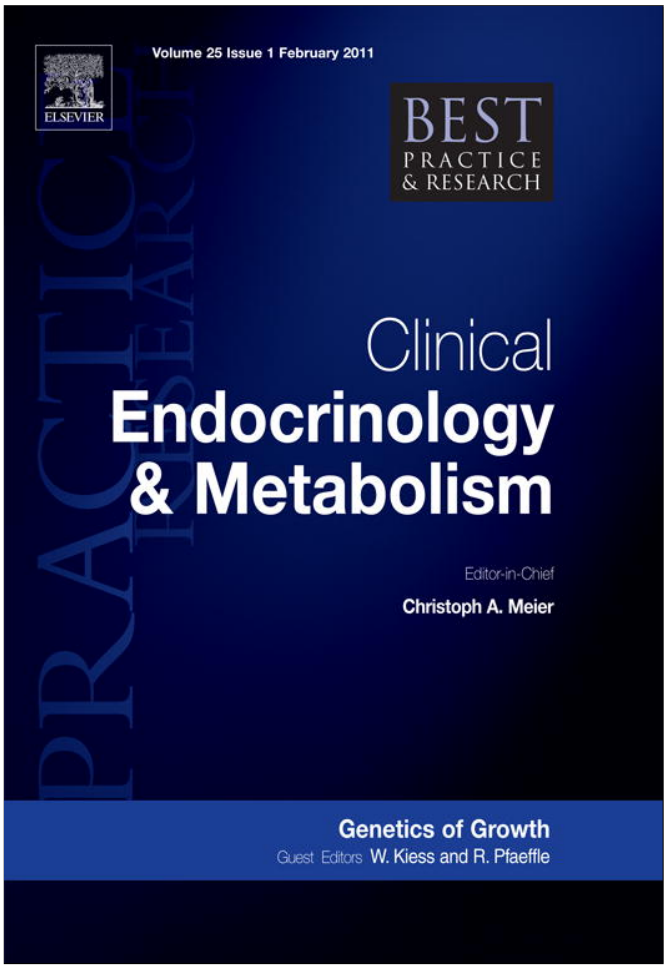

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Acid-labile subunit (ALS) deficiency

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The acid-labile subunit (ALS) protein is crucial for maintaining the integrity of the circulating IGF/IGFBP system. In humans, complete ALS deficiency is characterized by severely reduced serum IGF-I and IGFBP-3 concentrations that is incongruent with the associated mild growth retardation (height SDS -2 to -3 SDS before and during puberty). Twenty-one patients have been described with ALS deficiency, representing 16 unique homozygous or compound heterozygous inactivating mutations of the *IGFALS* gene. Pubertal delay in boys and insulin insensitivity are common findings. In the assessment of a child with short stature ALS deficiency should be consider in those patients presenting: 1) a normal response to GH stimulation test, 2) low IGF-I levels associated with more profoundly reduced IGFBP-3 levels, 3) a mild growth retardation, apparently out of proportion to the degree of IGF-I and IGFBP-3 deficits, 4) lack of response to an IGF generation test and 5) insulin insensitivity.

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The role of ALS in the IGF system

The insulin-like growth factor (IGF) system, consisting of two ligands (IGF-I and IGF-II), two receptors (IGF-I receptor, IGFIR; and IGF-II receptor, IGFIIR), and six high-affinity IGF binding proteins (IGFBP), plays a critical role in mammalian growth, as demonstrated in case studies in humans and in rodent models. Approximately 80–85% of circulating IGF-I is found in complex with IGFBP-3 (and, to a lesser extent, IGFBP-5), and an acid-labile subunit (ALS).[1] In clinical conditions of GH deficiency (GHD) and in some cases of GH insensitivity (GHI), all three components of the ternary complex are markedly reduced.

ALS is an 85 kilodalton glycoprotein that is produced almost exclusively by the liver and secreted into the circulation. The mature ALS protein belongs to the superfamily of leucine-rich repeats (LRR), a group of proteins characterized by their ability to participate in protein–protein interactions.[2] Molecular modeling of the ALS protein predicted 20 LRR motifs, arranged in a doughnut-like shape, with patches of electronegative regions within the center of the cavity capable of interacting with the IGF-I-IGFBP-3/5 binary complex.[2,3]

The main function of ALS appears to be to prolong the half-life of the IGF-IGFBP-3/IGFBP-5 binary complexes, as ALS has no affinity for free IGF-I, IGF-II or uncomplexed IGFBP-3 and IGFBP-5.[1] Free circulating IGF-I has a half-life of approximately 12 min, which is extended to greater than 12 hours when complexed to IGFBP-3 and ALS.[4] The identification of the first case of an inactivating mutation in the human *IGFALS* gene (located on chromosome 16p13.3; OMIM 601488), associated with short stature, insensitivity to GH and abnormally low serum IGF-I and IGFBP-3 levels, provided direct support for the importance of ALS in the maintenance of normal serum IGF-I and IGFBP-3 levels (5; see below).

