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RESEARCH ARTICLE

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# Downregulation of ACAN is Associated with the Growth hormone pathway and Induces short stature

**Huiping Wu | Chaoban Wang | Shiwen Yu | Xiaojun Ye | Yalan Jiang | Pingping He | Xiaoou Shan**

Department of Pediatric Endocrine, Yuying Children's Hospital, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

**Correspondence**
Xiaoou Shan. Department of Pediatric Endocrine, Yuying Children's Hospital, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, China.
Email: seagullshan@foxmail.com

## Abstract

**Background:** ACAN heterozygous mutations can cause short stature in patients with or without advanced bone age and have recently attracted researchers' attention. Growth hormone can be used to treat short stature induced by ACAN mutations; however, few studies have focused on the underlying mechanism of this treatment.

**Methods:** Four patients with new mutations were reported based on clinical data and genetic tests. We investigated the expression and Gene Ontology biological process enrichment of ACAN and GH pathways based on GTEx databases through bioinformatics analyses. The effect of ACAN on the growth hormone response evaluated in ATDC5 cells with a growth hormone stimulation test.

**Results:** Four mutations were reported in this study: c.619C > A, c.1967A > G, c.1888G > A, and c.1308_1309del. All patients' heights were under −2.5 SD, with one had advanced bone age, and two had GH deficiency.

Two individuals received growth hormone therapy acquired variable levels of height SD score improvement. ACAN and the GH pathway were strongly associated; ACAN does not affect GHR but regulates the response to GH. Downregulating ACAN inhibited ATDC5 cell proliferation induced by GH.

**Conclusion:** ACAN is associated with the GH pathway, revealing the potential mechanism underlying GH-targeted treatment for ACAN mutation-induced short stature. GH-promoting therapies may increase patients' heights.

KEYWORDS
ACAN, growth hormone, short stature, treatment

## 1 | INTRODUCTION

Height depends on the longitudinal growth of cartilage in the long bones, also known as the growth plate. Multiple hormones and signaling compounds regulate the high metabolic activity in the growth plate.[1] There are several phases in chondrocyte development during longitudinal growth: the resting, proliferative, prehypertrophic, hypertrophic, and terminal phases.[2]

Growth hormone (GH) is critical for promoting height. GH binds to growth hormone receptors (GHRs), activates Janus kinase 2

Huiping Wu and Chaoban Wang contributed equally to this study.

(JAK2) and signal transducer and activator of transcription (STATs), and induces changes in gene expression.[3] During bone growth, GH promotes chondrocyte proliferation and stimulates IGF-I local production, which increases chondrogenesis.[4] With age, chondrocyte proliferation decreases, slowing longitudinal bone growth.

Somatotropic axis activity regulation is associated with the feedback between hypothalamic neurohormones and somatostatin, GH, and insulin-like growth factor 1 (IGF-1), and the hypothalamic–pituitary–liver axis is involved in this process.[5]

Aggrecan, encoded by the ACAN gene, is synthesized by chondrocytes and participates in constructing the extracellular matrix.[6] Heterozygous mutations in ACAN have been reported to cause idiopathic short stature and bone age acceleration, with or without GH deficiency7. Patients with ACAN mutations are usually diagnosed by genetic screening and are treated with growth hormone (GH).[6,7]

However, few studies explain the association between ACAN and the GH pathway. In this study, we reported four new mutations in ACAN patients and treatment outcomes for 2 patients and summarize the effects of GH treatment on individuals with ACAN mutations reported in the literature. Then, we analyzed the characteristics of ACAN in the hypothalamic–pituitary–liver axis based on the GTEx database through bioinformatics analyses. Finally, we downregulated the expression of ACAN in ATDC5 cells to study the mechanism of the GH pathway.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients undergoing ACAN mutation screening procedures

General test: A total of 35 children with heights lower than −2.5 SD with and without advanced bone age were enrolled from January 2021 to June 2022 in the outpatient and inpatient departments of the pediatric endocrinology department at Yuying Children's Hospital, The Second Affiliated Hospital of Wenzhou Medical University. Several tests were conducted to assess the characteristics and causes of short stature, including growth hormone (GH) stimulation testing with clonidine, L-Dopa or arginine, serum GH level testing, routine blood analyses, full biochemical blood evaluations, blood gas analyses, thyroid function testing, hormone evaluations (pituitary, adrenal, and sex hormones), IGF 1 and IGF - BP3 level testing, peripheral blood chromosome karyotyping, pituitary MR imaging, adrenal b-ultrasound, uterine and ovarian B-ultrasound (girls), testicular B-ultrasound, and the Bone Age Measurement System of Greulich and Pyle Atlas (GP test).

