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Acid-Labile Subunit Deficiency and Growth Failure: Description of Two Novel Cases

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Key Words
Acid-labile subunit · Short stature · Insulin-like growth factor I deficiency · Mutations, acid-labile subunit · Linear growth

Abstract
Background/Aims: Mutations in the acid-labile subunit (ALS) gene *(IGFALS)* have been associated with circulating insulin-like growth factor I (IGF-I) deficiency and short stature. Whether severe pubertal delay is also part of the phenotype remains controversial due to the small number of cases reported. We report 2 children with a history of growth failure due to novel *IGFALS* mutations. ***Methods:*** The growth hormone receptor gene *(GHR)* and *IGFALS* were analyzed by direct sequencing. Ternary complex formation was studied by size exclusion chromatography. ***Results:*** Two boys of 13.3 and 10.6 years, with pubertal stages 2 and 1, had mild short stature (–3.2 and –2.8 SDS, respectively) and a biochemical profile suggestive of growth hormone resistance. No defects were identified in the *GHR*. Patient 1 was homozygous for the *IGFALS* missense mutation P73L. Patient 2 was a compound heterozygote for the missense mutation L134Q and a novel GGC to AG substitution at position 546–548 (546–548delGGCinsAG). The latter causes a frameshift and the appearance of a premature stop codon. Size exclusion chromatography showed no peaks corresponding to ternary and binary complexes in either patient. ***Conclusion:*** Screening of the *IGFALS* is important in children with short stature associated with low serum IGF-I, IGFBP-3 and ALS.

Introduction

Short stature in the presence of low insulin-like growth factor I (IGF-I) levels and normal or high growth hormone (GH) levels is generally caused by the inability of cells to respond to GH [1–3] or to the presence of a defective GH [4]. The description of the first case of short stature in a child with inactivation of the acid-labile subunit (ALS) gene *(IGFALS)* [5] has prompted reconsideration of the role of this protein in the GH-IGF-I axis and the promotion of linear growth.

The ALS is a GH-dependent protein [6]. Its main role is thought to be the regulation of circulating IGF-I bioavailability. When free or bound to IGFBP-3 in a binary complex (IGF-I/IGFBP-3), IGF-I has a half-life of minutes. Formation of the ternary complex IGF-I/IGFBP-3/ALS, however, limits IGF-I passage through the capillary

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barrier, reducing its clearance as well as its availability at the tissue level, thereby increasing its half-life to hours [7].

Since the first report in 2004, more cases of *IGFALS* inactivation in short stature children with IGF-I deficiency have been described, sometimes with controversial findings, such as pubertal delay [5, 8] or microcephaly [9]. We report novel homozygous and compound heterozygous mutations in the *IGFALS* in 2 unrelated children with short stature.

Patients and Methods

The 2 patients were referred to the Department of Paediatrics of Heartlands Hospital, Birmingham, UK, for short stature. Height and weight were measured using standard anthropometric techniques [10] and converted to standard deviation score (SDS). Pubertal status was assessed according to the criteria of Tanner [11, 12]. Boys were considered prepubertal if genitalia were stage 1 and testicular volume was <4 ml [13].

GH deficiency was excluded by detection of a GH peak of ≥3 ng/ml after stimulation test. The study was approved by the local ethical committee and informed consent was obtained.

Hormone Assays

Serum IGF-I, IGFBP-3 and ALS were measured from venous blood samples, using an enzyme-linked immunosorbent assay (ELISA kit; Diagnostic System Laboratories, Inc. Webster, Tex., USA). For IGF-I, the assay sensitivity was 0.01 ng/ml. The intra- and inter-assay coefficients of variation were 8.6 and 6.8% for mean serum concentrations of 104 and 90 ng/ml, respectively. For IGFBP-3 the assay sensitivity was 0.04 ng/ml. The mean intra- and inter-assay coefficients of variation were 7.2 and 8.3%, respectively. For ALS, the assay sensitivity was 0.7 ng/ml and the inter-assay coefficient of variation was 8%. For GH-binding protein (GHBP), the assay sensitivity was 1.69 pmol/l. The inter-assay coefficient of variation was 8.4%. Serum GH was measured with a fluoroimmunometric assay on the Immulite 2000 analyzer (DPC). The assay detects the 20- and 22-kDa GH isoforms.

