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A Novel Heterozygous ACAN Variant in an Iranian Family with Short Stature: A Case Report

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Abstract

Background: Short stature is estimated to account for half of the new visits to pediatric endocrine practices. Therefore, evaluating its underlying causes seems essential in order to choose the best treatment. Recently, some studies revealed the impact of ACAN, which encodes for aggrecan, mutations on growth ranging from mild idiopathic short stature to severe skeletal dysplasia.

Methods: Here, we describe clinical and molecular characteristics of an Iranian family with short stature using exome sequencing and co-segregation analysis through Sanger sequencing.

Results: A novel variant of ACAN mutation c.1604delG (p.Arg535fs) was identified in the heterozygote pattern which was confirmed through co-segregation analysis in family members.

Conclusion: We have found a novel variant within the ACAN gene in association with insignificant bone abnormality without a high incidence of familiar bone malformation. In order to achieve better clinical outcomes, we suggest genetic testing at an earlier age and also long-term GH treatment for children who are at risk of ACAN mutations. Children who are born small considering their gestational age, or who have persistent short stature, advanced bone age, midfacial hypoplasia, joint problems, or broad toes, can be candidates for ACAN sequencing.

Key Words: ACAN, Molecular Diagnostics, Pediatrics, Skeletal dysplasia.

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1- INTRODUCTION

Given the growing concern over the management of short stature, the attempt to define the underlying etiologies has become very topical these days attempting to improve the overall management of patients. It is believed that short stature, which affects 3% of the population, is still one of the most common referrals to pediatric endocrinologists. The majority of children with short stature, about 50 to 90%, are labeled as having constitutional delay of growth and puberty, familial short stature or idiopathic short stature (1). The expression of Idiopathic short stature (ISS) is used when the patient's height is below the expected height for a given gender, age, and population by a height of -2.25 standard deviations (SDS) in the absence of an identifiable cause (2). However, recent studies revealed that 25–40% of individuals diagnosed with ISS could receive a molecular diagnosis by the virtue of genetic studies namely, targeted gene panels and whole-exome sequencing (3). More than 700 genes are defined to be responsible for growth failure such as SHOX haploinsufficiency which is the most frequent monogenic cause of short stature accounting for 2-3% of ISS cases (1). Recently, some studies revealed that ACAN (MIM 155760) mutations are associated with growth failure in three distinct types of chondrodysplasia (**Table 1**). As can be seen in **Table 1,** short stature is the most obvious manifestation of ACAN mutations.

The ACAN gene encodes aggrecan, a chondroitin sulfate proteoglycan that is a major element in the structure of cartilage's extracellular matrix, such as articular and intervertebral disc cartilage and growth plates. The core protein is made up of three globular domains (G1, G2, and G3), interglobular domain (IGD), and centrally located glycosaminoglycan attachment region (GAG). Heretofore, some studies have found ACAN mutations among individuals with syndromic short statutes such as spondyloepiphyseal dysplasia, Kimberley type, and growth failure with early-onset osteoarthritis and/or osteochondritis dissecans. Although, the ACAN mutations seem to have a great impact on the matrix of cartilage and growth plate, a few numbers of patients with ACAN deficiency have been reported due to the wide phenotypic range of mutations. Thus, some affected individuals may remain undiagnosed and be categorized as ISS or other non-syndromic short statured (4).

Herein, we report a detailed case of ACAN deficiency in Iran due to a novel heterozygous deletion c.1604delG (p. Arg535fs) in a family with growth retardation and advanced bone maturation.

2- CASE PRESENTATION

The index case is a 6-year-old Iranian female with severe short stature and bone age advancement. She was born full term with a birth weight of 2940 g and birth length of 47 cm. Regarding the family history, the patient’s mother had short stature too, 132 cm, while the father was in the lower limit of normal height, 167cm. This patient had two normal siblings. Among maternal families the grandfather was known to have short stature, 135 cm, and also one of the aunts, two uncles and their children were significantly short (**Fig. 1**).

