Clinical Research Article

A High Proportion of Novel ACAN Mutations and Their Prevalence in a Large Cohort of Chinese Short Stature Children

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Received: 2 October 2020; Editorial Decision: 7 February 2021; First Published Online: 19 February 2021; Corrected and Typeset: 10 April 2021.

Abstract

Context: Aggrecan, encoded by the ACAN gene, is the main proteoglycan component in the extracellular cartilage matrix. Heterozygous mutations in ACAN have been reported to cause idiopathic short stature. However, the prevalence of ACAN pathogenic variants in Chinese short stature patients and clinical phenotypes remain to be evaluated.
Objective: We sought to determine the prevalence of ACAN pathogenic variants among Chinese short stature children and characterize the phenotypic spectrum and their responses to growth hormone therapies.

Patients and Methods: Over 1000 unrelated short stature patients ascertained across China were genetically evaluated by next-generation sequencing–based test.

Result: We identified 10 novel likely pathogenic variants and 2 recurrent pathogenic variants in this cohort. None of ACAN mutation carriers exhibited significant dysmorphic features or skeletal abnormalities. The prevalence of ACAN defect is estimated to be 1.2% in the whole cohort; it increased to 14.3% among those with advanced bone age and to 35.7% among those with both advanced bone age and family history of short stature. Nonetheless, 5 of 11 ACAN mutation carriers had no advanced bone age. Two individuals received growth hormone therapy with variable levels of height SD score improvement.

Conclusion: Our data suggest that ACAN mutation is 1 of the common causes of Chinese pediatric short stature. Although it has a higher detection rate among short stature patients with advanced bone age and family history, part of affected probands presented with delayed bone age in Chinese short stature population. The growth hormone treatment was moderately effective for both individuals.

Key Words: short stature, ACAN mutation, prevalence, growth hormone, genotype-phenotype correlation

The growth plate is a key organ for linear growth of human body. The endocrine, paracrine, and extracellular matrix, as well as intracellular mechanisms for chondrocyte proliferation and differentiation associated with growth plates are all known to be important regulators of human height (1-3). One of the most abundant molecules at the growth plate is a proteoglycan component in the extracellular cartilage matrix aggrecan, which plays a key role in the morphogenesis of cartilage and bone. The mutations in ACAN gene is a major cause of idiopathic short stature (ISS) (4-7), even though it was initially found to be associated with several short stature syndromes such as spondyloepiphyseal dysplasia, Kimberley type (OMIM 608361), and Spondyloepimetaphyseal dysplasia (SEMD), aggrecan type (OMIM 612813) (8). While many individuals with ACAN mutation presented with short stature and advanced bone age (4,5,9), still many others presented with short stature and normal or even delayed bone age (7,10-13). Thus, ACAN mutations can be responsible for ISS in general. High ACAN mutation rates have been reported for ISS patients with advanced bone age (4-7,9,14-16), and variable detection rates of ACAN mutation have been reported for small cohorts of ISS patients of different ethnic backgrounds (7,10,11,13,17), but the detection rate for general ISS patients had not been assessed in a large cohort.

Here we ascertained over 1000 Chinese ISS children and performed exome sequencing for genetic etiological assessment for short stature. We examine the clinical characteristics and the mutation spectrum of Chinese patients with ACAN mutation and compare to the cases that had been reported so far. We also review the effectiveness and safety of growth hormone (GH) treatment for patients with ACAN mutations.

Materials and Methods

Patients

Individuals who met 1 or more of the following criteria were included in our cohort: (i) multiple pituitary hormone deficiency; (ii) unequivocal GH insensitivity; (iii) small for gestational age without catch-up growth; (iv) additional congenital anomalies or dysmorphic features; (v) evidence of a skeletal dysplasia; (vi) associated intellectual disability; (vii) microcephaly; and (viii) height below −3 SD (18). A total of 1005 unrelated Chinese pediatric short stature patients with likely mendelian disorders were recruited from 11 medical centers (18) (Supplementary Tables 1 and 2 (19)). Of these cases, 229 had parental short stature (either 1 or both parents had short stature (<−2 SD)]. All patients were referred to pediatric endocrinologists for clinical evaluation of short stature (<−2 SD]. The project was approved by the ethics committee of the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region. Informed consent was obtained from the parents of the patients.