Genetic Analysis

Genomic DNA was extracted from peripheral blood leukocytes. The coding exons and the intronic boundaries of the *GHR* and *IGFALS* were amplified by polymerase chain reaction (PCR) using specific primers (sequences available on request). For the *GHR*, cycling conditions were: 95°C for 2 min (1 cycle); 95°C for 30 s, 55°C for 30 s and 72°C for 30 s (30 cycles) and 72°C for 5 min. For the *IGFALS* cycling conditions were: 95°C for 2 min (1 cycle); 95°C for 30 s, 63°C for 30 s and 72°C for 30 s (30 cycles) and 72°C for 5 min. PCR products were visualized on a 1% agarose gel and sequenced using the ABI Prism Big Dye Sequencing kit and an ABI 3700 automated DNA sequencer (Applied Biosystems), in accordance with the manufacturer's instructions. A 1,290-bp PCR fragment corresponding to part of the *IGFALS* exon 2 (*IGFALS* ID: ENST00000215539 from 1,254 to 2,544 bp, www.ensembl.org) was ligated into the pGEM T-easy vector (Promega) and amplified in JM109 bacterial cells. Colonies were selected after an overnight incubation. Plasmid DNA was isolated using the QIAprep miniprep kit (Qiagen) and sequenced on the ABI 3700 DNA sequencer.

Size exclusion Chromatography

Samples were fractionated on a HiPrep 16/60 Sephacryl S-200HR column (Amersham Biosciences, GE Healthcare, USA). Serum samples (100 μl) were incubated overnight at 22°C with 3.5×10^6 counts per minute of ^{125}I-labeled IGF-I, and were then cross-linked with 5 mM disuccinimidyl suberate (Sigma Aldrich). After 30 min the cross-linking reaction was stopped by adding 1 M Tris HCl. Five hundred microliters were loaded into the column and one-milliliter fractions were collected and counted on a gamma counter [14].

Results

Growth Development and Hormonal Profile

Both patients were referred for a history of growth failure (fig. 1). Patient 1 was a 13.3-year-old boy from a consanguineous family. At the time of evaluation, his height was 3.2 SDS below the mean for age and sex, whereas the rest of his medical history and physical examination were unremarkable. His bone age was 12.5 years and he was Tanner stage II. The patient had low IGF-I (40, normal range 41–2,604 ng/ml) and IGFBP-3 (0.4, normal range 2.0–9.2 mg/l) and undetectable ALS levels (normal range 16.3–32.1 mg/l). The patient had an exaggerated GH response (61.7 ng/ml) after stimulation test. GHBP levels were normal. His father and mother were consanguineous and their height SDS were –1.1 and –2.2, respectively.

Patient 2 was a 10.6-year-old boy from a non-consanguineous marriage. At the time of evaluation, his height was 2.8 SDS below the mean for age and sex. He was prepubertal and had a bone age of 6.5 years. His medical history and physical examination were otherwise unremarkable. Biochemical evaluation demonstrated low IGF-I levels (33, normal range 20–855 ng/ml) and very low IGFBP-3 (0.2, normal range 1.4–7.6 mg/l) and ALS levels (0.3, normal range 12.3–26.0 mg/l). GH levels increased markedly after provocation test (38.5 ng/ml). GHBP levels were normal. His mother and father had mild short stature (–1.98 and –1.67 SDS, respectively).

Molecular and Genetic Analysis

The *GHR* was analyzed in both patients. Direct sequencing did not reveal any defect in the *GHR* coding region or in the pseudoexon 6Ψ.