Diagnostic cutoffs: Patients with a GH peak <5 ng/ml were diagnosed with severe growth hormone deficiency (SGHD) and those with a GH peak between 5 ng/ml (including) and 10 ng/ml (excluding) were diagnosed with partial growth hormone deficiency (PGHD).[8]

Gene identification: With the informed consent of the patients' families, next-generation sequencing (NGS) was performed using HiSeq2000 (Illumina). Rare variants were evaluated and classified following the ACMG/AMP standards and guidelines. Then, ACAN candidate variants were determined by targeted Sanger sequencing, including the parents, and exclusion of growth hormone deficiencies (such as pituitary dysplasia and growth hormone receptor defects), malnutrition, constitutional youth retardation, tumor, chronic wasting disease, and diseases such as chromosome aberration. Clinical information included clinical characteristics, birth weight and length, family stature history, biochemical and radiological materials, and treatment history. All affected patients' heights were evaluated according to the "Height and weight standardized growth charts for Chinese children and adolescents aged 0~18 years." In this study, we found 4 patients with ACAN mutations.

The research project was submitted to the Wenzhou Science and Technology Bureau in advance and passed the ethical review of the hospital (project number: Y2020464, ethical review number: LCKY2020-367-01).

### 2.2 | Bioinformatics analyses

Data source: ACAN mRNA expression data for multiple tissues were obtained from NCBI (www.ncbi.nlm.nih.gov/) and HPA (http://www.proteinatlas.org/) databases. GTEx data were downloaded from the UCSC Xena database (http://xena.ucsc.edu/).

Data processing: After downloading the GTEx data, the expression matrices for the hypothalamus, pituitary, and liver were obtained using R language. The R code is shown in Appendix S1.

Co-correlation analysis: The correlation between ACAN and other genes was calculated using R language. The R code is shown in Appendix S1.

GO (BP) enrichment analysis: A gene set with a corrected $p$ Value <0.001 was selected to conduct the GO (BP) enrichment analysis in Metascape (http://metascape.org/).[9]

GSVA: GSVA was performed to show the quantitative pathway analysis of each sample, and the pathways associated with growth were selected to analyze correlations with the ACAN gene using R language. The R code is shown in Appendix S1.

### 2.3 | Cell culture

ATDC5 cells were cultured in DMEM with 100 mg/ml streptomycin, 100 U/ml penicillin, and 10% fetal bovine serum. After cell seeding overnight, the medium was changed to Opti-DMEM with ACAN siRNA or the negative control for 24 h; subsequently, the medium was changed to DMEM or DMEM containing growth hormone for 24 h.

ACAN silencing: siRNA was used to downregulate the expression of ACAN. Sequence: UUCGAUAGUCCUGUCAUUCTT (siACAN-as); GAAUGACAGGACUAUCGAATT (siACAN-ss). RNAIMAX (13,778,150, Thermo Fisher) was used for this process, with all steps performed according to the instructions.

TABLE 1 Characteristics of patients with ACAN mutation.

| Patient | P1 | P2 | p3 | p4 |
|---|---|---|---|---|
| Gene mutation characteristics | | | | |
| cDNA | c.619C > A | c.1967A > G | c.1888G > A | c.1308_1309del |
| Protein | p.Q207K | p.Y656C | p.G630S | p.Gly437Argfs*22 |
| Inherited | Paternal | Paternal | Paternal | Maternal |
| Exon | 4 | 10 | 10 | Frameshift mutation |
| ACMG/AMP classification | Uncertain | Uncertain | Uncertain | Likely pathogenic |
| Birth characteristics and growth history | | | | |
| Gestational age (week) | 40 | 39 | 40 | 39 |
| Birth weight (SDS) | −1.895 | −0.56 | −3.26 | −0.98 |
| Birth length (SDS) | −0.47 | 0.07 | −3.41 | 0.076 |
| GV (cm/y) | 2.6 | 3.1 | 4.3 | 3.6 |
| Primary diagnosis Characteristics | | | | |
| Gender | Male | Female | Male | Female |
| Age (year,month) | 8y,7m | 5y,1m | 5y,8m | 4y,7m |
| Height (cm) | 102 | 95.2 | 102.4 | 96 |
| Height (SD) | −5.6 | −3.6 | −3.0 | −2.8 |
| Weight (kg) | 20 | 12.7 | 16.5 | 18 |
| IGF-1 level (ng/ml)[SD] | - | 96.8 [−0.907SDS] | 63.4 [−1.22SDS] | 132 [0.14SDS] |
| GH peak (ng/ml) | 13.1 | 10.06 | 2.58 | 8.72 |
| Pituitary | Normal | Normal | Normal | Normal |
| Bone age | 5.5 | 5 | 5.5 | 5.5 |
| Parental height (cm) | | | | |
| Father | 158 | 167 | 170 | 175 |
| Mother | 155 | 150 | 157 | 146 |

### 2.4 | RNA isolation and qPCR

After collecting ATDC5 cells from 6-well plates, total RNA was extracted using the TRIzol protocol (Invitrogen). Three to five micrograms of total RNA was reverse transcribed into cDNA using HiScript III-RT SuperMix for qPCR (+gDNA wiper) (Vazyme), and ChamQ Universal SYBR qPCR Master Mix (Vazyme) was used for qPCR. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative fold change in mRNA expression. Primer sequences for qPCR are provided in Table S1.