There has been little evidence of other biological functions of ALS, although, interestingly, the recently identified fly ortholog of the mammalian ALS, the *Drosophila dALS*, was shown to regulate carbohydrate and fat metabolism, as well as growth.[6] In rodents, genetic targeted ablation of the *Igfals* gene appeared to increase sensitivity to insulin (7; see below). Hence, it is of note that some of the subjects severely deficient in ALS[8], presented phenotypes suggestive of abnormal carbohydrate metabolism (see below).

The ALS-KO (*Igfals*$^{-/-}$) mouse model

To evaluate the physiological role of ALS, a mouse model with inactivation of the *Igfals* gene was developed.[9] Unexpectedly, ALSKO mice presented with a mild growth impairment of only 13–20% at 10 weeks of age[9,10], despite a marked reduction of 62% and 88% in the circulating levels of IGF-I and IGFBP-3, respectively. Since no differences in liver IGF-I and liver and kidney IGFBP-3 mRNA abundance were observed in ALSKO mice, the reduction in IGF-I and IGFBP-3 plasma levels appears to result from an accelerated turnover, rather than a decrease in their synthesis. Although the modest effect on growth is surprising, given the central role of IGF-I in promoting linear growth, it is likely that a substantial fraction of postnatal growth could be maintained by locally produced IGF-I that does not require transportation in the circulation to exert its effects by autocrine or paracrine mechanisms.

ALS protein was absent in *Igfals*$^{-/-}$ mice and result in inability of serum to form ternary complexes after incubation with [^{125}I]IGF-I and IGFBP-3. These results emphasize the importance of ALS in the maintenance of high circulating IGF-I levels, by delaying its passage to the extravascular compartment, and preventing negative effects such as hypoglycemia and cell proliferation.[1] The unusual finding that the severity of IGFBP-3 deficiency is more pronounced than that of IGF-I, sustains the concept that ALS has a protective role on IGFBP-3, preventing its degradation by the action of proteases. Interestingly, mice with a single null ALS allele (*Igfals*$^{+/-}$) presented with low ALS levels, which result in significant reduction in serum IGF-I (17%) and IGFBP-3 (40%) concentrations, indicating a distinct phenotype for heterozygous carriers.[1,9] ALSKO mice have both normal glucose and insulin levels[9], opposite to what is observed in mice with conditional deletion of the *Igf1* exclusively in the liver (LID), where normal glucose with elevated insulin levels was observed, indicating some degree of insulin insensitivity.[7] A likely explanation for this discrepancy in insulin levels is that while GH levels are clearly elevated in the LID mice, they remain normal in the ALSKO.[7] Studies of carbohydrate metabolism in ALSKO mice also

revealed that, not only were plasma fasting levels of glucose, insulin and free-fatty acids all normal, but also a faster glucose clearance was observed during an intraperitoneal glucose tolerance test, and a trend towards higher whole body utilization and muscle glucose uptake during hyperinsulinemic-euglycemic clamping experiments.[7] These findings are indicators of a higher sensitivity to insulin in ALSKO mice, compared to controls.

The ALS-KO mice also presented retardation of bone development, characterized by a reduction in femoral periosteal circumference and cortical thickness and a lower total bone mineral density (BMD). A 7.5% reduction in femoral length was observed, with a 24% reduction in femoral cortical bone volume. The ALS-KO mice also showed a 37% reduction in trabecular bone.[10]

In addition, *in vitro* culture of marrow-derived mesenchymal stromal cells, showed a preferential differentiation to adipocytes, which might indicate that impairment of IGF-I integration into ternary complex formation in bone marrow alters cell fate, leading to increased adipogenesis.[11] However, it is not yet clear if all these alterations are a direct or indirect result of a total ALS deficiency.

Human ALS deficiency

The first description of complete ALS deficiency in human was performed in a 16 year-old boy submitted for evaluation of short stature associated with pubertal delay.[5] The finding of normal GH stimulation tests and the severe reduction of serum IGF-I and IGFBP-3 concentrations, with a lack of response to rhGH administration, prompted an investigation of the IGF system. The disproportionate severity of the IGF-I deficiency in relation to the mild growth deficit, resembling that observed in ALSKO mice, drove the investigation to the characterization of the *IGFALS* gene. A homozygous frameshift *IGFALS* gene mutation was found (p.E35KfsX87), establishing the molecular defect responsible for the ALS deficiency.[5] The absence of ALS in the circulation resulted in the generalized deficiency of the circulating, so called "endocrine IGF system," with reduction of total IGF-I and IGF-II, as well as free-IGF-I and bioactive IGF-I.[12] While IGFBP-3 levels were even more profoundly reduced, IGFBP-1 and -2 were found to be also reduced, although to a lesser extent.[12] The patient also exhibited insulin insensitivity and a reduction in bone mineral density (BMD).[13] However, the description of ALS deficiency in this unique patient made it difficult to ascertain whether these findings, as well as the association with pubertal delay, were all part of the syndrome or just coincidental, non-related features.