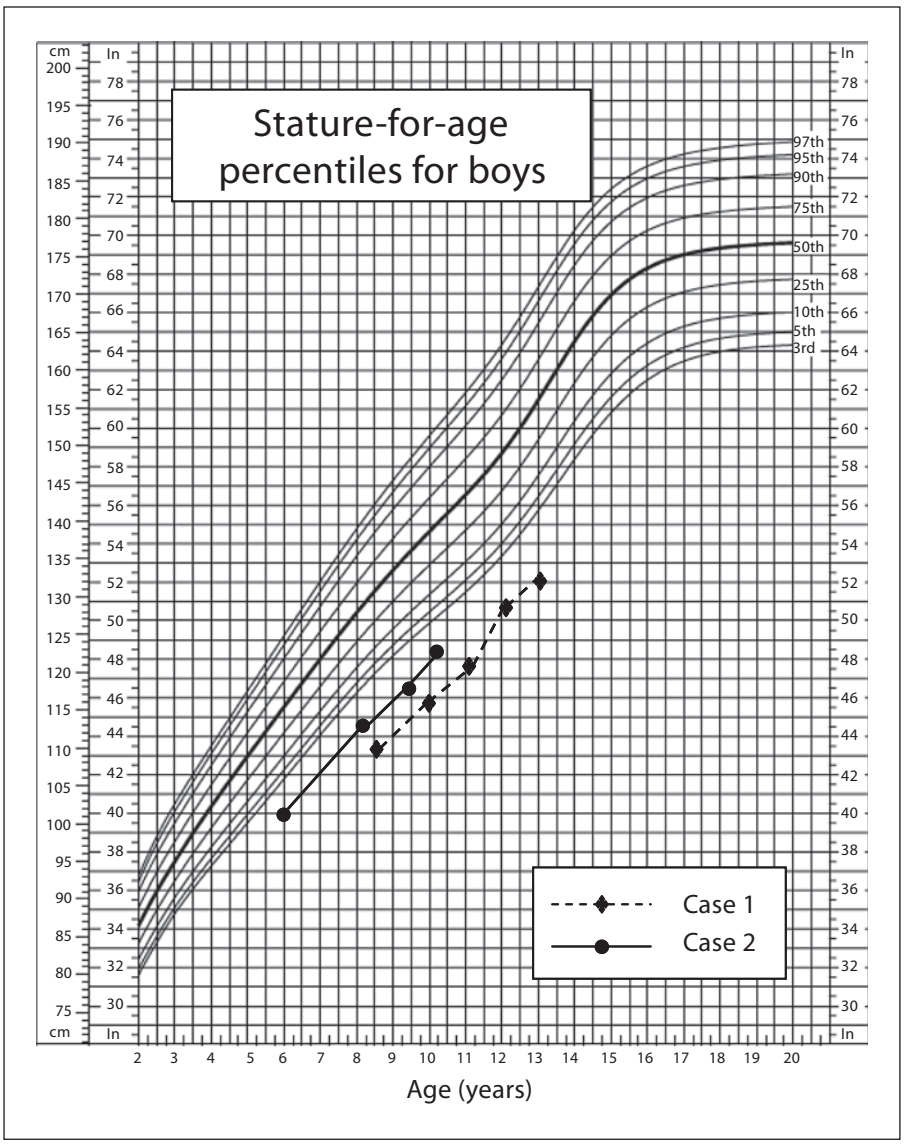

Fig. 1. Growth chart for patients 1 and 2.

Sequencing of the *IGFALS* in patient 1 revealed a homozygous base change from C to T at position 218. This defect resulted in a missense mutation causing a proline to leucine substitution (P73L; fig. 2A).

Sequencing of the *IGFALS* in patient 2 revealed two heterozygous defects: a base change from T to A at position 401 causing a leucine to glutamine substitution (L134Q) and the replacement of the GGC triplet at position 546–548, with AG (546–548delGGCinsAG; fig. 2A). The latter causes a frameshift and the appearance of a premature stop codon. In the absence of parental DNA, the location of both defects on the same allele was excluded by cloning of the *IGFALS* PCR fragment carrying the two heterozygous defects. Sequencing of plasmid DNA isolated from different bacterial colonies demonstrated that the two defects were located on different alleles (fig. 2B).

Size Exclusion Chromatography

The ability of patients' serum to support formation of the ternary complex IGF-I/IGFBP-3/ALS was studied by means of size exclusion chromatography. No peak corresponding to the ternary complex was identified. No peak corresponding to the binary complex IGF-I/IGFBP-3 was present in either patient (fig. 3).