There was no history of short stature among the paternal family members and they seemed normal. The patient was referred to the Akbar hospital pediatric endocrinology department, Mashhad, Iran, for diagnosis and counseling at 3 $^{4/12}$ years due to failure to thrive. Her height was 84 cm (below the 3rd percentile, Height SDS -3.18), weight 13 kg (Weight SDS -0.97) and her bone age was accelerated (4 $^{6/12}$). One of her cousins was also referred to the endocrine department due to growth retardation.

Table-1: Comparison between different ACAN related phenotypes

Phenotype	Phenotype MIM number	Prevalence	Inheritance	Short stature	Midface hypoplasia	Brachydactyly / broad thumbs	Vertebral deformity	Epiphyseal changes	Additional features
Spondyloepimetaphyseal dysplasia ,aggrecan type(SEMDAG)	612813	<1 / 1 000 000	AR	+	+	+/+	-Kyphosis -lordosis -Platyspondyly -multiple cervical-vertebral clefts	irregular epiphyses of long bones with widened metaphyses	-absent nasal cartilage -prognathism -macrocephaly -rhizomelia and mesomelia -horizontal nails -telescoping interphalangeal joints -low-set ears
Short stature and advanced bone age, with or without early-onset osteoarthritis and/or osteochondritis dissecans (SSOAOD)	165800	Unknown	AD	+	+	+/+	-Lumbar lordosis -intervertebral disc disease -multiple lumbar disc herniation	-	-advanced bone maturation -early-onset osteoarthritis (OA) -osteochondritis dissecans -flat nasal bridge
Spondyloepiphyseal dysplasia, Kimberley type (SEDK)	608361	<1 / 1 000 000	AD	+	-	-/-	-sclerosis of the vertebral bodies	mild and variable	-Stocky habitus -progressive osteoarthropathy of the weight-bearing joints