### 2.5 | Immunofluorescence

After the cells were cultured in an 8-well chamber slide, EdU was added to the medium before the endpoint time of a single test for 1 h. An EdU Cell Proliferation Kit (Sangon Biotech) was used to test the cell proliferation rate. The proliferation rate was calculated by counting the number of EdU marker cells and Dapi marker cells in each group.

### 2.6 | Statistical analysis

Associations between ACAN and other genes were calculated using the Pearson method in R. The statistical results of the enrichment analysis were obtained from Metascape. All laboratory data were analyzed using a *t* test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

## 3 | RESULTS

### 3.1 | Characteristics of patients with ACAN mutations

We identified four novel mutations in ACAN: c.619C > A, c.1967A > G, c.1888G > A, and c.1308_1309del. The cDNA and protein mutation site details, inheritance mechanisms, exon sites, and ACMG/AMP classifications are shown in Table 1. Patients included 2 males and 2 females, with an age range of 5.1–13.2 years. Patients with ACAN mutations also showed low birth length, with a range of 45–50 cm [−3.43 SDS ~ 0.07 SDS], and birth weight, with a range of 2.24–3.0 kg [−3.26 SDS ~ -0.56 SDS]. The range of the height SDS and weight SDS at the first visit was −5.6 SDS to −2.8 SDS and − 3 SDS to M (Table 1). All affected patients showed no significant dysmorphic features or skeletal abnormities and demonstrated normal intelligence and motor development.

The genetic family tree, protein structure prediction, and radiographs of bone age of all patients are shown in Figure 1. Three of these patients' bone ages were approximately matched (P1-3), but P4 showed significantly advanced bone age (Table 1).

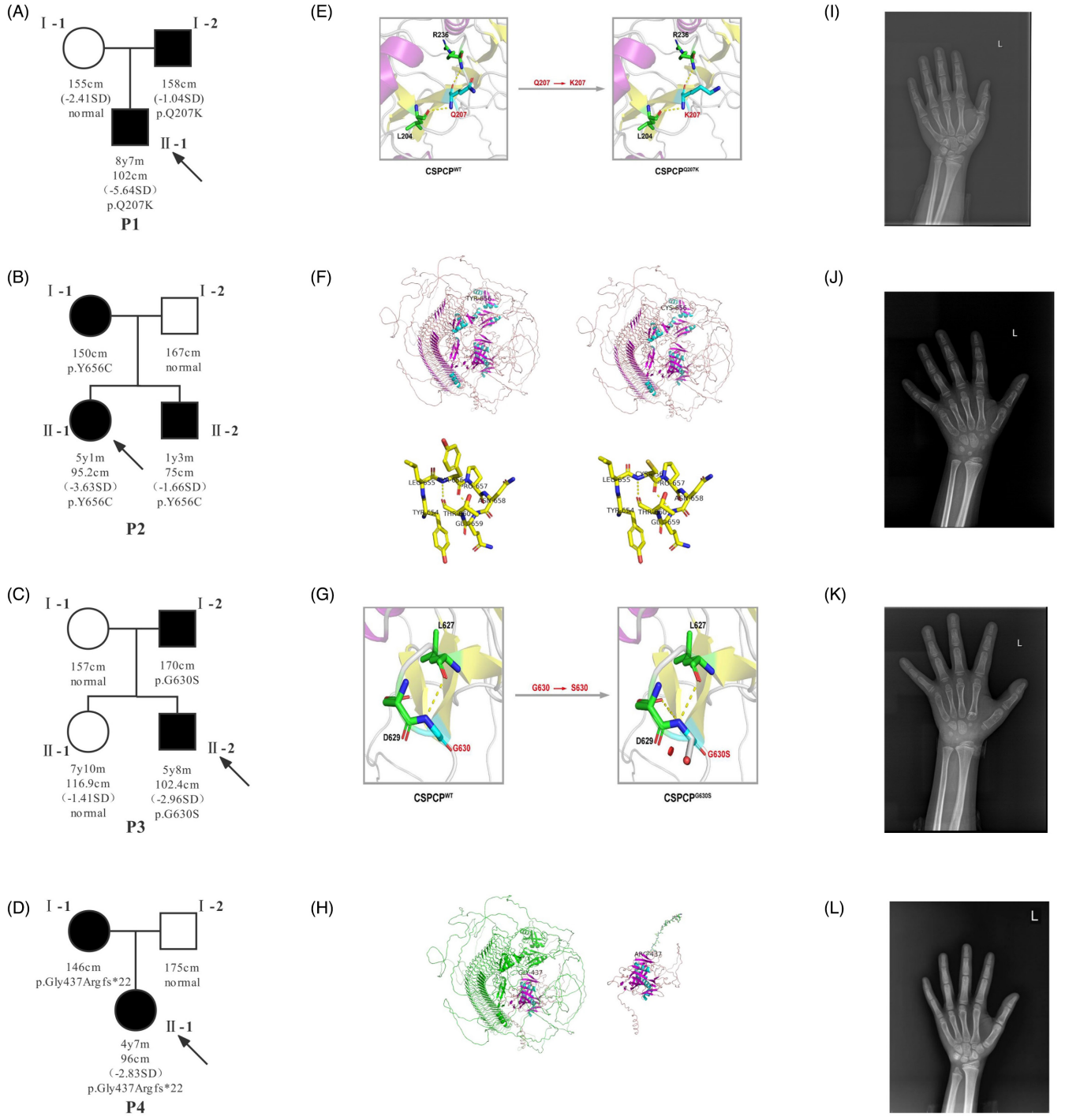



**FIGURE 1** Genetic family tree, protein structure prediction, and radiographs of the bone age of all patients. Genetic family tree: A–D; protein structure prediction: E–H; radiographs of bone age: I–L.

