HHS Public Access

Author manuscript

J Pediatr Endocrinol Metab. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

J Pediatr Endocrinol Metab. 2015 July 1; 28(0): 927–932. doi:10.1515/jpem-2014-0450.

Idiopathic Short Stature due to Novel Heterozygous Mutation of the Aggrecan Gene

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Abstract

Background—Recently, whole exome sequencing identified heterozygous defects in the Aggrecan gene (ACAN) in three families with short stature and advanced bone age.

Objective—We report a novel frameshift mutation in ACAN in a family with dominantly inherited short stature, advanced bone age, and premature growth cessation. This is the first case of targeted sequencing of ACAN in this phenotype and confirms that ACAN sequencing is warranted in patients with this rare constellation of findings.

Results—We present a 5 1/2 year old male with a family history of short stature in 3 generations. The maternal grandfather stands 144.5 cm (Ht SDS -4.7), mother 147.7 cm (Ht SDS -2.6), and index case 99.2 cm (Ht SDS -2.7). Our prepubertal patient has significant bone age advancement (bone age 8 years at chronologic age 5 1/2 years) resulting in a poor predicted adult height of 142 cm (Ht SDS -5.1). DNA sequencing identified a novel heterozygous variant in *ACAN*, which encodes aggrecan, a proteoglycan in the extracellular matrix of growth plate and other cartilaginous tissues. The mutation (p.Gly1797Glyfs*52) results in premature truncation and presumed loss of protein function.

Conclusion—Mutations in aggrecan gene should be included in the differential diagnosis of the child with idiopathic short stature or familial short stature and bone age advancement.

Keywords

Short stature; bone age advancement; aggrecan; mutation

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Financial disclosure: None

Conflict of Interest: None

Contributors' Statement: Jose Bernardo Quintos: Dr. Quintos drafted the initial manuscript with further revisions, approved the final manuscript and was involved in the direct care of the patient described.

Michael H. Guo: Mr. Guo performed the genetic analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Andrew Dauber: Dr. Dauber conceptualized, reviewed, revised, and approved the final manuscript for submission.

Introduction

The initial evaluation of the child with short stature involves screening for pathological causes of poor growth and routine assessment of bone age for correlation with chronologic age and height age.[1] Radiography of the left hand or bone age x-ray generally narrows the differential diagnosis of short stature usually into two broad categories: 1) short stature with significant bone age delay (CA>BA) which can be due to endocrine causes (growth hormone deficiency, hypothyroidism, Cushing syndrome) or constitutional delay in growth and puberty, and 2) short stature with minimal bone age delay or bone age equal to chronologic age which can be due to familial short statute, genetic or syndromic causes e.g. Turner syndrome or Noonan syndrome, and idiopathic short stature (ISS).

ISS is a heterogeneous condition defined as height that is at least two standard deviation scores (SDS) below the mean for age, sex and population without evidence of systemic, endocrine, nutritional or genetic/chromosomal abnormalities. [2] ISS is a diagnosis of exclusion and includes many etiologies such as familial short stature and constitutional delay in growth and puberty.

Recently, genetic causes of ISS including mutations and deletions in the short stature homeobox gene (*SHOX*), heterozygous mutations in the natriuretic peptide receptor-2 (*NPR2*) gene, protein-tyrosine phosphatase, nonreceptor type 11 (*PTPN11*), and mutations in the aggrecan gene have been identified. [3-5]

Aggrecan is a protein encoded by the *ACAN* gene. The encoded protein is an important part of the extracellular matrix. [6, 7] Recently, we reported 3 families with autosomal dominant short stature, bone age advancement, and premature growth cessation due to aggrecan mutations. [4] Here, we report three individuals from a family with idiopathic/familial short stature marked by accelerated bone age maturation and premature growth cessation due to a novel heterozygous variant in *ACAN*. Our current family is the first family to have targeted *ACAN* sequencing performed in the setting of dominant short stature with advanced bone age.

Case Report

The index case is a 5 1/2 year old Asian/African American male with proportionate short stature and bone age advancement. He was born full term with birth weight of 3.6 kg and birth length of 50 cm. He was initially evaluated in our outpatient endocrine clinic at 2 ½ years for short stature. His height was 82.9 cm (below the 3rd percentile, Ht SDS -3.2), weight 11.6 kg (6th percentile), head circumference 49 cm (50th percentile), and BMI 16.8 kg/m^2 (69th percentile).

He was noted to have proportionate short stature, mid-face hypoplasia, flat nasal bridge, normal 4th metacarpals, prepubertal genitalia and no evidence of gynecomastia. He had normal screening laboratory examination including complete blood count, sedimentation rate, electrolytes, tissue transglutaminase IgA Antibody, total IgA, and TSH. Growth hormone markers were normal: Insulin- like growth factor 1 (IGF1) level was 128 ng/ml

(17-248) and IGF binding protein 3 (IGFBP3) was 3.1 mg/L (0.8-3.9). Skeletal survey showed no evidence of skeletal dysplasia.