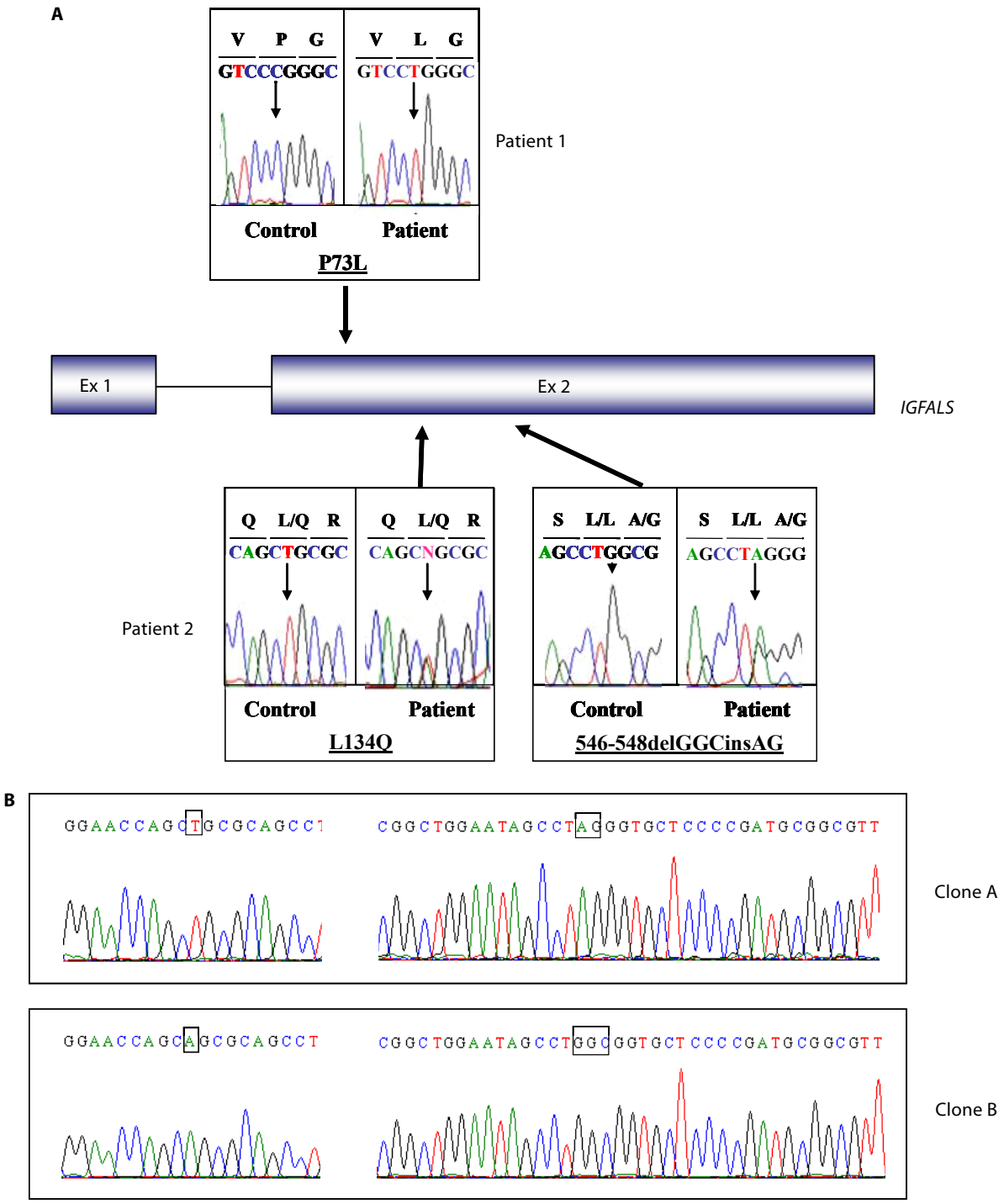

Fig. 2. Partial DNA and amino acid sequence for patients and controls. **A** Sequencing of the *IGFALS* revealed that patient 1 was homozygous for the missense mutation C to T at 218 (P73L) and patient 2 compound heterozygous for a missense mutation T to A at 401 (L134Q) and a GGC to AG substitution at position 546–548 (546–548delGGCinsAG). **B** A 1,290-bp *IGFALS* PCR product containing the two heterozygous defects identified in patient 2 was cloned and sequenced, showing that the two defects were located on separate alleles.