In the past 6 years, another 20 patients with complete ALS deficiency have been characterized at the molecular level, involving children and adolescents from diverse ethnic backgrounds[14–21]; thus, this condition appears to be more frequent than previously expected (Table 1). The analysis of auxological, biochemical and genetic data from 16 ALS-deficient patients by The International ALS Collaborative Group[8], permitted a more precise definition of the main characteristics of this condition.

It was consistently found that ALS deficiency was characterized by:

- A severe reduction in IGF-I levels
- An even more marked reduction in IGFBP-3 levels (Fig. 1)
- An out of proportion reduction in IGF-I and IGFBP-3 deficits compared to the lesser effect on postnatal growth
- An impairment of *in vitro* ternary complex formation
- Insulin insensitivity characterized by normal glucose and increased insulin levels.
- Pubertal delay that was found in about half of the male subjects
- A poor response to rhGH treatment, both in term of growth acceleration and increase in IGF-I and IGFBP-3 levels
- Reduced BMD was not consistently present

To date, four different genetic causes resulting in GH insensitivity, also known as primary IGF deficiency have been identified in human patients[22,23]: GH receptor (*GHR*) gene deficiency, or Laron Syndrome[24]; *IGF1* gene deficiency[25]; signal transducer and activator of transcription 5b (*STAT5B*) gene deficiency[26] and acid-labile subunit (ALS) gene deficiency[5] (Table 2).

Table 1
Reported mutations in the *IGFALS* gene in patients with complete ALS deficiency.

Mutation N	Protein	Type of mutation	Homozygous/ heterozygous	Ethnic origin	Reference number
1.	p.E35KfsX87	Frameshift, premature stop codon	Homozygous	Argentinean	5
2.	p.D440N	Missense	Homozygous	Turkish	15
3.	p.C540R	Missense	Compound heterozygous	Norwegian/German	16
4.	p.S195_R197dup	In-frame duplication of 3 amino acids, SLR			
5.	p.N276S	Missense	Homozygous	Spanish	17
6.	p.Q320X	Nonsense	Homozygous	Spanish	
7.	p.L497FfsX40	Frameshift, premature stop codon	Homozygous	Kurdish	18
8.	p.L437_L439dup	In-frame duplication of 3 amino acids, LEL.	Homozygous	Mayan	19
9.	p.C60S	Missense	Compound heterozygous	Jewish/Eastern European (Polish, Russian, Austrian-Hungarian)/Icelandic/ European (French, English)	
10.	p.L244F	Missense			
11.	p.L134Q	Missense	Homozygous	Indian/Pakistani	
12.	p.P73L	Missense	Compound heterozygous	Ashkenazi Jewish	
13.	p.L241P	Missense			
11.	p.L134Q	Missense	Compound heterozygous	British Asian	20
14.	p.A183SfsX149	Frameshift, premature stop codon			
12.	p.P73L	Missense	Homozygous	British Asian	
15.	p.L172F	Missense	Homozygous	Swedish	21
15.	p.L172F	Missense	Compound heterozygous	Swedish	
4.	p.S195_R197dup	In-frame insertion of 3 amino acids, SLR	Compound heterozygous		
5.	p.N276S	Missense	Compound	Spanish	22
16.	p.E35GfsX17	Frameshift, premature stop codon	Heterozygous		

The amino acid numbering system was based on the precursor protein, with the initial methionine numbered as +1.

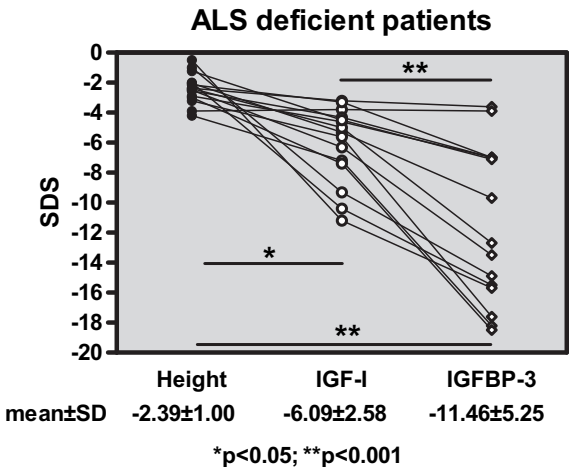


Fig. 1. Height and IGF-I, IGFBP-3 and ALS levels are expressed in SDS in 21 children and adolescent with ALS deficiency.

Table 2
Growth hormone insensitivity syndromes.