Genetic analysis

Both targeted panels consisted of 705 short stature–related genes using Nimble Design (Roche, Madison, WI, USA)
and exome capture using Sure Select Human All Exon Kit (Agilent Technologies, Santa Clara, CA, USA) were used for the cohort study. Next generation sequencing was done using Hiseq2500 platform (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. Sequence coverages for the ACAN genomic interval were at least 20× except for the interval GRCh37/hg19, chr15: 89298700-89399950, which are highly enriched for simple repeats. Variants were annotated by Genome Analysis Toolkit and filtered by Ingenuity Pathway Analysis (https://variants.ingenius.com) and TGex (http://tgex.genecards.cn/). The candidate variants were validated by Sanger sequencing and its pathogenicity classified following American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines (8).

**Results**

**Genetic findings**

A total of 12 ACAN pathogenic variants was detected (Fig. 1, Table 1), thus, the overall prevalence of pathogenic ACAN variants in this cohort was 1.2% (12/1005). Among them, 10 variants were novel, including 3 nonsense and 7 frameshift variants. Parental testing using Sanger sequencing on 7 patients revealed 1 de novo variant c.1467C>G (p.Y489*); the remainder were inherited from affected parents. The ACAN mutation rate was 3.5% (8/229) among individuals with parental short stature. These variants distributed across the whole protein, including 7 located in 3 globular domains (G1, G2, G3), 2 in chondroitin sulfate attachment region, 2 in interglobular domain, and 1 in keratan sulfate attachment region (22). All cases showed a dominant inheritance pattern. 16 of 17 individuals with pathogenic variants from the 12 families (Fig. 2) exhibited short stature (<−2 SD), indicating a high penetrance.

![Schematic of the aggrecan proteoglycan and the locations of reported pathogenic variants and height SDS changes. The novel mutations identified in this study are highlighted in red, de novo variants are underlined. Abbreviations: CLD, C-type lectin domain; CRP, complement regulatory like domain; CS, chondroitin sulfate attachment domain; EGFI, 2, epidermal growth factor-like domain 1; 2; G1, globular domain 1; G2, globular domain 2; G3, globular domain 3; IGD, interglobular domain; KS, keratin sulfate attachment domain. # Early-onset osteoarthritis (OA). & Osteochondritis dissecans (OD).](image-url)
Clinical phenotypes

GH stimulation test of ACAN probands revealed that 3 had no GH deficiency (GH level > 10 ng/mL), 5 had partially GH deficiency (GH level = 5-10 ng/mL), and 2 had GH deficient (GH level <5 ng/mL; 1 had severely deficient) (23). Eleven probands had reliable bone age assessment data; among them, 6 presented with advanced bone age, whereas the rest presented with either delayed bone age (4) or equivalent to the chronological age (1)(Fig. 3A). The height SDS score (SDS) of probands ranged from −5.51 to −2.16 [average height (n = 12): −3.46], including 2 probands showing severe short stature (−4 SD) (Fig. 3B). The height SDS of affected adults ranged from −4.54 to −1.59 [average height (n = 5): −3.33]. Nine individuals underwent insulin-like growth factor 1 (IGF-1) testing; the IGF-1 levels were <−2 SD in 3, <-1 SD in 3, <0 SD in 2 individuals. One had >0 SD IGF-1 level. In addition, of the 837 patients for whom bone age data were available, 752 (89.8%, 752/837) showed delayed bone age, and 42 (5%, 42/837) presented with advanced bone age. Among them, the proportion of ACAN mutation in short stature patient with advanced bone age was 14.3% (6/42), and 35.7% (5/14) in familial short stature with advanced bone age. The phenotypes of these patients are summarized in Table 2.