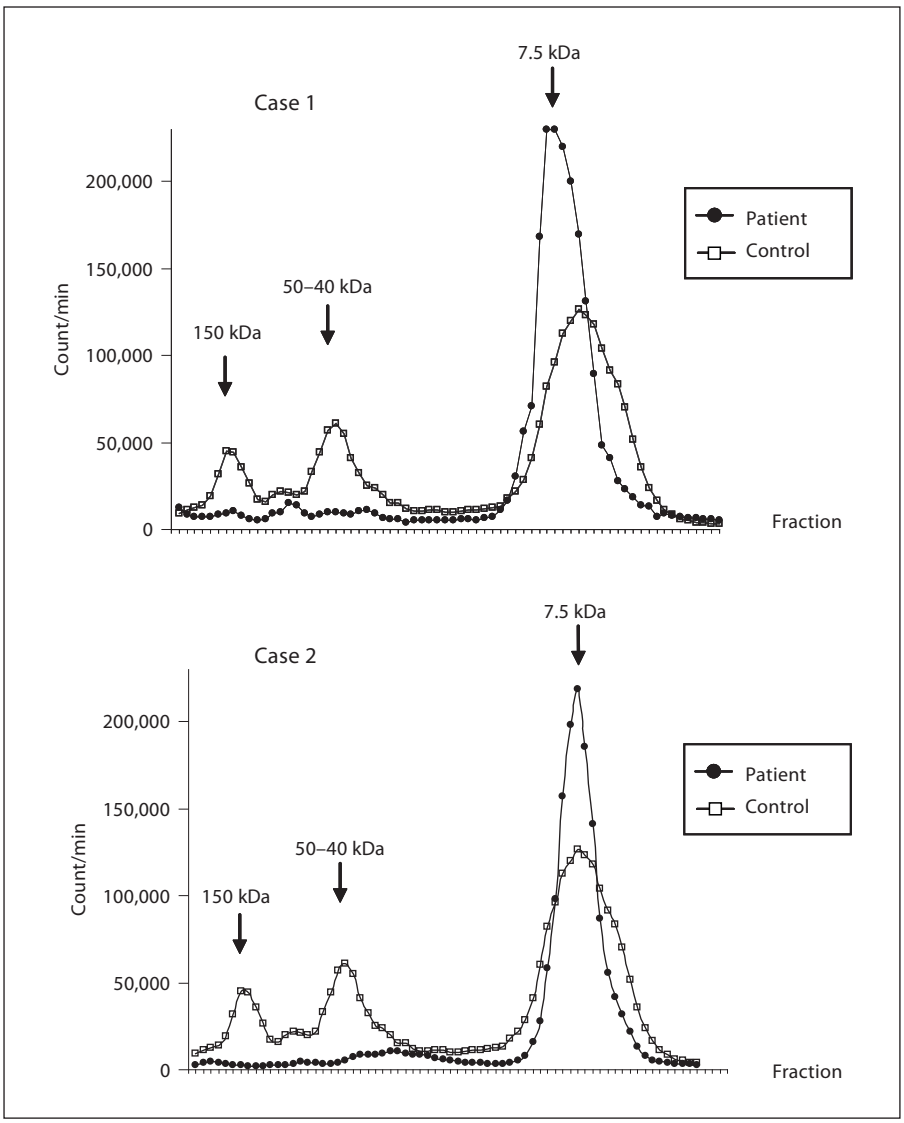

Fig. 3. Size exclusion chromatography. The position of the ternary complex (predicted molecular weight 150 kDa), binary complex (predicted molecular weight 40–50 kDa) and unbound IGF-I (predicted molecular weight 7.5 kDa) are indicated.

