 0.91^	−2.44 ± 1.22^^	0.024

*$p = 0.334$. **$p = 0.766$.
#$p = 0.352$.
##$p = 0.618$.
^$p = 0.211$.
^^$p = 0.373$.

Chinese children with ISS, with a result of 1.4% (Hauer et al., 2017; Hu et al., 2017). Another two recent Chinese studies revealed similar results of 1.1% and 1.2% (Lin et al., 2021; Yang et al., 2018). These studies suggest that *ACAN* pathogenic variants are common causes of ISS.

Isolated short stature induced by *ACAN* variant was typically associated with advanced BA and premature growth cessation at first (Nilsson et al., 2014). Individuals with advanced BA at the prepubertal stage may lead to early growth cessation after the start of puberty due to premature hypertrophic chondrocyte maturation-induced vascular invasion and ossification of growth cartilage (Domowicz et al., 2009). Advanced BA was not a reliable indicator for *ACAN* variant since delayed BA was reported frequently (Tatsi et al., 2017). Over a half of the pediatric patients had accelerated BA compared to CA, with a range of 0–4 years in our research. In general, short stature is most accompanied by delayed BA in endocrine disorders and ISS (Baron et al., 2015). Hypothesized that delayed BA in individuals with *ACAN* variant may be related to growth hormone deficiency (GHD) or SGA as a result of the balance between growth hormone function and the effect of individuals with *ACAN* variant on the epiphysis (Liang et al., 2020). However, some patients with normal growth hormone still had delayed BA. Therefore, clinicians should pay attention to patients diagnosed with short stature with advanced BA, and *ACAN* variants need to be taken into consideration even with delayed BA.

4.2 | Early-onset OA and OCD

ACAN variant can affect both the growth plate and articular cartilage. The etiology of OCD is undetermined but may be a multifactorial combination of genetic factors, spontaneous osteonecrosis, vascular deficiency, ischemia, and repetitive trauma. OCD is characterized by the separation of an articular cartilage and subchondral bone fragment from the articular surface (Edmonds & Polousky, 2013). Stattin et al. first reported 15 *ACAN* mutation-related familial OCD patients in a large family with early-onset OA and disproportionate short stature (Stattin et al., 2008, 2010). To date, 8 *ACAN* variants (5 missense, 2 nonsense, and 1 frameshift) have been associated with OCD in 24 individuals (Table 1). Most probands started with pain in joints as chief complaints in their early teens without family history and had involved joints such as knees, hips, elbows, ankles, and patella (Florio et al., 2019; Hauer et al., 2017; Mancioppi et al., 2021; Nilsson et al., 2014; Stattin et al., 2008, 2010; Stavber et al., 2020; van der Steen et al., 2017). Five variants occurred in the G3 domain, which is a structural and functional fundament for aggrecan's interaction with the components of the extracellular matrix (ECM). The G3 domain of aggrecan is a protein region that is highly conserved between different species. Stattin et al found that perturbed function in the G3 domain of aggrecan was a key factor in the etiology of OCD. As the first two missense variants were located at the C-terminal C-type lectin domain within the G3 domain, a relationship between individuals with *ACAN* variant in the C-type lectin domain and a specific articular phenotype was proposed (Dateki, 2017; Nilsson et al., 2014). New OCD-related variants located in the IGD, G2 domain, and CS domain were not suitable for the hypothesis. Plain radiographs can make the diagnosis, and magnetic resonance imaging (MRI) can further characterize the lesion's stability. No consensus has been achieved in the treatment of OCD, and nonoperative treatment and surgical treatment should be based on skeletal maturity, lesion stability, and the individual situation (Edmonds & Polousky, 2013).

The sporadic and familial forms of OCD had different responses to secondary OA when lesions occurred before closure of the epiphyseal growth plate; those individuals with familial OCD were associated with secondary OA, while sporadic patients spontaneously healed (Stattin et al., 2010). However, OA was also a primary symptom of *ACAN* variant.

Early-onset OA was the second most common clinical manifestation and has been reported by several articles (Anderson et al., 1990; Gkourogianni et al., 2017; Gleghorn et al., 2005; Hu et al., 2017; Mancioppi et al., 2021; Nilsson et al., 2014; Stavber et al., 2020; Tatsi et al., 2017; van der Steen et al., 2017). Most of them had a family history and

started in late adolescence. Some severe individuals suffer from knee pain beginning at 5 years of age (van der Steen et al., 2017). The knees were the most commonly affected joints but also included elbows, spines, ankles, hips, and hands. There is no clear correlation between genotype and specific OA phenotype since the associated frameshift, missense, deletion, and nonsense variants are located in various regions of *ACAN* (Table 1).

