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ALS deficiency caused by an exon 2 deletion and a novel missense variant in the gene encoding ALS

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ABSTRACT

Context: ALS deficiency (ACLSD), caused by mutations in *IGFALS,* is characterized by a mild short stature, low concentrations of IGF-I and IGFBP-3, and a normal growth hormone (GH) stimulation test response. To our knowledge, no larger deletions have been reported.

Case description: A 17-year-old adolescent male was evaluated due to delayed puberty and short stature. He had a height of 154.4 cm (SDS -2.84), a weight of 53.3 kg (SDS -1.41), a BMI of 22.4 kg/m2 (SDS +0.31), a Tanner 2 pubertal stage with a testicular volume of 10 mL, and a bone age of 16 years (SDS -1.33). After biochemical evaluation, low IGF-I levels, undetectable IGFBP-3 levels, and a normal response to the GH stimulation test were observed, suggesting GH insensitivity. ACLSD was confirmed by ALS measurement (116 ng/mL, SDS -3.19) and genetic analysis of *IGFALS*. An apparently homozygous missense variant, p. Pro624Leu, was found in exon 2 of the proband; this mutation was observed on one allele of the proband's father but was absent in the mother and siblings. Deletion/duplication analysis by multiplex ligation-dependent probe amplification (MLPA) was consistent with a deletion encompassing a significant part of exon 2 on one allele in the proband and in his mother and siblings.

Conclusion: This is the first report of a large deletion in a patient with ACLSD. Deletion/duplication analysis should be considered in the genetic study of ACLSD, especially when homozygosity for a pathogenic variant cannot be confirmed by the study of the parents or when no variants are found but ALS concentrations are very low.

1. Introduction

The acid-labile subunit (ALS) is a glycoprotein whose primary function is to prolong the half-lives of insulin-like growth factor (IGF)-I, IGF-II, insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-5 by forming a ternary complex [1]. ALS deficiency (ACLSD; OMIM #615961) has been described in patients with mild short stature, low concentrations of IGF-I and IGFBP-3, and a normal response to the growth hormone (GH) stimulation test. Other clinical findings associated with ALS deficiency are pubertal delay, insulin insensitivity and low bone mineral density [2]. Patients with ACLSD do not respond adequately to treatment with recombinant GH.

The first case was described in 2004 [3], and at least 62 patients have thus far been diagnosed with ACLSD [4]. ALS is encoded by *IGFALS*, located at chromosome 16, which consists of 2 exons, with exon 2 encoding a major part of the protein [5]. Different genetic variants have been found in *IGFALS*; however, to our knowledge, no larger deletions have been reported.

2. Case

A seventeen-year-old adolescent male was evaluated at the pediatric endocrine clinic. The main concern was delayed puberty and loss of growth velocity. He is the third son of nonconsanguineous parents and had no past medical history. He was born at term (38 gestational weeks) with a newborn weight of 3150 g (standard deviation score (SDS)

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+0.18) and a newborn length of 49 cm (SDS +0.24). Upon physical examination, he showed a height of 154.4 cm (SDS -2.84, 0.91 SDS units below his target height of 162 cm (SDS -1.93)), a weight of 53.3 kg (SDS -1.41), and a BMI of 22.4 kg/m2 (SDS +0.31). He had a Tanner 2 pubertal stage with a testicular volume of 10 mL bilaterally. The patient's bone age (Greulich-Pyle method) was 16 years, and his chronological age was 17 years and 6 months (SDS -1.33).

The first-line biochemical evaluation for short stature showed no relevant results, showing a negative celiac disease screening, normal liver function test, normal complete blood count, normal venous blood gases and normal serum electrolytes. Upon further investigation, low IGF-I and undetectable IGFBP-3 concentrations were found together with a normal pituitary MRI. The patient responded normally to a GH stimulation test with clonidine (basal, 11.2 ng/mL; 60 min, 19.3 ng/mL; 90 min, 10.7 ng/mL; and 120 min, 7.25 ng/mL), suggesting GH insensitivity. No changes were observed regarding the IGF-I and IGFBP-3 concentrations after 4 weeks of treatment with recombinant human GH (0.1 and 0.2 U/kg per day for 2 weeks each). ACLSD was suspected because of low IGF-I concentrations, undetectable IGFBP-3, an normal response to the GH stimulation test and absence of a biochemical response to treatment with GH. The ALS concentration was determined to be 116 ng/mL (SDS -3.19) as measured by ELISA (Mediagnost, Reutlingen, Germany) [6,7], confirming ACLSD. Available family members, including siblings, parents and paternal family members, were also studied. The maternal family was not available. Clinical characteristics as well as IGF-I [8], IGFBP-3 [9], and ALS concentrations are summarized in Table 1.