Discussion

We describe 2 cases of ALS deficiency caused by novel *IGFALS* mutations. Similar to previous reports, the absence of the ALS protein resulted in growth failure and reduction of IGF-I and IGFBP-3 serum levels [5, 15–17].

Circulating IGFBP-3 and IGF-I have a short half-life when in the free form or bound together in the relatively unstable binary complex IGF-I/IGFBP-3. Binding to ALS to form a ternary complex stabilizes the complex increasing its half-life [7, 18, 19]. The *IGFALS* defects described in this study caused ALS deficiency and the absence of ternary complex formation [5]. Serum samples also suggested a lack of binary complex. This is not surprising, since extremely low concentrations of IGFBP-3 were present in the serum of these patients.

In both subjects, ALS deficiency resulted in a biochemical profile resembling GH resistance. Similar to previously reported cases [5, 8, 9], the low levels of GH-dependent proteins were associated with an increased GH response to stimulation. Patients with ALS deficiency have been shown to have a reduction in total as well as free circulating IGF-I [16]. It is widely accepted that free IGF-I is biologically active and even a minimal reduction may be sufficient to trigger an increased GH release from the pituitary and affect metabolism at the tissue level.

Delayed puberty has been suggested to be part of the novel ALS-deficient phenotype, but this is still controver-

sial due to the few cases reported in the literature. No pubertal delay was documented in patient 1, and patient 2 was prepubertal at 10 years of age. This may suggest that pubertal delay is the result of an individual genetic background rather than of *IGFALS* defects.

Both patients reported here were male, as are the majority of reported cases with ALS deficiency. The reason for this gender diversity is still unknown, however, it is possible that, for social reasons, the presence of short stature drives more attention in boys than girls.

In this study, we have shown the deleterious effect of novel defects in the *IGFALS*. In the first case, ALS deficiency resulted from a homozygous proline to leucine substitution. Because of the different chemical properties of these two amino acids, this defect is likely to cause a profound change in the secondary structure of the ALS protein possibly leading to protein misfolding and intracellular degradation. In the second patient, a leucine to glutamine substitution at position 134 was present in compound heterozygosity, with a novel and unusual defect resulting in the replacement of the GGC triplet with an AG. The latter defect causes a premature ALS truncation. The ALS protein is characterized by a 20 leucine-rich repeat domain that contributes to its complex doughnut structure [20]. The substitution of the leucine residue at position 134 can be predicted to disrupt the ALS structure and function. In homozygosity it has been shown to be associated with undetectable ALS levels [21].

There are, to date, less than 20 reported cases of patients with ALS deficiency, many of which are siblings. This is obviously too small a number to allow conclusions on a genotype/phenotype relationship. Nevertheless, the presence of GH insensitivity characterized by mild growth failure, undetectable ALS levels, low IGF-I and IGFBP3 and normal GHBP levels, with or without pubertal delay, should call for the screening of *IGFALS*. However, it has to be noted that while, to date, all reported *IGFALS* defects result in absence of the ALS protein, mutations leading to a biologically inactive, but immunologically active protein may be present. Population screening to ascertain the frequency of *IGFALS* variants and their impact on prepubertal height would be desirable. This could aid in assessing whether a wider range of phenotypes exists and also the extent to which ALS deficiency affects final height. This is particularly relevant in view of the achievement of a near-normal final height in patients with delayed puberty [8, 22].

An important observation from this study is that parents of the index cases, obligate heterozygous carriers of the above *IGFALS* mutations, also had short stature, but to a lesser degree than their homozygote children. The same can be said for other reported cases [8, 16] and seems to suggest that single heterozygote *IGFALS* defects might be sufficient to cause growth retardation and may affect final height in selected individuals.

In conclusion, we identified novel mutations in the *IGFALS* causing ALS deficiency. This reinforces the importance of screening this gene in children with mild growth failure and a biochemical phenotype resembling GH resistance. Clinicians should be aware of this emerging new phenotype, since further studies and especially follow-up data are needed to precisely delineate the condition associated with ALS deficiency.

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