1) Congenital defects
GH receptor (*GHR*) gene defects
Extracellular domain mutations
Transmembrane domain mutations
Intracelular domain mutations
GH signal transduction defects
STAT5b gene mutations
IGF transport defects
IGFALS gene defects
IGF-I synthesis or action defects
IGF1 mutations (including bioinactive IGF-I)
IGF1R gene mutations
2) Acquired defects
Malnutrition
Parenchymal liver diseases
Type 1 diabetes
Catabolic states
Chronic inflammatory
GH neutralizing antibodies

Molecular defects at the *IGFALS* gene

Spectrum of IGFALS *gene mutations*

The human *IGFALS* gene maps to chromosome 16, at position 16p13.3. Exon 1 encodes the first 5 amino acids of the signal peptide and the first base of the codon corresponding to the sixth amino acid of the protein, exon 2 encodes the two last bases of codon six and the remaining 599 amino acids.[27] The signal peptide comprises the first 27 amino acids and the mature protein consists of 578 amino acids organized in 20 repeating leucine-rich domains (LRR) of 24 amino acids each, flanked by two amino- and carboxy-terminal regions containing 13 cysteine residues.[2] The protein contains 7 asparagine residues that are potential glycosylation sites.[3] The leucine-rich domains account for 75% of the protein and are organized into a doughnut-shaped structure.[2] The center hole of the doughnut is enriched in negative charges and is likely to be involved in binding to positive charges present in the carboxyl-terminal region of IGFBP-3, rich in basic residues.[2]

To date, 21 patients with complete acid-labile subunit deficiency have been characterized at the molecular level[5,14–21] (Table 1). Only three patients are female, probably the result of a more frequent concern of parents for the height of their male offspring. Sixteen different mutations of the human *IGFALS* gene have been identified in the twenty-one patients studied from sixteen different families (Fig. 2). Twelve patients were found to be homozygous and nine were compound heterozygous. Approximately one third of the patients (8 patients from 3 families) were familial cases. When the parents of the affected subjects were available for the characterization of the *IGFALS* gene, they presented the same mutations as their affected children, indicating an autosomal recessive pattern of inheritance. Consanguinity was present in three families, absent in eleven families (including eight patients, three of them siblings), and unknown in the other two families.

All the mutations were located in exon 2, including missense (56%), frameshifts with premature stop codon (25%), in-frame insertions (13%), and nonsense (6%) (Table 1, Fig. 2). The majority of *IGFALS* gene mutations produce defects in the leucine-rich repeat region of the protein (13 out of 16): 8 were missense, 1 nonsense, 2 frameshift, and 2 were in-frame duplication mutations. Two frameshift mutations were detected in the region encoding the amino terminal flanking domain, and one missense mutation in the region encoding the carboxyl-terminal flanking domain.

Two mutations (Cys60Ser in the amino-terminal domain, and Cys450Arg in the carboxy-terminal domain), result in the loss of two highly conserved cysteine residues in LRR proteins, most likely involved in disulfide bonds. Thus, it is very likely that these mutations preclude formation of disulfide

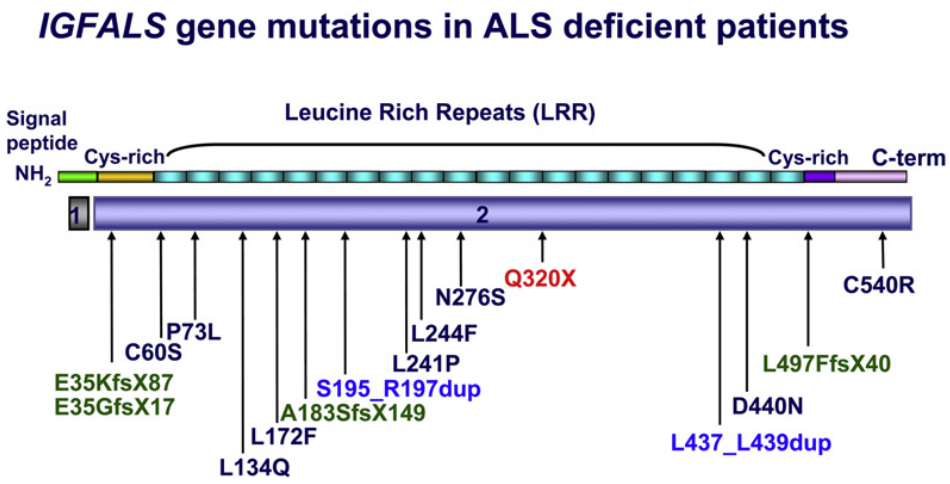


Fig. 2. Schematic representation of the ALS protein indicating the location of the 16 identified human mutations. Black: missense; green: frameshift; red: nonsense; light blue: duplication.

bridges or lead to erroneous pairing of cysteines, thereby disturbing the integrity of the spatial arrangement of the ALS protein. The ALS protein is unique among the RI-like subfamily (porcine ribonuclease inhibitor, the only LRR protein with a solved crystal structure) in having 20 LRR domains, 5 more than the other members of this subfamily. This extension makes it possible for ALS to adopt a doughnut-like structure with the amino- and carboxy- terminal domains in close proximity and able to form disulfide bridges.[2] An interesting aspect of *IGFALS* mutations is the finding of two different nine base in-frame duplications (c.583_591dup and c.1308_1316dup) in the seventh and seventeenth LRR, respectively, resulting in the insertion of three amino acid residues (p.Ser195_Arg197dup and p.Leu437_Leu439dup). These duplication mutations alter the length of one LRR repeat, thereby impairing the alignment of the hydrophobic residues and probably disrupting the spatial conformation of the protein. Similar mutations involving insertion or deletions of 3 to 8 amino acids have been reported in the gene encoding nyctalopin.[28] The large stretch of similar and repeated amino acid composition, that implies a similar nucleotide sequence, might represent a potential source for mistakes in the positioning of DNA polymerase at the time of DNA replication.