3. Genetic analysis

Sanger sequencing of *IGFALS* was performed on the proband and available relatives. For the proband, an apparently homozygous missense variant, c.1871C > T (p. Pro624Leu, RNA not analyzed, NP_001139478.1, rs756451070), was found in exon 2. This missense variant has been reported at very low frequencies in different databases (GnomAD 2/219992, TOPMED 1/125568, ExAC 1/44672) but not in a clinical context. *In silico* analysis predicts this variant as probably damaging with PolyPhen2 (score 1) and as disease-causing with Mutation Taster (probability 0.999994). The variant was found on one allele in his father but not in the mother or siblings. Therefore, multiplex ligation-dependent probe (MLPA) analysis, which utilizes 3 probes targeting *IGFALS* (SALSA® MLPA® P217-B2 IGF1R, MRC Holland), was performed. For the proband, his mother and his siblings, half of the probe signals were directed to exon 2 in *IGFALS*, which is consistent with a deletion encompassing at least a significant part of exon 2 on one allele (c.16_1929del, p. Lys5_Cys643del, RNA not analyzed, NP_001139478.1), resulting in the following genotype for the proband: p.[Pro624Leu];[Lys5_Cys643del]. The family pedigree and the identified variants as well as the MLPA results of the proband are shown in Fig. 1. Additionally, the results in Table 1 are displayed according to genotype.

4. Discussion

Here, we present a novel genetic mechanism as a cause of ACLSD in the first reported Chilean patient. In the proband, a large deletion involving exon 2 of *IGFALS* was observed on one allele, as demonstrated by MLPA, and a missense variant not previously described in a clinical context was observed on the other allele. Relatives with one affected allele showed lower ALS concentrations (SDS below −1) than those with no affected alleles, indicating a gene-dosage effect, which has been already reported [10]. These results indicate that both gene alleles are required to maintain optimal ALS levels, sustain normal circulating levels of IGF-I and IGFBP-3 and fulfill growth potential.

Sequencing identified a missense variant (p. Pro624Leu) that has not been previously reported in a patient with ACLSD but is present at very low frequencies in public databases. This finding is not surprising because the continued expansion of genomic databases will increase the probability of pathogenic variants being present. The deleterious effect of this variant on the protein is supported by two bioinformatics tools, and the effect on the phenotype is best demonstrated by the differences in ALS concentrations observed in subjects with two, one or no alleles affected. Based on American College of Medical Genetics recommendations, we conclude that p.Pro624Leu is likely a pathogenic variant [11].

To date, only point mutations have been reported in *IGFALS*. In our patient, we suspected the presence of a deletion, which was confirmed by MLPA. Although this finding was unexpected because it had not been previously reported in this gene, deletions are a common mechanism implicated in genetic variation and a possible cause of genetic diseases. The ALS concentrations in the subjects presenting the deletion on one allele were similar to those in subjects with the missense variant

Table 1
Clinical and biochemical characteristics.

	Sex	Age years	Height cm (SDS)	BMI kg/m^2 (SDS)	Pubertal delay	IGF-I ng/mL (L,N,H)[a]	IGFBP-3 ug/mL (L,N,H)[a]	ALS ng/mL (SDS)[b]
p.[Pro624Leu];[Lys5_Cys643del]								
Index case (III-7)	M	17.4	154.4 (−2.79)	22.4 (0.63)	Yes	45 (L)	< 0.5 (L)	116 (−3.19)
p.[Pro624Leu];[=]								
Father (II-3)	M	44.7	161.2 (−2.05)	27.4 (1.39)	Yes	80 (N)	2.4 (L)	5623 (−1.18)
Grandmother (I-2)	F	66.5	148.0 (−2.33)	33.1 (1.89)	Unknown	44 (N)	2.5 (L)	5434 (−1.52)
Cousin (III-2)	M	13.4	154.2 (−0.58)	23.3 (1.58)	No	172 (N)	4.4 (N)	7202 (−1.23)
Uncle (II-1)	M	31.4	170.7 (−0.82)	40.1 (2.76)	No	64 (L)	2.6 (L)	4831 (−1.80)
Cousin (III-1)	M	16.9	151.0 (−0.52)	29.4 (2.77)	No	62 (L)	2.8 (L)	4009 (−2.17)
p.[Lys5_Cys643del];[=]								
Mother (II-4)	F	44.3	149.9 (−2.18)	28.1 (1.40)	Unknown	39 (L)	2.5 (L)	5049 (−1.72)
Brother (III-8)	M	16.9	165.0 (−1.39)	20.9 (−0.11)	No	171 (L)	3.6 (N)	6883 (−1.41)
Sister (III-6)	F	6.3	107.7 (−1.64)	14.1 (−0.79)	Prepuberal	35 (L)	2.0 (N)	3253 (−1.98)
Brother (III-9)	M	21.0	165.0 (−1.49)	N/A	Yes	153 (N)	2.9 (L)	5569 (−1.23)
p.[=];[=]								
Grandfather(I-1)	M	77.0	157.7 (−2.61)	25.4 (1.03)	Unknown	52 (N)	2.2 (L)	3360 (N/A)
Cousin (III-3)	M	16.5	166.9 (−0.94)	22.7 (0.65)	No	150 (N)	5.1 (N)	12,137 (0.01)
Cousin (III-4)	F	5.8	113.3 (−0.12)	20.3 (2.46)	Prepuberal	81 (N)	4.5 (N)	9381 (0.34)
Cousin (III-5)	F	5.5	103.5 (−1.72)	14.6 (−0.47)	Prepuberal	42 (L)	2.8 (N)	6470 (−0.76)

Abbreviations: BMI, body mass index; SDS, standard deviation score; M, male; F, female; N/A, not available; L, low; N, normal; H, high.