Growth hormone treatment

We recorded the therapeutic effect of recombinant human GH in 2 ACAN patients. The ages of initial treatment initiation were 4 years, 10 months (case 10) and 9 years, 6 months (case 11); the treatment duration was 30 months and 19 months, respectively. They were given a recombinant human GH dose of 48 ug/kg/day. Case 10 had an improvement in height SDS of 0.23 and 1.07, respectively, in 2 years, and case 11’s SDS improvement was 0.48 and 0.13, respectively. Their yearly height velocity ranged from 7.5 to 9 cm/year (Fig. 3B).

Discussion

Human height is a highlyheritable trait that involves many genes. Previously, ACAN mutation had been reported as a cause of short stature with frequency of 1.4% to 37.5% in short stature populations (7,10,11,17). In our cohort, we demonstrate for the first time that the prevalence of ACAN mutation in Chinese short stature patients and familial subcohort is 1.2% and 3.5%, respectively. This is the largest ISS cohort examined so far for assessing the genetic causes of pediatric short stature. The samples were ascertained from multiple clinics across China and thus are

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Table 1. ACAN mutations identified in this study

| Patient | cDNA | Protein | Inherited | Type | Exon | Domain | Evidence for ACMG/AMP classification | ACMG/AMP classification | Reference |
|---------|------|---------|-----------|------|------|--------|-------------------------------------|----------------------------|-----------|
| P1      | c.560dupA | p.Leu188fs*13 | Maternal | Frameshift | 4 | G1 | PVS1+PM2 | Likely pathogenic | No |
| P2      | c.631_632insA | p.Tyr211fs | — | Frameshift | 5 | G1 | PVS1+PM2 | Likely pathogenic | No |
| P3      | c.661delT | p.Tyr221fs*10 | Maternal | Frameshift | 5 | G1 | PVS1+PM2 | Likely pathogenic | Hu X et al (10) |
| P4      | c.1117_1120delCAGA | p.Thr374* | Paternal | Nonsense | 7 | IGD | PVS1+PM2 | Likely pathogenic | Hu X et al (10) |
| P5      | c.1411C>T | p.Gln471* | Paternal | Nonsense | 7 | IGD | PVS1+PM2 | Likely pathogenic | |
| P6      | c.1467C>G | p.Tyr489* | De novo | Nonsense | 8 | G2 | PVS1+PM2 | Likely pathogenic | No |
| P7      | c.1861A>T | p.Lys621* | — | Nonsense | 10 | G2 | PVS1+PM2 | Likely pathogenic | No |
| P8      | c.1880_1883dupTGGC | p.Asp629fs | Paternal | Frameshift | 10 | G2 | PVS1+PM2 | Likely pathogenic | No |
| P9      | c.2173delG | p.Glu725fs | Maternal | Frameshift | 11 | KS | PVS1+PM2 | Likely pathogenic | |
| P10     | c.5443delC | p.Leu1815fs | — | Frameshift | 12 | CS | PVS1+PM2 | Likely pathogenic | No |
| P11     | c.5579delC | p.Gly1861fs | — | Frameshift | 12 | CS | PVS1+PM2 | Likely pathogenic | No |
| P12     | c.6861delC | p.Cys2288fs*28 | — | Frameshift | 13 | G3 | PVS1+PM2 | Likely pathogenic | No |