In addition, all affected patients were diagnosed with SGHD or PGHD by twice growth hormone stimulation tests. P4 had PGHD (GH level = 8.72 ng/ml), and P3 had SGHD (GH level = 2.58 ng/ml). Two patients' (P2 and P3) had IGF-1 levels around -1SD; P4 had levels higher than 0 SD (Table 1). Unfortunately, no IGF-1 data were collected before and after treatment for P1.

We investigated the response of two ACAN variants to GH treatment. The major characteristics of children who received GH treatment are shown in Table 2. The treatment duration was 6 years and 4 years, P1 had an improvement in height SDS of 2.4, with height increase rate ranged from 2–3 to 5–6 cm/year. Before presenting at our hospital, P1 had received GH treatment for 4.5 years. When presented at our hospital, he was nearly 13 years old, with a pubertal stage of G3/PH2, testicular volume of 12 ml, and bone age of 13 years. We supplemented G therapy with GnRHa to inhibit development, but this treatment was only administered for 1 year

**TABLE 2** Growth hormone treatment follow-up.

| | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| Therapeutic Regimen | rhGH+GnRHa | - | - | rhGH |
| Start Age (y.o.) | 8.7 | - | - | 4.6 |
| Treatment Duration (years) | 5.83 | - | - | 4 |
| Height SDS after Treatment | −3.2SDS | - | - | −1.3SDS |
| Change in Height SDS | 2.4SDS | - | - | 1.5SDS |
| IGF-1 (ng/ml) [SDS] | | - | - | 276 ng/ml [1.208SDS] |

for financial reasons. P4 received GH therapy when she was diagnosed with PGHD at 4 years and 7 months of age with a bone age of 5.5 years. It is important to note that although the peak growth hormone of P4 was 8.72 ng/ml and her IGF-1 levels were not low, her bone age was advanced; these findings are atypical of true growth hormone deficiency. However, after the growth hormone treatment, her height SDS was significantly improved. The patient demonstrated an improvement in height SDS of 1.5 SD, and the height increase ranged from 4–5 to 8–10 cm/year. IGF-1 SDS also increased significantly, indicating a positive response to treatment.

In addition to reporting on our current cases, we reviewed the other available studies on children with an ACAN variant, which involved 20 patients and 8 out of patients received treatment with GH, and data are shown in Table 3.[6,10,11] the overall yearly height change during GH treatment was −0.01 SDS to 1.3 SDS. Among these children, there was a general trend of a gradual reduction in yearly height SDS growth over the course of GH treatment.