[a] IGF-I and IGFBP-3 values reported by the manufacturer [8,9].

[b] ALS values are based on ref. [6] for patients 18 years old and younger and on values reported by the manufacturer for adults [7].

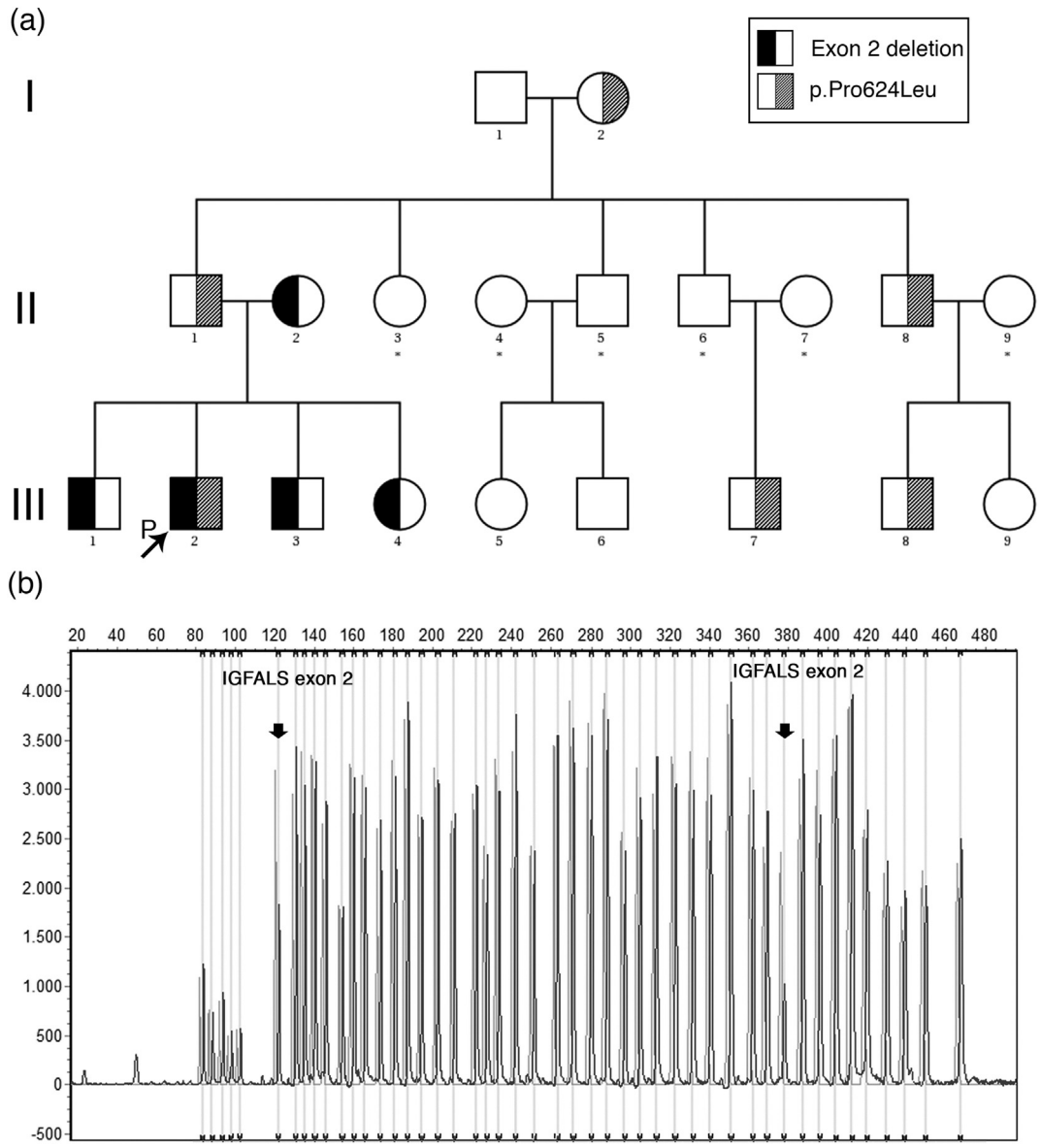

Fig. 1. (a) Family pedigree.
(b) MLPA results of the proband. Light gray lines: control; dark gray lines: proband.

and lower than those in subjects with no variants.

In the assessment of children with short stature, ALS deficiency should be considered in patients presenting with a mild short stature and delayed puberty, which is associated with low IGF-I and extremely low IGFBP-3 levels, but normal responses to GH stimulation and no response to an IGF generation test. To confirm the diagnosis, ALS should be measured, and if possible, genetic testing should also be performed. We suggest that deletion/duplication analysis should be considered in the genetic analysis of ACLSD when homozygosity for a pathogenic variant cannot be confirmed by studying the parents or when no variants are found but ALS levels are very low.

Disclosure summary

The authors have nothing to disclose.

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Declaration of Competing Interest

None.

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