Abbreviations: American College of Medical Genetics and Genomics; AMP, Association for Molecular Pathology; cDNA, complementary DNA; CS, chondroitin sulfate attachment domain; IGD, interglobular domain; KS, keratin sulfate attachment domain.
representative of Chinese pediatric short stature population. We suggest that our prevalence data are more reliable than those based on small cohorts (7,10,11,17). Due to the lack of genetic segregation and functional evidence in this study, we cannot upgrade some missense variants to likely pathogenic or pathogenic (Supplementary Table 3 (19)); therefore, it is an underestimation of ACAN variant in height determination in Chinese short stature population. The ACAN mutation rate certainly should be further confirmed in even larger cohorts in the future, incorporating all lines of evidence. In addition, we identified 10 novel pathogenic variants for short stature phenotype. The high proportion of novel pathogenic ACAN variants supported the notion that the Chinese patient population is understudied and more novel pathogenic variants are yet to be uncovered, particularly from independent populations. Our findings significantly expanded the ACAN mutation spectrum.

We reviewed all ACAN pathogenic variants reported so far (Fig. 1). The majority of variants (60/81) are associated with ISS. The variants are distributed across all domains, and no domain specific phenotype was observed; some domains such as the C-type lectin domain were associated with multiple types of conditions (ISS, OD, OA, and SEMD) (Fig. 1). In addition, frameshift (n = 20), missense (n = 40), and nonsense (n = 17) mutations are the main mutation types in short stature patients; splicing (n = 3) and deletion (n = 1) mutations are relatively rare. The severity of short stature is not associated with mutation types (Supplementary Figure 1 (19)). Thus, different types of mutations across all domains of aggregcan had similar effects in causing short stature. Interestingly we noticed that the bone age characteristics of ACAN mutation carriers are significantly different across populations. While 87.5% (7/8) of the American population (4,5,9,15,24) and 62.5% (30/48) of the European population showed
advanced bone age (6,7,11,12,14,25), only 25.0% (4/16) of the Asian population showed advanced bone age by existing data (10, 13,16,26,27). In our cohort, 54.5% (6/11) of probands presented with advanced bone age. Thus, so far, among the 19 ACAN carriers found in China (including our data) (10, 16,27), 47.4% (9/19) showed advanced bone age. Subjective nature of bone age assessment and potential patient ascertainment bias could contribute to the finding regarding bone age differences in ACAN mutation carriers of different ethnicity. The true nature of the effect of ACAN mutation on bone age warrant further study.

We reviewed the responses of GH treatment of 26 (12 females, 14 male) ACAN mutation carriers, and we plotted the height SDS during the first year of treatment (6,9,11,14,27) (Supplementary Figure 2 (19)). Of these, 10 patients (5 females, 5 male) received additional treatment (1 aromatase inhibitor, 9 gonadotropin-releasing hormone antagonist). The changes in height SDS during the first year of treatment ranged from −0.5 SDS to 0.8 SDS, and the total growth height SDS change during GH treatment was −0.5 SDS to 1.6 SDS. Half of patients (13/26; 7 females, 6 male) exhibited moderate to good responses during the first year of the treatment (growth SDS > 0.35). It seems that more individuals showed poor responses after age of 10, indicating the benefit of early treatment, especially for those with advanced bone age. No significant side effects have been reported, but data for longer treatment are currently limited.

In conclusion, we reported the ACAN mutation rate in Chinese ISS patients based on a large and representative cohort and further uncovered a high proportion of novel pathogenic ACAN mutation. While no genotype-phenotype correlation is observed based all reported cases, we confirmed a relative higher rate of advanced bone age among Chinese ACAN mutation carries. The response of GH treatment should be further examined with long-term outcomes.
Table 2. Phenotype of patients with ACAN mutations