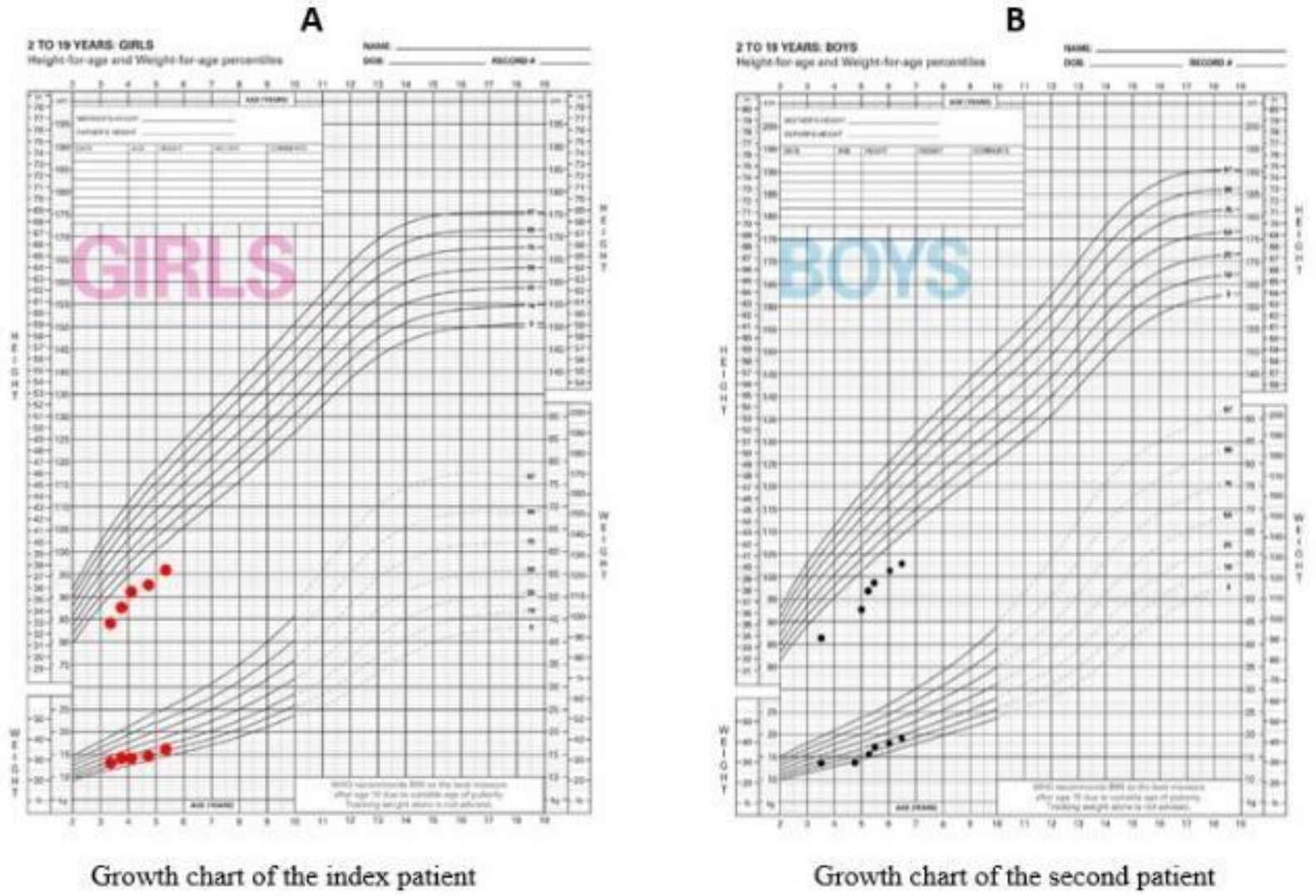

Fig. 1: A) Shows the growth chart of the index patient. B) Shows the growth chart of the index patient.

The strong family history of short stature among her maternal family members was suspected to have a genetic source, thus the patient was referred to a medical genetic research center for further evaluations. Height, weight and bone age (GP atlas) were measured. SDS values for height and weight were calculated every three months using the national references.

2-1. Whole Exome Sequencing

We performed Whole Exome Sequencing (WES) by Target Enrichment Preparation Kit for Agilent (Version V6, February 2018). The captured libraries underwent 101 bp paired-end sequencing using the Illumina HiSeq 4000 system (Illumina, Inc., San Diego, CA, USA). Resultant FASTQ files were aligned to the human reference sequence (hg19) by Burrows-Wheeler Aligner (BWA); and SAM files were produced. Further, SAM to BAM conversion, BAM file sorting, and removal of duplicate reads were carried out by Picard (http://picard.sourceforge.net), followed by local realignment and variant calling by Genome Analysis Toolkit (GATK) to generate VCF files, and then annotation was performed with Annovar32, for identification of pathogenic variants in affected individuals. Variants are classified on the basis of multiple genomic databases – including, NCBI Exome Sequencing Project, Exome Aggregation Consortium and HGMD® Professional – and in accordance with the American College of Medical Genetics and Genomics (ACMG) 2015 guideline (5). Then, to verify the candidate variant in patients and parents, specific primers were designed which were followed by polymerase chain reaction (PCR) and Sanger sequencing.

2-2. Clinical Findings

The index case is a 6-year-and-1-month-old Iranian female with familial short stature and markedly advanced bone age. The endocrine evaluations have been normal with no sign of GH deficiency, hypothyroidism or early puberty. At the chronologic age of 3 years 4 months, her bone age was markedly advanced at 4 years 6 months. Skeletal survey performed at 3 years and 4 months of age was normal with no signs of skeletal dysplasia. However, during the two years of follow up and treatment, mild lordosis and Varus valgus had appeared.

The second patient, a cousin of the index patient, is a 6-year-and-7-month-old Iranian male. He was born full term with a birth weight of 2900 g and birth length of 48 cm. He was referred to the endocrine department when he was about 3 years and 7 months, because of growth retardation. His height was 86 cm (below the 3rd percentile, Height SDS -3.48), his weight 14 kg (Weight SDS -0.90). The endocrine evaluations have been normal with no sign of GH deficiency, hypothyroidism or early puberty. At chronologic age of 5 years 3 months, his bone age was not markedly advanced. Skeletal survey performed at 5 years and 3 months of age was normal with no signs of skeletal dysplasia. However, in radiographic evaluation of the left hand, mild clinodactyly was noticed. Both patients are shown in **Fig. 2**.