All the described mutations result in either completely absent or barely detectable ALS levels, suggesting that the mutated proteins are unable to be normally expressed or secreted. Alternatively, they could be unstable and rapidly degraded after secretion, or have structural changes which alter their recognition by antibodies. The *in vitro* expression and determination of ternary complex formation by the mutant proteins is required to characterize their biological impact. However, only the p.Asp440Asn (mature mutant peptide designated p.Asp413Asn-ALS) has been characterized by *in vitro* expression.[29] This mutant ALS was inefficiently secreted, remaining trapped inside the cells. The recombinant p.Asp440Asn protein also displayed a reduced ability for *in vitro* ternary complex formation when incubated with IGF-I and IGFBP-3, probably because the p.Asp440Asn substitution resulted in a new potential N-glycosylation site, that disrupted the acidic internal surface, a proposed IGFBP-3 binding site. Except for the mutations that predict a truncated and probably inactive protein, the *in vitro* expression of other mutated ALS-proteins is essential to determine their impact on the levels of expression and secretion, as well as to characterize the effects on ternary complex formation with IGF-I and IGFBP-3.

Impact on growth and development

Although homozygous and compound heterozygous mutations of *IGFALS* result in marked reductions of serum concentrations of both IGF-I and IGFBP-3, growth retardation is surprisingly modest. Indeed, since adequate studies of the prevalence of *IGFALS* mutations in the normal population have not yet been performed, it is conceivable that the reports of poor growth reflect some degree of

ascertainment bias, i.e., children with short stature have serum levels of IGF-I and IGFBP-3 measured and, when the patients are found to have low levels, the possibility of ALS deficiency is entertained.

Given these caveats, it is, nevertheless, worthwhile to evaluate the 21 patients identified to date. Data concerning prenatal growth are relatively scarce, although are suggestive of a mild reduction in weight at birth.[8] Growth attenuation has been reported to primarily affect postnatal growth. The mean height SDS at diagnosis was -2.31 ± 0.87, with 62% having heights below -2SD. Heights ranged from: -3.61 to -0.39 SD.[8] Pubertal delay has been noted in approximately 50% of the patients, so that short stature in childhood is, at least on occasion, partially compensated by a prolonged adolescent growth phase.[8] When homozygous or compound heterozygote patients are compared with their wild-type relatives, they appear to be approximately 2 SD shorter during childhood, but only 1 SD shorter as adults.[8]

Metabolic consequences

Insulin insensitivity is a common finding in ALS deficiency

Carbohydrate metabolism has been described in 11 ALS-deficient patients.[8] While fasting glucose levels were normal, all but one presented with elevated basal or glucose-stimulated insulin levels, elevated HOMA index (homeostasis model assessment index) or low IGFBP-1 levels, an indirect marker for insulin insensitivity.[30] These data suggest that patients with ALS deficiency exhibit some degree of insulin resistance. Although, the pathophysiological mechanism involved is not completely understood, moderately elevated GH levels reported in these patients may contribute to impaired insulin action by multiple mechanisms (increased levels of free-fatty acids by the lipolytic effect of GH excess[31,32], impairment of insulin signaling by cross talk between GH receptor and insulin receptor signaling pathways[33], among others). The diabetogenic properties of GH were first described in the 1930's when Houssay reported that hypophysectomy reduced the hyperglycemia of experimental diabetes in dogs.[34] However, it seems unlikely that the mild elevation of GH levels observed in ALS deficient patients can be the only cause of insulin insensitivity. Since IGF-I is able to improve glucose uptake, particularly by skeletal muscle[35], it is likely that the marked decrease in IGF-I levels may also contribute to the insulin insensitivity observed in these patients. The increase in GH secretion, reduction in circulating IGF-I levels or the interaction between these factors could be involved, but the relationship between the GH axis and carbohydrate metabolism is complex and only partially understood.[36]

Marked insulin resistance is observed in the liver-specific IGF-I knock-out mouse (LID)[37,38], presenting with a 75% reduction in circulating IGF-I and fivefold increase in GH levels. However, the ALS-KO mice presenting similar degree of IGF-I deficiency but normal GH levels, demonstrate comparable insulin sensitivity as controls.[7] The double KO mice (LID + ALSKO) presented unexpected results. They showed the most profound changes in the GH/IGF-I axis, having an 85% reduction in IGF-I and a 15-fold increase in GH. But despite presenting with elevated insulin levels, they showed an improved glucose uptake at muscle and fat levels, with no improvement in hepatic glucose production compared with the LID mice.[7] These findings underline the complexity in the regulation of carbohydrate metabolism by the GH/IGF-I system and demonstrate the difficulties involved in the direct extrapolation of data from animal models to an understanding of human physiology. More dynamics studies on insulin sensitivity in ALS deficient patients are required to characterize the mechanisms determining insulin resistance in this condition.