At 5 ½ years of age his bone age was 8 years and height was 99 cm (height SDS -2.5). His physical examination was unchanged and he remained prepubertal (Fig 1). His predicted adult height using Bayley-Pinneau method was 148 cm (- 5 Ht SDS). Review of the skeletal survey done at 3 years of age showed that his bone age was advanced to 5 years.

To elucidate the cause of his bone age advancement, hormonal work up performed by mass spectrometry at 5 ½ years were normal: total serum testosterone <2.5 ng/dl (2.5-10), DHEA-Sulfate 24 ug/dL (<57), 17-alpha-hydroxyprogesterone 13 ng/dl (<91), and estradiol <1 pg/ml (<15). Repeat IGF1 137 ng/ml (30-174) and IGFBP3 2.9 mg/L (1.5-3.4) were normal. Growth velocity was 6 cm/year. (Fig 2)

His mother stands 147.7 cm (- 2.6 Ht SDS). She had menarche at 12 years of age but reported growth cessation prior to menarche. She denies history of arthritis but had “bone chips” in her knee which were removed by arthroscopy and also has sacroiliac joint inflammation. His father stands 169.8 cm (-1 Ht SDS) and is healthy. He has a 3 year old unaffected brother who stands 91.8 cm (-0.8 Ht SDS). The maternal grandfather stands 144.5 cm, (-4.7 Ht SDS) and denies arthritis and back pain. The clinical characteristics of the family are summarized in Table 1.

Sequencing

The proband and all family members provided written informed consent and assent for research sequencing. This protocol was approved by the Institutional Review Board of Boston Children's Hospital. Sanger sequencing of *ACAN* was performed as previously described[8] and with an additional set of PCR primers: ACAN_Exon12H_FWD: AGGGGAACCATTGGCATCAG and ACAN_Exon12H_REV: CCACCATCCCAGATTTGCCT.

For the sequencing of Exon 12H, the PCR mix was comprised of 5 uL genomic DNA (10 ng/uL), 5 uL of primer mix (each at 1 uM), 3 uL of 10X PCR Buffer, 0.3 uL of 10mM dNTPs, 3.6 uL of 25 mM $MgCl_2$, 0.3 uL of HotStarTaq Polymerase (Qiagen, Hilden, Germany), and 12.8 uL of H_2O. PCR was performed in a BioRad T100 Thermocycler (Bio-Rad, Hercules, CA) with the following cycling conditions: a single denaturing step at 95°C for 10 min, 35 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min, followed by primer extension at 72°C for 10 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen). Dideoxy Sanger method was performed from both directions using the ACAN_Exon12H_FWD and ACAN_Exon12H_REV primers.

We identified a single novel heterozygous frameshift variant chr15:89401207delG (reference human genome build 19 coordinates), c.5391delG (NM_013227.3) (Supplemental Figure 1). The variant was present in the proband, his mother, and maternal grandfather but not in the father or unaffected brother. The variant is not present in the1000 Genomes Project (November 2012 release) or the National Heart, Lung, and Blood Institute ESP6500 exome variant server. The variant is located in exon 12 of the gene and is predicted to lead

to premature truncation of the protein and complete loss-of-function. There were no other rare (MAF <1%) nonsynonymous variants in *ACAN* segregating with the phenotype.

Discussion

Longitudinal bone growth is a complex process involving numerous endocrine hormones including growth hormone, insulin like growth factor 1, glucocorticoid, thyroid hormone, sex steroids (estrogen and androgen), vitamin D, and leptin. Growth of long bones occurs at the growth plate by endochondral ossification, a process where cartilage is formed and remodeled into bone tissue. Chondrogenesis is the process involving chondrocyte proliferation, hypertrophy, and extracellular matrix secretion.[9] We report a novel heterozygous variant in the aggrecan gene (*ACAN*) in a family with a rare autosomal dominant form of idiopathic/familial short stature with bone age advancement and premature growth cessation.

This report highlights critical clinical lessons. First, it is important to obtain a 3 generation pedigree of heights of family members in cases that appear to be “familial short stature”. Our patient's mother's height of 147.7 cm (-2.6 Ht SDS) may be considered as mild short stature. However, our patient's maternal grandfather has severe short stature with -4.7 Ht SDS. Secondly, our patient demonstrated bone age advancement in the absence of central precocious puberty, congenital adrenal hyperplasia, estrogen or androgen excess or exposure. His bone age advancement resulted in a poor height prediction of 142 cms, which is significantly below his midparental target height of 165 cm. Calculation of our patient's midparental target height is inaccurate, however, since his mother also carries the heterozygous mutation in the aggrecan gene. The history of autosomal dominant short stature and bone age advancement ultimately led to the analysis of *ACAN*.