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 |
|---------|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| Birth characteristics | | | | | | | | | | | | |
| Weight (g) | 2800 | 3500 | — | — | 2400 | 3200 | 2100 | — | 3400 | 2300 | 3950 | — |
| Length (cm) | 47 | 50 | — | — | 45 | 49 | 48 | — | — | 45 | 50 | — |
| Circumference (cm) | — | — | — | — | 32 | — | — | — | — | 32 | — | — |
| First visit | | | | | | | | | | | | |
| cDNA | c.560dupA | c.631_632insA | c.661delT | c.1117_1120delCAGA | c.1411C>T | c.1467C>G | c.1861A>T | c.1880_1883dupTGGC | c.2173delG | c.5443delC | c.5579delC | c.6861delC |
| Gender | Male | Male | Male | Male | Male | Male | Female | Male | Male | Female | Female | Female |
| Age (y) | 10.8 | 11 | 12 | 15 | 5 | 8.4 | 6.5 | 4 | 10 | 4 | 9.4 | 9.3 |
| Height (cm) | 120.7 | 131 | 130 | 133.4 | 98 | 113.6 | 102.5 | 92 | 118.5 | 87 | 119.5 | 115.2 |
| Height (±SD) | -3.47 | -2.16 | -3 | -5.51 | -3.16 | -3.47 | -3.67 | -2.95 | -3.5 | -4.38 | -2.91 | -3.31 |
| Weight (kg) | 24 | 41.5 | — | 29 | 14 | — | 17.6 | 12.9 | 23 | 11 | 22.2 | 19.5 |
| IGF-1 level (ng/mL) | — | 154 | — | 139 | 150 | — | 9.34 | 2.92 | 23.23 | 10.3 | 5.91 | 7.2 |
| GH peak (ng/mL) | — | 0.09 | 5.96 | 5.94 | 11.25 | — | 9.34 | 2.92 | 23.23 | 10.3 | 5.91 | 7.2 |
| Pituitary height | Normal | Normal | Normal | Normal | — | — | Normal | Normal | Normal | — | Normal | — |
| Skeletal system | | | | | | | | | | | | |
| Chronologic age (y) | 12.2 | 12 | — | 15 | 7 | 8.4 | 8 | 4 | 10 | 13 | 9.9 | 9.3 |
| Bone age (Greulich/Pyle) | 11.5 | 12.5 | — | 12.5 | 7 | 9 | 11 | 6 | 11.5 | 12.5 | 10.7 | 6.5 |
| RUS Bone age (TW-C) | 11.2 | 12.5 | — | 12.2 | 7 | 8.8 | 10.2 | 5.8 | 11 | 12.8 | 10.5 | 6.8 |
| Physical examination | | | | | | | | | | | | |
| Age (y) | 14 | 14 | — | — | 10.3 | 12 | 8.8 | — | — | — | — | 13.6 |
| Circumference (cm) | 54 | 58 | — | — | — | 51 | — | — | — | — | — | 52 |
| Sit height/height (cm) | 76/149 | 79/153 | — | — | 78.6/129 | 72/135 | 67.4/120.3 | — | — | — | — | 76/140 |
| Arm span/height (cm) | 137/149 | 158/153 | — | — | 124/129 | 131/135 | — | — | — | — | 135/140 |
| Parental height (cm) | | | | | | | | | | | | |
| Father | 170 | 173 | — | 145 | 163 | 150 | 166 | 150 | 171 | 176 | 160 | 160 |
| Mother | 140 | 159 | — | 161 | Normal | 158 | 142 | 157 | 141 | 153 | 153 | 150 |

Abbreviation: cDNA, complementary DNA; TW-C, Tanner-Whitehouse-Chinese.
Acknowledgments

We are grateful to the all the patients, their families, and clinical research coordinators of short stature cohort for their participation in this study.

Financial Support: The “Eastern Scholar” Fund; the “Guangxi Bagui Scholar” fund; the National Science Foundation of China (Grant No.81873633); National key research and development program (2018YFC1002501), the Major Research Plan of the Provincial Science and Technology Foundation of Guangxi (Grant No.AB16380214), the Health Department of Guangxi Province (Z20190692), National Key Research and Development Program of China (SQ2018YFC100101), Medical and Health Appropriate Technology Development and Application Project of Guangxi (S2020060).

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Disclosures: The authors report no conflicts of interest.

Data Availability: The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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