Reduced bone mineral density (BMD) is not frequent in ALS deficiency

Given that ALSKO mice exhibit reduced bone mineral density[10], it was not surprising that the first reported case with ALS deficiency presented at 16 years of age with a marked reduction in BMD (-4.7 SDS) at the lumbar spine level. Partial recovery (to -2.1 SDS) was found by the end of puberty at 19 years of age.[5,13] After the finding that two brothers with complete ALS deficiency and a reduced BMD presented with fractures after minor trauma, the involvement of ALS deficiency in the etiology of osteopenia evoked further interest.[17] However, in this consanguineous family from the Kurdistan, low BMD was also noticed in heterozygous and wild-type carriers, suggesting that this effect was

independent of the ALS defect. More recently BMD was found to be normal in other patients having ALS deficiency.[16,18] Because pubertal delay was present in some ALS deficient patients, slow pubertal development could be considered a negative factor in the process to achieve normal bone mineralization. The impact of chronically deficient circulating IGF-I levels, the lack of ternary complexed IGF-I, or even the absence of ALS by itself, are all potential etiologic factors for the BMD alterations observed in some, although not all, ALS deficient patients. Here again, more in depth studies are needed to improve our understanding about the role of ALS, not only on bone mineralization, but also on the architectural structure of both trabecular and cortical bone.

Diagnosis of ALS deficiency

If an ALS determination is not readily available, during the evaluation of a child with short stature, the determination of IGF-I, IGFBP-3, and GH provocative tests may be enough to select potential candidates for the characterization of the *IGFALS* gene. Alternatively, when [^{125}I]-IGF-I is available, *in vitro* ternary complex formation resolved by size exclusion chromatography, may well be the most specific method to display functional inability for 150 kDa ternary complex formation, the main feature of ALS absence.[5]

ALS deficiency appears to be the only clinical condition in which IGFBP-3 levels are more markedly affected than IGF-I. In addition, the deficit of these two markers appear out of proportion in relation to the growth deficit. The finding that all described mutations in the *IGFALS* gene reside in exon 2, makes the molecular assessment relatively accessible in terms of cost/benefit relationship.

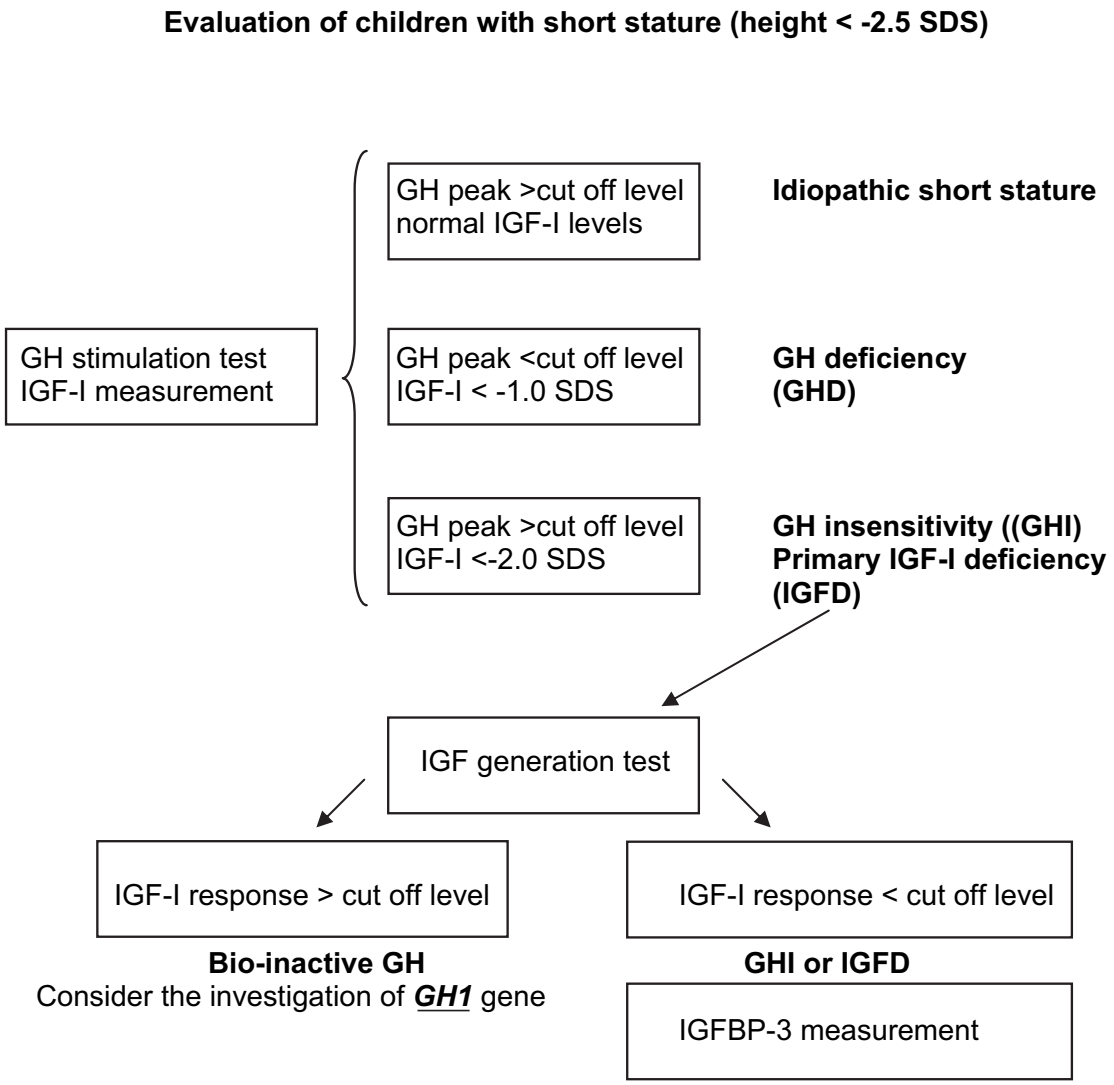
Table 3 illustrates a flow-chart for the study of children with short stature, with special attention to the characterization of already known gene defects associated with GH insensitivity/primary IGF-I deficiency syndromes, particularly ALS deficiency.