Aggrecan is a proteoglycan component in the matrix of both articular and growth plate cartilage. [6, 7] Articular cartilage lines the gliding surfaces of synovial joints. Aggrecan is crucial in articular cartilage as it provides the hydrated gel structure important for load bearing properties of joints as well as chondrocyte and bone morphogenesis.

Studies in mice and chicks with homozygous mutation in *ACAN* resulted in impairment of bone elongation and growth plate formation. [10, 11] The mechanism of premature growth cessation may be due to premature hypertrophic chondrocyte maturation due to abnormal indian hedgehog, fibroblast growth factor, and bone morphogenetic protein signaling. [10] Hypertrophic differentiation induces vascular invasion and ossification of growth plate cartilage.[4]

ACAN mutations have been reported in children with skeletal dysplasias and in 3 families with idiopathic short stature. The families with skeletal dysplasia with *ACAN* mutations were classified as having three distinct syndromes: spondyloepimetaphyseal dysplasia, aggrecan type (SEMD), familial osteochondritis dessicans, and spondyloepiphyseal dysplasia, Kimberly type (SEDK).[8, 12-15] The affected patients are mildly dysmorphic and have variable heights from mild to extreme short stature (Ht SDS −2 to -15).[4] Using whole exome sequencing, we recently reported 3 families with autosomal dominant short stature,

advanced bone age and premature growth cessation due to heterozygous aggrecan mutations and affected individuals had adult heights of -2.6 to -4.2 SDS, similar to those seen in our report. Our family is the fourth family with this rare genetic cause of idiopathic short stature without evidence of skeletal dysplasia.

Our current family is the first family to have targeted *ACAN* sequencing performed in the setting of dominant short stature with advanced bone age. The mutation (p.Gly1797Glyfs*52) results in premature truncation and presumed loss of protein function. Although some of the protein is preserved with this truncating mutation, the mutation does lead to loss of the C-terminal globular domains (G3 domains). These C-terminal globular domains are needed to link the aggrecan molecule to components of the extracellular matrix (ECM)[16]. Thus, this mutation is likely to significantly perturb protein function. Another frameshift mutation mapping closely upstream (p.Gly1330Trpfs*221) has been known to cause spondyloepiphyseal dysplasia, Kimberly type)[13]. The exact mechanism of how each distinct mutation in *ACAN* leads to a range of phenotypes is unknown.

Recently, we performed whole exome sequencing of 14 children with severe short stature (-3 Ht SDS) many of whom had additional dysmorphic features and found a monogenic disorder in 5 individuals including two cases of 3-M syndrome.[17] Thus, genetic testing has been recommended in selected patients with heights below – 3 SDS, heights below – 2.5 SDS with congenital anomalies, dysmorphic features, skeletal dysplasia, intellectual disability, and in individuals whose predicted adult height is more than 2 SD below their midparental target height.[3]

In conclusion, our report expands the differential diagnosis of the short child with bone age advancement and highlights the need for routine radiography of the left hand in families with autosomal dominant short stature especially with history of early growth cessation. It also emphasizes the role of genetics in revolutionizing the diagnostic work up of individuals with short stature. Future studies should elucidate the mechanism of premature growth plate closure in these families as well as the potential role of growth hormone treatment and aromatase inhibitors to delay epiphyseal closure in pubertal patients with this disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Source: This work was supported by National Institutes of Health (NIH) Grant1K23HD073351 (to A.D.), the Pediatric Endocrine Society Clinical Scholar Award (to A.D.), NIH Grant 5T32GM096911–03 (to M.G.) and March of Dimes Grant 6-FY12-393(to M.G.).

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Abbreviations

ISS	Idiopathic short stature
Ht SDS	height standard deviation score
BA	bone age
CA	chronologic age
N/A	Not applicable/available