2-3. Measurement of responses to growth hormone treatment

The index patient was a candidate for growth hormone therapy due to her failure to thrive. As it can be seen in **Fig. 1**, the growth response to the GH therapy was greater in the first year of treatment and it has been gradually decreased during the second year.

The second patient has recently become a candidate for growth hormone therapy after the positive impacts of this treatment on his cousin.

2-4. Molecular findings

We diagnosed a heterozygous variant in exon 8 of the ACAN gene c.1604delG

(p.Arg535fs) in proband, her mother and her cousin who had similar clinical findings, using bidirectional sequencing. The ACAN gene is proposed to be associated with autosomal dominant forms of short stature and advanced bone age, with or without early-onset osteoarthritis and/or osteochondritis dissecans (MIM number: 165800). However, this mutation was absent in her father and one of her siblings (**Fig. 3**). Likewise, this variant was absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genome-Project, or Exome Aggregation Based Consortium. Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc). Based on ACMG 2015 guidelines, this variant can be classified as a likely pathogenic variant (6).

Fig. 2: A) The index patient appearance. Valgus deformity can be seen in the patient. Micrognathia is another clinical finding in this patient. B) The second patient appearance.

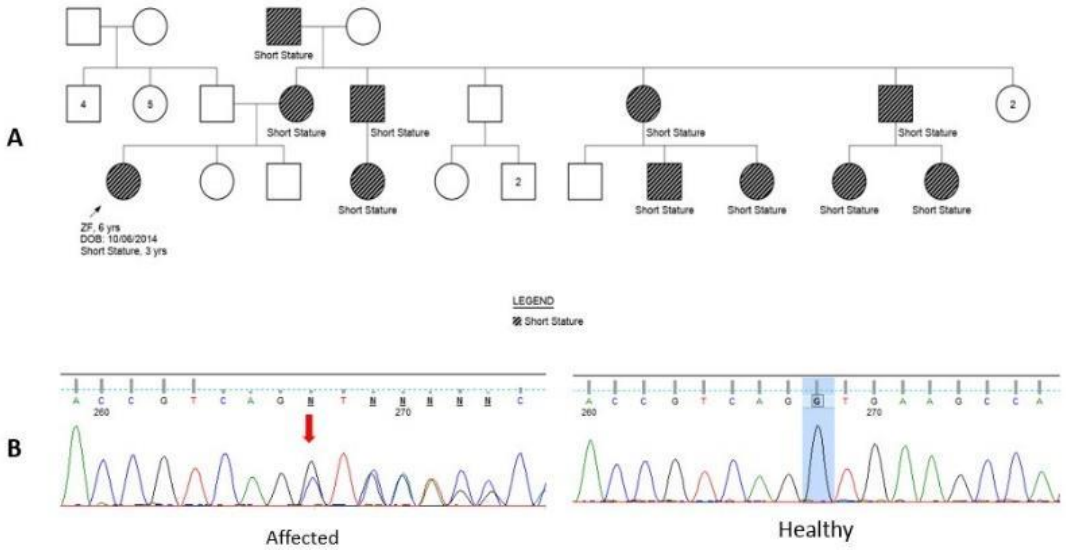

Fig. 3: Segregation analysis in the family. A) Three generation family pedigree. Black shapes show all affected individuals. B) Chromatogram of heterozygote and normal homozygote family members.