4.3 | Intervertebral disc diseases

Aggrecan is a major component of intervertebral disc cartilage, and its variants may lead to intervertebral disc diseases and short necks. Degradation and loss of aggrecan can result in impairment of disc function and the onset of degeneration (Sivan et al., 2014). The arm spans of individuals with *ACAN* variant were commonly greater than the heights, which demonstrated the impaired ability of aggrecan to withstand compressive loads (Gkourogianni et al., 2017; Sivan et al., 2014).

Back pain was also a common chief complaint in affected individuals from 18 families who were diagnosed with intervertebral disc diseases. Most of them suffered from the diseases beginning in their forties and fifties later to joint diseases and slowly progressed to need surgery (Gkourogianni et al., 2017). However, 3 girls showed symptoms in their teens. The mean height of the adults was −3.59 SD and may reflect the severity of impairment of their disc cartilages. A correlation may exist between their early-onset IDD and severe short stature.

4.4 | Small for gestational age (SGA)

In our research, most patients were born within the normal length and weight ranges, and only 14 patients were born SGA, accounting for 4.5% overall. The prevalence of SGA in the Japanese population is near that in the Chinese population, which is 3.5% (Fujita et al., 2016). Van der Steen et al identified 4 patients with an *ACAN* variant in 290 SGA children accounting for 1.4%, while the ratio became 13.8% in children ($n = 29$) with advanced BA (≥0.5 years compared to CA) and an *ACAN* variant (van der Steen et al., 2017). Freire et al found 1 individual with *ACAN* variant in 55 patients born SGA with persistent short stature, accounting for 1.8%, and defined 8 pathogenic genetic variants in genes already associated with growth disorders in 55 patients (14.5%), while the *ACAN* gene made up 12.5% (Freire et al., 2019). We can conclude that *ACAN* is to some extent related to SGA. Therefore, genetic screening is necessary for ISS patients born SGA.

4.5 | SEDK

Four heterozygous *ACAN* variants associated with SEDK were found in 30 patients. Their height ranged from nearly normal to severe short stature, −4.2 SD to −0.76 SD in children, and −5.36 SD to −2.89 SD in adults. The c.7465 T > C mutation (p.Gln2364Pro) is located in a highly conserved region and was predicted to be deleterious (Xu et al., 2018). The carrier relatives even had early-onset OA and IDD. We hypothesize that the same genotype may result in different phenotypes in a family with a similar living environment. No correlation seems to exist between the genotype and SEDK.

4.6 | SEMDAG

SEMDAG is generally caused by a homozygous *ACAN* variant first reported by Tompson SW et al., and a compound heterozygous variant also led to SEMDAG later reported (Deng, 2018; Fukuhara et al., 2019; Tompson et al., 2009). Compared with the other 2 types of variants, SEMDAG patients seem to be worse overall; for example, SEMDAG patients were far shorter than other variants, and the nasal bridge was absent in SEMDAG but was flat in others.

The compound heterozygous missense variants were separately located in the CS1 and CS2 domains, consisting of a large number of repeats (Fukuhara et al., 2019). Both of them failed to obtain samples from their parents for segregation analysis. The homozygous missense variant was proven to influence the binding and kinetics of the interactions between the aggrecan G3 domain and tenascin-C (Tompson et al., 2009). The carrier families showed a height of nearly 150 cm without facial dysmorphism or joint disease. Tompson et al thought this phenomenon raised the possibility that there was a carrier phenotype of mild, proportionate short stature, and SEMDAG carriers did not have OA or IDD, which was different from SEDK carriers; the mechanisms of disease in the two disorders were likely to be distinct (Tompson et al., 2009). Based on these data, we hypothesize that heterozygous variants may lead to SEDK or OCD due to haploinsufficiency, and homozygous variants can result in SEMDAG due to a lack of cartilage genesis.

4.7 | Diagnosis

Van der et al found that midface hypoplasia, joint problems and broad great toes were characteristics of *ACAN* variants in pediatric patients born SGA with advanced BA (≥0.5 years compared with CA). If they had two additional

characteristics, 50% had a variant; if they had all three additional characteristics, 100% had a variant (van der Steen et al., 2017). The three features are different from our phenotypic spectrum, and some deviations may exist due to their small sample size.