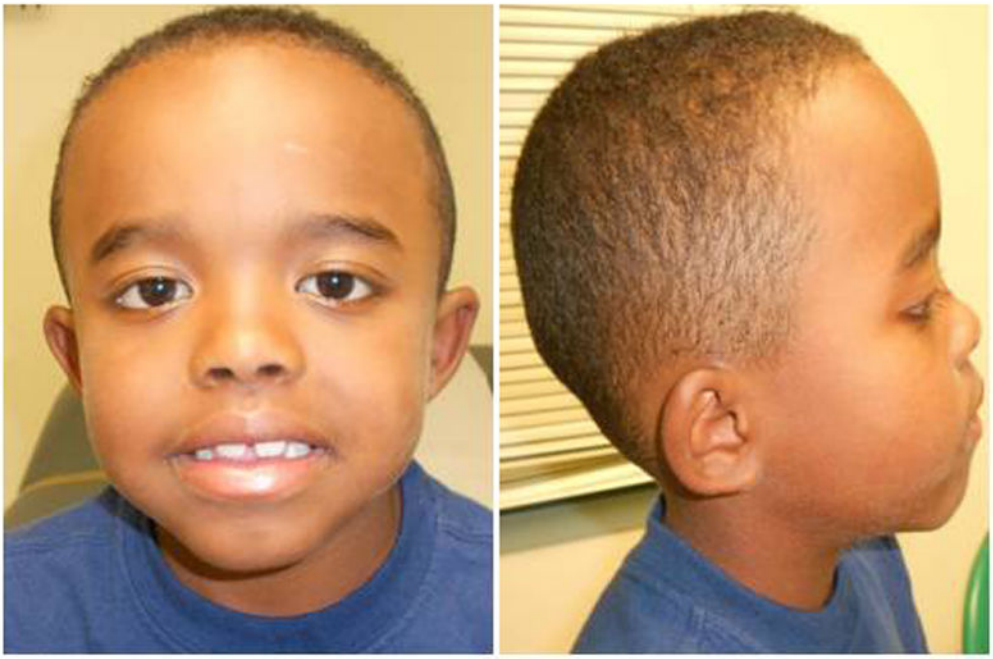

Figure 1.
Photograph of patient showing mid-face hypoplasia and flat nasal bridge.

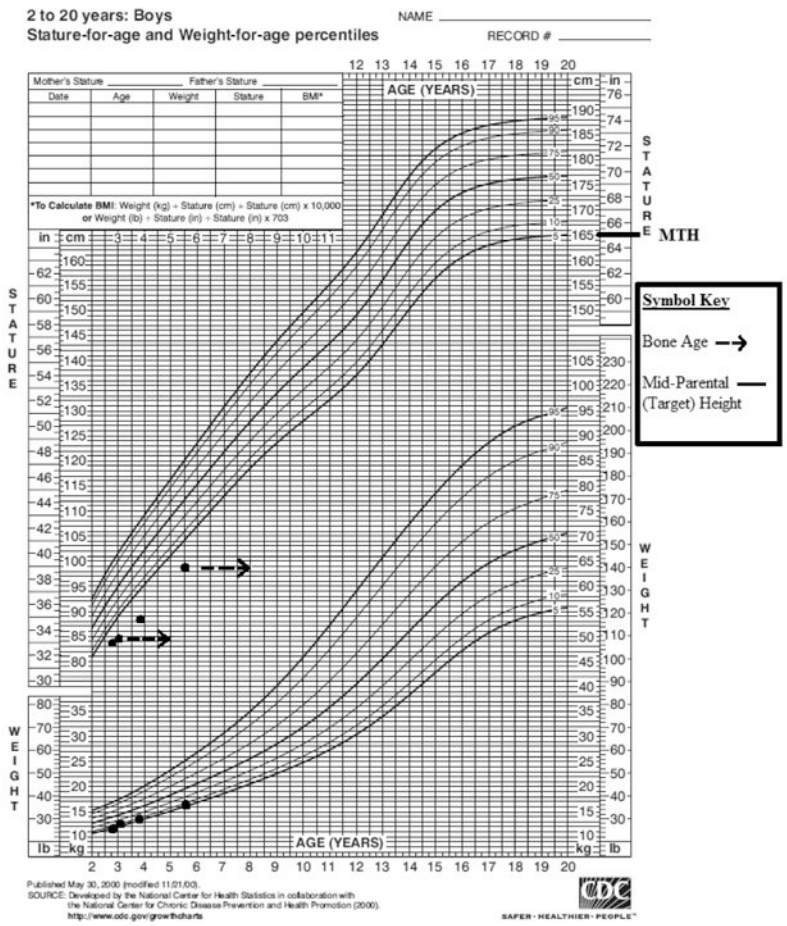

Figure 2.
Growth chart

Table1

Clinical characteristics of family with ACAN mutation

Individual	Proband	Mother	Grandfather
Sex	Male	Female	Male
Age (years:months)	5:6	34	68
Height (cm)	99.2	147.7	144.5
Height SDS	-2.8	-2.6	-4.7
Weight (kg)	16.6	67.4	52
Arm Span (cm)	105	N/A	N/A
Sitting height	52.8	78	77
Head circumference (cm)	51	53.5	53.5
Bone age (years-months)	8	N/A	N/A
Predicted adult height (cm)	142	N/A	N/A
Midparental target height (cm)	165	N/A	N/A
Midface hypoplasia	Yes	No	No
Early onset arthritis	N/A	No	No

N/A: Not applicable or not available