Effect of heterozygosity for IGFALS *gene mutations*

ALS has a lower association constant for binary complexes of IGF-I and IGFBP-3 in comparison to the high affinity of IGF-I for IGFBP-3, and under normal circumstances circulates in a large molar excess in comparison to IGF-I and IGFBP-3.[1] As a result of this, mice with a single null ALS allele, that secrete less ALS, showed significant reductions of IGF-I (17%) and IGFBP-3 (40%) levels.[1,9] In humans, first degree relatives of ALS deficient patients, who are heterozygous (HC) carriers for a mutated *IGFALS* gene allele, also presented with subnormal levels of ALS, IGFBP-3 and IGF-I.[12,15,17] Furthermore, in HC relatives of ALS deficient patients, height was found to be approximately 1.0 SDS lower than family members who were wild type for both alleles of the *IGFALS* gene.[39] These findings indicate that two functional active *IGFALS* gene alleles are required to maintain normal ALS levels and to fulfill growth potential. Since low IGF-I levels have been reported in about 30% of idiopathic short stature (ISS) subjects, the *IGFALS* gene could be involved in the etiology of short stature in a subset of these children, specially in those presenting with diminished levels of both IGF-I and IGFBP-3. It is likely that mild cases of ALS deficiency, resulting from less detrimental gene mutations affecting both *IGFALS* alleles and even one severe gene mutation in heterozygosis may account for a subset of ISS children.

The *IGFALS* gene is highly polymorphic and both synonymous and non-synonymous single nucleotide polymorphisms (SNPs) have been reported in the general population. Although the impact of these SNPs on circulating levels of components of the IGF system has not been completely characterized, preliminary findings have found that non-synonymous *IGFALS* SNPs are more frequently found in ISS compared to children of normal height. Indeed, heterozygous defects of the *IGFALS* gene have been reported in an important percentage of ISS children: 9.6% in 52 ISS children by David et al[40] and 10.1% in 89 ISS by Scaglia et al.[41] In addition, prevalence for heterozygous mutations increased in children presenting with low IGF-I levels (17.8%) and even further in those showing concomitant reduction of both IGF-I and IGFBP-3 levels (55.6%). In families of ISS children, heterozygous carriers for *IGFALS* gene mutations were shorter and presented lower levels of IGF-I, IGFBP-3 and ALS compared to relatives having two wild type alleles.[42] These findings suggest that both *IGFALS* gene alleles are required to maintain a normal circulating IGF system and to fulfill growth potential. Functional characterization of mutant ALS-proteins is required to confirm the involvement of this gene in the etiology of short stature in a subset of ISS children.

Table 3
Flow chart for the diagnosis of gene defects resulting in GH insensitivity (GHI) or primary IGF deficiency (IGFD).



1) If IGF-I SDS is more reduced than IGFBP-3 SDS
and height SDS deficit is proportional to IGF-I SDS or IGFBP-3 SDS deficiencies,
a) consider the investigation of ***GHR*** **(GH receptor) gene**
b) if in addition a history of respiratory infections and evidence of cellular immunodeficiency is present, consider the investigation of ***STAT5B*** **molecular defects**

2) If IGFBP-3 SDS is more reduced than IGF-I SDS
and height SDS deficit is less pronounced than IGF-I SDS or IGFBP-3 SDS deficiencies consider the investigation of ***IGFALS*** **gene defects**

Summary and perspective

ALS deficiency constitutes the first monogenic defect involving an IGF binding protein. From the original description in 2004, human ALS deficiency have been described in 21 subjects from South and North America and in Europe in patients from diverse ethnical background, indicating that this condition may be more prevalent that previously expected. In contrast to other autosomal recessive diseases, most of ALS deficient patients belong to non-consanguineous families and, in addition, near fifty percent are compound heterozygous for inactivating mutations in the *IGFALS* gene. These findings suggest that heterozygous carriers could be present in the general population, given that these mutations are unlikely to be under a strong negative selection pressure, and are the source of complete ALS deficiency in non-consanguineous families.