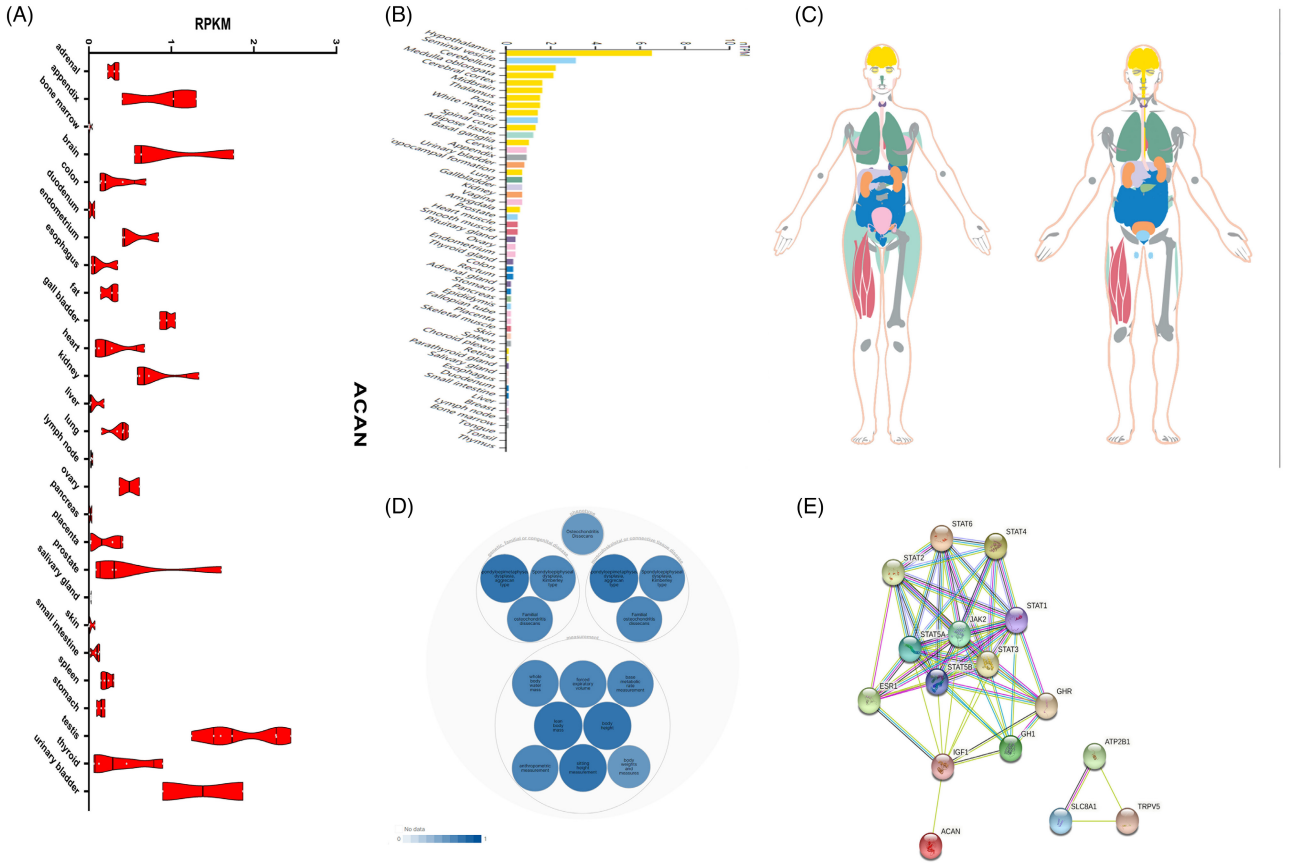
These results indicate that ACAN mutations result in different patient phenotypes and responses to GH treatment. Even in patients without GH deficiency, GH treatment plays a significant role in height improvement.

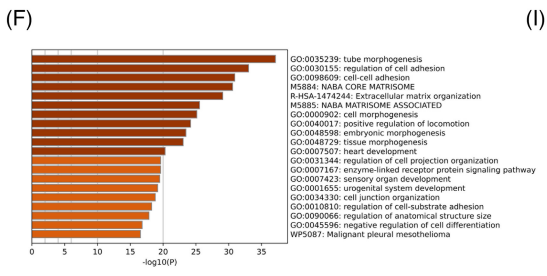
## 3.2 | The characteristics and pathways associated with ACAN expression in multiple tissues

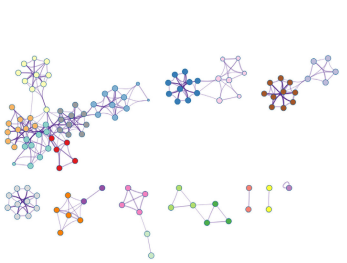
First, we analyzed the expression levels of ACAN mRNA in multiple tissues using NCBI-gene and HPA databases. The results showed that ACAN mRNA is widely expressed in various tissues and is highly expressed in the brain and testes, while the HPA data revealed that ACAN expression levels are highest in the hypothalamus (Figure 2A–C). Because patients with ACAN mutations often present with short stature and advanced bone age, we analyzed the disease association between ACAN levels and the GH and estrogen pathways by PPI analysis. The results demonstrated that ACAN is highly correlated with musculoskeletal and genetic diseases, consistent with the clinical features of those diseases (Figure 2D). ACAN was found to be associated with IGF1 in the PPI analysis (Figure 2E). These results

**TABLE 3** Response to GH treatment in ACAN patients reported in the previous literature

| PMID | References | ACAN mutation | patients | treatment | treatment duration (month) | height SDS before treatment (SDS) | height SDS after treatment | height SDS changes |
|---|---|---|---|---|---|---|---|---|
| 29,769,040 | Dandan Xu, et al 2018 | c.7465T>C (p.Gln2364Pro) | P1 | GH+GnRha | 96 | −2.09 | −1.07 | 1.02 |
| | | c.7465T>C (p.Gln2364Pro) | P2 | GH+GnRha | 96 | −0.88 | −0.31 | 0.57 |
| | | c.7465T>C (p.Gln2364Pro) | P3 | GH | 96 | −3.74 | −3.75 | −0.01 |
| 32,658,585 | Liang H, et al 2020 | c.1817 C>A | p2 | GH | 30 | −2.88 | −1.91 | 0.97 |
| | | c.2266 G>C | p4 | GH | 11 | −2.92 | −2.16 | 0.76 |
| | | c.1733G>A | p5 | GH+GnRha | 2 | −4.53 | −4.37 | 0.16 |
| 33,606,014 | Lin L, et al 2021 | c.5443delC(p.Leu1815fs) | p10 | GH | 30 | −4.38 | −3.08 | 1.3 |
| | | c.5579delC(p.Gly1861fs) | p11 | GH | 19 | −2.91 | −2.3 | 0.61 |