Although most patients have a short stature, in general, *ACAN* variants cause short stature in only a small proportion of ISS patients. Advanced BA was once thought to be a reliable indicator; now, it still has to some extent indicative value. No clinical feature was highly specific in the diagnosis of *ACAN* variants.

NGS for the proband and Sanger sequencing for families is a recommended strategy for patients and the "gold standard" in diagnosis. The gene sequencing reports and pathogenicity prediction results should be interpreted with caution. All individuals recruited in our study were confirmed by genetic testing, and dozens of possibly affected relatives were excluded due to lack of gene identification. As a result, the actual prevalence may be higher.

4.8 | Management

Many children have been reported to receive rhGH treatment to increase adult height and GnRHa and/or aromatase inhibitor therapy to block puberty at the prepubertal stage, but most of them are sporadic and receive varied doses of rhGH and GnRHa (Crippa et al., 2018; Dateki et al., 2017; Florio et al., 2019; Gkourogianni et al., 2017; Hauer et al., 2017; Hu et al., 2017; Kim et al., 2021; Liang et al., 2020; Lin et al., 2021; Ma, 2021; Mancioppi et al., 2021; Nilsson et al., 2014; Stavber et al., 2020; Tatsi et al., 2017; van der Steen et al., 2017; Wang et al., 2020; Xu et al., 2018; Zhao et al., 2021). Wei et al. reported that a Chinese boy gained a height of 9–10 cm after 1 year of rhGH treatment but without detailed therapeutic data (Wei et al., 2021). Only one patient experienced an adverse event (rash) after initiation for 1 month and discontinued the therapy, and no other side effects were reported (Hu et al., 2017). Financial reasons were common in those patients who stopped rhGH therapy early.

Compared with untreated probands' diagnosed parents, the heights of probands who received rhGH (and GnRHa) therapy showed marked improvement. However, some probands did not reach adult height when reported.

Appropriate rhGH therapy can improve the adult height of affected pediatric patients caused by *ACAN* variants, while some patients showed a poor response to rhGH therapy. Adding GnRHa therapy was not superior to rhGH therapy only, and efficacy was independent of sex, therapy regimen, variant type, and whether they had a diagnosis of GHD. However, most patients do not respond satisfactorily to growth-promoting treatments, which may be related to the fact that they have not reached adult height at the time of the study and started treatment late.

4.9 | Limitations

We only collected articles with full text. The data were extracted from the literature and may not be as accurate as the primary data. No data on untreated patients' child heights were successfully collected to compare with those of treated patients. The efficacy of rhGH and GnRHa therapy still needs large sample and random studies.

5 | CONCLUSION

In summary, we identified 100 heterozygous *ACAN* variants, 3 homozygous variants, and 1 compound heterozygous variants in *ACAN* from literature and our 3 cases associated with short stature, advanced/equal BA, variable dysmorphic features, OA, OCD, and intervertebral disc diseases. Our study provides a new understanding of the phenotypic spectrum, diagnosis, and management of individuals with *ACAN* variants. C.532A>T, c.1411C>T, c.1608C>A, c.2026+1G>A, and c.7276G>T may be hot variants. Gene sequencing is necessary to diagnose *ACAN* variants that cause short stature. In general, appropriate rhGH and/or GnRHa therapy can improve the adult height of affected pediatric patients caused by *ACAN* variants. The exact pathogenesis and molecular treatment mechanism still need to be studied further.

AUTHOR CONTRIBUTIONS

Prof. Chao-Chun Zou conceptualized and designed the study, and Jun-Fen Fu and Guan-Ping Dong reviewed and revised the manuscript. Wei Tang drafted the initial manuscript. Ke-Mi Wu collected data. Qiong Zhou and Yan-Fei Tang were responsible for the follow-up. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

There is no competing interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

ETHICS STATEMENT

This study protocol was reviewed and approved by the ethics committee of the Children's Hospital, Zhejiang University, School of Medicine, approval number 2022-IRB-230, which means informed consent from patient guardians can be exempted. All methods were carried out in accordance with relevant guidelines and regulations.

CONSENT TO PUBLISH STATEMENT

All authors gave their consent for the publication of this paper.

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