3- DISCUSSION

ACAN gene has been previously described to be related to short stature and advanced bone age, with or without early onset osteoarthritis and/or osteoarthritis-dissecans, autosomal recessive (7-11). Pathogenic variants in ACAN are a joint reason for familial short stature in both autosomal dominant and autosomal recessive inheritance patterns. Aggrecan is encoded by ACAN which is located at 15q26.1. As a result of genetic mutations within the ACAN gene, low aggrecan levels, reduced chondrocyte proliferation, accelerated hypertrophy of chondrocytes, and abnormal structure of the cartilage extracellular matrix differentiation are observed. Furthermore, Spondyloepimetaphyseal dysplasia, which is a distinctive aggrecan-type phenotype, is caused by homozygous ACAN mutations (SEMD, OMIM#612813). Some prominent clinical features of SEMD are Kyphosis, lordosis, Platyspondyly, multiple cervical-vertebral clefts, and irregular epiphyses of long bones with widened metaphyses. We have found deletion of "G" nucleotide at chr15-88847416 which has been predicted as a frameshift change after arginine codon 535. This change can create a premature stop codon at four amino acids later on the new reading frame and consequent truncated protein.

Homozygous c.7141G > A (p. Asp2381Asn) was diagnosed in a family affected by SEMD. Meanwhile, there are two distinct phenotypes caused by heterozygous mutations: spondyloepiphyseal dysplasia, Kimberley type (SEDK, OMIM#608361) as well as short stature with or without accelerated bone age (BA); and an osteoarthritis and/or osteoarthritis dissecans that develops at an early age (SSOAOD, OMIM#165800) (1, 12). Some key clinical features regarding SEDK are sclerosis of the vertebral bodies, mild and variable epiphyseal changes, Stocky habitus, and progressive osteoarthropathy of the weight-bearing joints. In respect to the SSOAOD, clinical features are considered as lumbar lordosis, intervertebral disc disease, and multiple lumbar disc herniation. c.3758dupC (p.Gly1254Trpfs*175) was diagnosed in a family affected by SEDK and c.7249G > A (p.Val2417Met) was detected in patients affected by familial SSOAOD (8). Moreover, ACAN mutations could be identified in patients with non-syndromic short stature without any known etiology, as X. Hu et al. reported three novel variants c.661delT (p.Tyr221Metfs*10), c.6_13delCACTTTAC (p.Thr3Leufs*21), and c.1117_1120delCAGA (p.Thr374*) all categorized as pathogenic variants (8). Sentchordi-Montané et al. also revealed that brachydactyly is associated with ACAN mutations in patients with short stature and mild skeletal defects (13). Some less common findings such as severe inflammatory elbow involvement are also detected in patients with heterozygous mutations of the ACAN gene (14).

We detected a novel heterozygous likely pathogenic NM_001135 c.1604delG (p.Arg535fs) variant in the exon 8 of the ACAN in a 5-year-old symptomatic girl with growth retardation. Short stature and advanced bone age, without early onset osteoarthritis or osteochondritis dissecans, 5 (phenotype MIM number: 165800), is confirmed in the studied proband. Likewise, we detected the same mutation in a heterozygous form in her mother and cousin (son of her mother's brother). Her father and siblings were healthy; therefore, the new frameshift (fs) mutation found was followed by an autosomal dominant inheritance pattern.

Exon 8 along with exons 9 & 10 code for globular domain 2 (G2), is one of the Core domains of ACAN protein. Most of the fs mutations are considered to introduce the premature stop codon (PTC), leading to a loss of the functional domains of the

protein (1). However, one limitation of our study is that we could not include functional analyses such as mRNA expression analysis or protein expression and rate analysis in the body of the studied subjects.

4- CONCLUSIONS

A novel variant within the ACAN gene has been identified in association with insignificant bone abnormality without a high incidence of familiar bone malformation. Although treatment with GH didn't show any promising results compared to the effect in children with GHD, according to our finding and the result of other similar studies, investigations on similar cases are needed to confirm the theory that children with ACAN mutations may benefit from long-term GH treatment by slowing down the cumulative deterioration of growth loss. In order to achieve better clinical outcomes, genetic testing using whole exome sequencing at an earlier age is recommended.

5- CONFLICTS OF INTEREST

None.

6- FUNDING

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