(A)
RPKM
adrenal
appendix
bone marrow
brain
colon
duodenum
endometrium
esophagus
fat
gall bladder
heart
kidney
liver
lung
lymph node
ovary
pancreas
placenta
prostate
salivary gland
skin
small intestine
spleen
stomach
testis
thyroid
urinary bladder
(B)
ACAN
(C)
(D)
(E)
STAT6
STAT4
STAT2
STAT1
JAK2
STAT5A
STAT3
STAT5B
ESR1
GHR
GH1
IGF1
ACAN
ATP2B1
SLC8A1
TRPV5


(F)
GO:0035239: tube morphogenesis
GO:0030155: regulation of cell adhesion
GO:0098609: cell-cell adhesion
M5884: NABA CORE MATRISOME
R-HSA-1474244: Extracellular matrix organization
M5885: NABA MATRISOME ASSOCIATED
GO:0000902: cell morphogenesis
GO:0040017: positive regulation of locomotion
GO:0048598: embryonic morphogenesis
GO:0048729: tissue morphogenesis
GO:0007507: heart development
GO:0031344: regulation of cell projection organization
GO:0007167: enzyme-linked receptor protein signaling pathway
GO:0007423: sensory organ development
GO:0001655: urogenital system development
GO:0034330: cell junction organization
GO:0010810: regulation of cell-substrate adhesion
GO:0090066: regulation of anatomical structure size
GO:0045596: negative regulation of cell differentiation
WP5087: Malignant pleural mesothelioma
-log10(P)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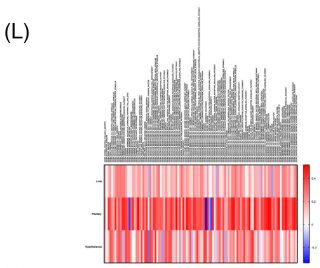
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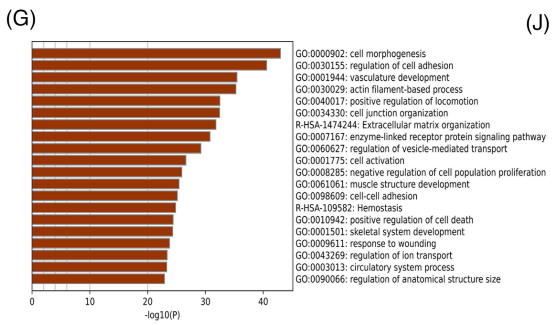
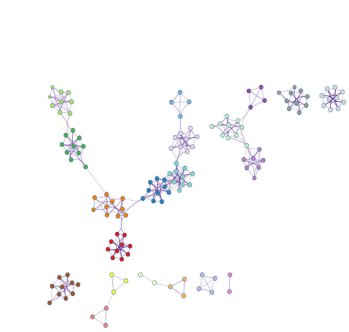

(G)
GO:0000902: cell morphogenesis
GO:0030155: regulation of cell adhesion
GO:0001944: vasculature development
GO:0030029: actin filament-based process
GO:0040017: positive regulation of locomotion
GO:0034330: cell junction organization
R-HSA-1474244: Extracellular matrix organization
GO:0007167: enzyme-linked receptor protein signaling pathway
GO:0060627: regulation of vesicle-mediated transport
GO:0001775: cell activation
GO:0008285: negative regulation of cell population proliferation
GO:0061061: muscle structure development
GO:0098609: cell-cell adhesion
R-HSA-109582: Hemostasis
GO:0010942: positive regulation of cell death
GO:0001501: skeletal system development
GO:0009611: response to wounding
GO:0043269: regulation of ion transport
GO:0003013: circulatory system process
GO:0090066: regulation of anatomical structure size
-log10(P)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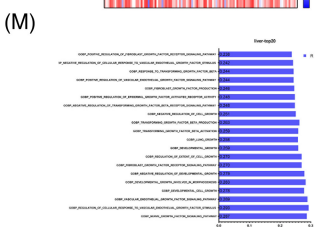
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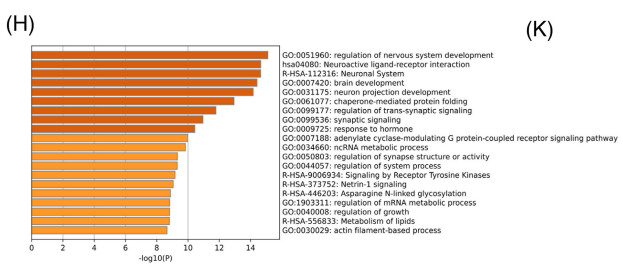
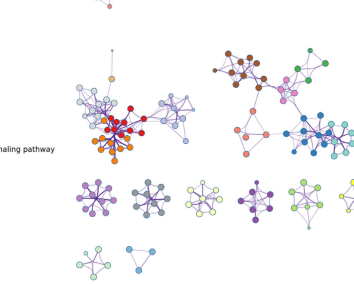