Complete ALS deficiency result in a peculiar type of GH insensitivity/primary IGF-I deficiency. While the lack of ALS protein resulted in the absolute disruption of the entire circulating IGF system and a severe deficiency of circulating IGF-I ("endocrine IGF-I"), local production of IGF-I ("autocrine/paracrine IGF-I") would remain unaffected. The magnitude of postnatal growth retardation in ALS deficient patients is less pronounced than might be predicted from the observed IGF-I deficit, indicating that other mechanisms may partly compensate for the circulating IGF-I deficiency. Locally produced IGF-I, moderately increased levels of GH, and rapid efflux of circulating IGF-I to the extravascular compartment may all be involved in maintaining linear growth near or even within normal limits.

It should be noted that not a single patient with a complete inactivation of other IGF binding proteins (IGFBP-1 to -6) has yet been described, suggesting that due to the overlapping and relatively redundant actions of these proteins, a deficiency in one IGFBP may well be compensated for by the remaining IGFBPs. The requirement for ALS in ternary complex formation, and the resulting effect in the extension of IGF-I and IGFBP-3 half-life, however, could not be compensated by other members of the IGFBP family.

Other important lessons from the study of ALS deficient families is the observation that heterozygous carriers are approximately 1.0 SD shorter than wild type relatives, and also presented with lower serum levels of IGF-I, IGFBP-3 and ALS. These observations are in agreement with the concept that expression from both *IGFALS* gene alleles are required to maintain a molar excess of circulating ALS in order to stabilize IGF-IGFBP-3 binary complexes, due, in part, to the relatively low affinity of ALS for these complexes, and that normal levels of circulating IGF-I are required to fulfill growth potential. In support of this hypothesis, heterozygous carriers for presumably deleterious genetic variants in a subset of children with idiopathic short stature presented low levels of the three components of the IGF ternary complex: IGF-I, IGFBP-3 and ALS.[42]

Although ALS deficient patients have shown poor clinical response to GH treatment, it is likely that heterozygous carriers presenting short stature may benefit with GH treatment, considering that by acting on the remaining intact *IGFALS* allele, an increase in circulating ALS would be expected, and consequently, incremental increases in both IGF-I and IGFBP-3. Whether genotyping the *IGFALS* gene in ISS children would be a good predictor of growth acceleration in these children, however, is something that should be determined.

The other clinical concern in ALS deficient patients is the effect of long term circulating IGF-I deficiencies on carbohydrate metabolism and bone mineralization. Although the pathophysiological mechanisms of insulin insensitivity, frequently observed in these patients, are not completely understood, preliminary observations indicated that they positively respond to treatment with metformin by normalizing both basal and stimulated glucose and insulin levels (unpublished observation in the ALS deficient patient described in reference 5). Further studies are required for a better characterization of this insulin insensitivity and prospective studies to determine the best therapeutic approaches. Although osteopenia has been described in only a few ALS deficient patients, the deleterious consequences of long-term reduction in BMD point to the necessity for careful studies evaluating the impact of chronic IGF-I deficiency on bone architectural and mineralization.

Finally, it is unknown if all ALS actions are exerted through the modulation of IGF-I bioavailability, or if IGF-independent ALS actions are involved.

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Practice points

Diagnosis of ALS deficiency

Although there is no information regarding prevalence of ALS deficiency in short children, this condition should be investigated when they present most of the following characteristics:

- A normal response to GH stimulation test
- Low levels of IGF-I associated to more profoundly reduced IGFBP-3 levels
- A mild growth retardation, apparently out of proportion to the degree of IGF-I and IGFBP-3 deficits
- Lack of response of IGF-I and IGFBP-3 to an IGF generation test
- Insulin insensitivity characterized by normal glucose levels in the face of elevated insulin levels
- Pubertal delay, specially in male patients

Research agenda

- The mechanism involved in maintaining linear growth near normal limits despite profound circulating IGF-I deficiency needs to be clarified (free- or locally-produced IGF-I, increased IGF-I efflux, etc).
- When and how insulin resistance should be treated in these patients?
- More studies are required to determine the effect of chronic IGF-I deficiency on bone architecture and mineralization.
- The characterization of heterozygosity for *IGFALS* gene mutations in idiopathic short stature and its impact on the IGF system and height need to be determined.
- Whether all physiological consequences of ALS deficiency are related to circulating IGF-I deficiency or if there is a direct effect of a lack of ALS itself, should be further investigated.
- Should those children presenting short stature having complete or partial ALS deficiency (homozygous or heterozygous for *IGFALS* gene mutations) be treated with GH, IGF-I or the combination of both?

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