(H)
GO:0051960: regulation of nervous system development
hsa04080: Neuroactive ligand-receptor interaction
R-HSA-112316: Neuronal System
GO:0007420: brain development
GO:0031175: neuron projection development
GO:0061077: chaperone-mediated protein folding
GO:0099177: regulation of trans-synaptic signaling
GO:0099536: synaptic signaling
GO:0009725: response to hormone
GO:0007188: adenylate cyclase-modulating G protein-coupled receptor signaling pathway
GO:0034660: ncRNA metabolic process
GO:0050803: regulation of synapse structure or activity
GO:0044057: regulation of system process
R-HSA-9006934: Signaling by Receptor Tyrosine Kinases
R-HSA-373752: Netrin-1 signaling
R-HSA-446203: Asparagine N-linked glycosylation
GO:1903311: regulation of mRNA metabolic process
GO:0040008: regulation of growth
R-HSA-556833: Metabolism of lipids
GO:0030029: actin filament-based process
-log10(P)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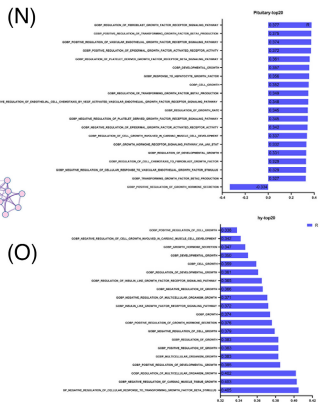
(N)
(O)

**FIGURE 2** Bioinformatics analysis of ACAN in multiple tissues. (A). ACAN mRNA expression levels in multiple tissues based on NCBI gene data. (B, C). ACAN mRNA expression levels in multiple tissues based on the HPA database. (D) ACAN-associated disease. (E) PPI analysis of ACAN and growth pathway proteins. (F–K) Enrichment of genes associated with ACAN identified through Metascape in the hypothalamus–pituitary–liver axis. (L–O) GSVA of the growth pathway associated with ACAN in the hypothalamus–pituitary–liver axis.

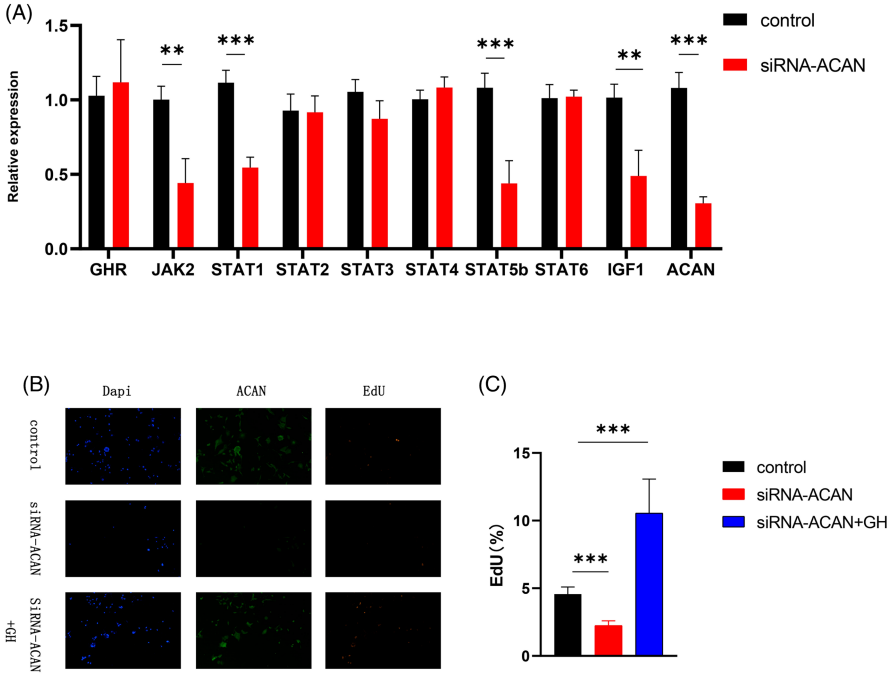


**FIGURE 3** Downregulation of ACAN in ATDC5 cells inhibits the GH pathway and cell proliferation. (A). mRNA expression of the GH pathway under the control and downregulation of ACAN. (B). EdU marks ATDC5 proliferation under control, siRNA-ACAN and siRNA-ACAN conditions with GH, DAPI (blue), ACAN (green), and EdU (red). (C). The proliferation rate of ATDC5 cells at 1 h with control siRNA, siRNA-ACAN and siRNA-ACAN with GH.

suggest that ACAN may be related to the GH pathway or the GH axis.

Subsequently, we analyzed the genes associated with ACAN in the hypothalamus (2F, I), pituitary (2G, J), and liver (Figure 2H,K). The association analysis between ACAN and the growth pathway (GSVA) revealed that ACAN might also affect BP in the hypothalamus–pituitary–liver axis (Figure 2L–O). Overall, the results of the bioinformatics analyses suggest that ACAN is potentially related to the growth hormone signaling pathway and that it plays a role in the GH axis.

### 3.3 | Downregulation of ACAN in ATDC5 cells inhibits the GH pathway and cell proliferation

The ACAN sequence is highly repetitive ( Appendix S2); thus, it is not possible to sequence the whole gene. We used siRNA to silence the ACAN gene in ATDC5 cells to study the effect of ACAN on bone and evaluated the expression levels of GHR, JAK2, STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6), and IGF1. The results showed no differences in GHR and STAT2/3/4/6 after silencing ACAN at the mRNA level in ATDC5 cells, but JAK2, STAT1, STAT5b, and IGF1 were significantly downregulated, and we could not detect stat5a expression (Figure 3A). This finding suggests that patients with ACAN mutations may have a relative deficiency of GH. Furthermore, we determined the effect of ACAN silencing on GH reactivity, and the results showed that downregulation of ACAN inhibited the proliferation of ATDC5 cells, while GH reversed this effect (Figure 3B,C). These results show that ACAN may affect chondrocyte proliferation, regulating height development.

## 4 | DISCUSSION

In this study, we reported four new mutations in ACAN-induced short stature. Patients with ACAN mutation show different phenotypes, with GH deficiency, advanced bone age, or short stature alone, consistent with previous research. Patient phenotypes vary even among those with the same mutation; additionally, the parent phenotypes vary. Thus, we wondered whether the negative effects of the mutation or the relative deficiency resulting from the ACAN mutation were associated with the patients' short stature.

In subsequent studies, we found a high degree of repeatability within the ACAN gene sequence; thus, it is impossible to measure the complete sequence of the ACAN gene based on existing sequencing technology. This finding provided answers as to why (1) mutations cannot be detected in some patients who have mutations in the repetitive sequence region, and (2) there is limited literature available; as it is not possible to accurately sequence the full ACAN sequence, the reliability of ACAN mutation studies is reduced.

Because of the difficulty in sequencing ACAN and the different phenotypes that arise among patients with the same mutation, we

focused on the relative inadequacy of ACAN expression. First, we analyzed ACAN mRNA expression in multiple tissues based on the NCBI-gene and HPA databases, revealing that ACAN mRNA is expressed in several tissues, including high ACAN mRNA expression levels in the hypothalamus. Thus, we further analyzed the association between ACAN expression and the hypothalamus–pituitary–liver axis using the GTEx database. The enrichment analysis based on genes associated with ACAN revealed that ACAN mRNA expression might influence the biological function of the hypothalamus–pituitary–liver axis. We subsequently analyzed the relationship between ACAN and growth by GSVA. The GSVA results also showed a high association between ACAN and the hypothalamus–pituitary–liver axis, especially in the pituitary gland. These results indicate that the ACAN mRNA expression levels could affect growth hormone-related biological processes.

More importantly, we silenced ACAN expression in ATDC5 cells and studied the potential influence of ACAN on bone. Downregulating ACAN did not affect GHR but inhibited JAK2, STAT1, STAT5b, and IGF1.

ACAN downregulation also inhibited the proliferation of ATDC5 cells. These results indicate that insufficient ACAN expression inhibits bone growth and diminishes height growth potency.

GH treatment in 2 ACAN patients demonstrated a trend of improved height SDS. Although the number of cases was small, these results indicate GH can effectively treat ACAN-induced short stature, whether these affected individuals need to use rhGH with/without GnRHa to improve their final adult heights remains controversial.[12] According to our statistical results, we hold the view that growth promoting, and formal treatment can promote affected individuals' heights significantly by using rhGH and/or GnRHa. However, a larger sample size is required to prove this observation.[11]

This study has some limitations. Due to the limited number of patients, it is difficult to evaluate the effect of treatment. Based on research on ATDC5, the results may vary in humans. In particular, analyzing differences in expression levels of ACAN can help determine the reason why patients with the same mutation demonstrate different phenotypes, ACAN expression levels cannot be measured directly in patients. Direct studies of mutations may be more revealing, but sequencing techniques for highly repetitive genes limit this approach. The subsequent development of new techniques may address this issue.

Overall, we reported four new mutations in ACAN, and this study represents the first attempt to explain the mechanism underlying ACAN-induced short stature, thus contributing vital knowledge regarding this disease, and providing a basic theory for the mechanisms underlying the efficacy of GH treatment for ACAN mutation-induced short stature. Our findings indicate that GH-targeted therapies may be beneficial for ACAN mutation patients, increasing their height. However, whether there is a difference in response to growth hormone treatment among patients with ACAN mutations with or without growth hormone deficiency remains to be determined. More data on GH therapy in patients with these mutations are required.

## AUTHOR CONTRIBUTIONS

Huiping Wu and Chaoban Wang contributed to conception and design, collection and assembly of data, data analysis, and interpretation. Shiwen Yu, Xiaojun Ye, Pingping He and Yalan Jiang contributed to collection and assembly of data, data analysis, and interpretation; Xiaoou Shan contributed to conception and design and administrative support. All authors contributed to article writing and final approval of the article.

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Not applicable.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

*Xiaoou Shan* https://orcid.org/0000-0003-2768-7562

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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