

## Analysis of acid-labile subunit and its usefulness in pediatrics

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### ABSTRACT

The acid-labile subunit (ALS) is an 85 kDa glycoprotein that belongs to the leucine-rich repeat superfamily. It mainly circulates in serum bound to a high molecular weight ternary complex.

The main and most widely studied function of ALS is to prolong the half-life of the binary complex formed by insulin-like growth factors type 1 and 2 and its transport proteins 3 and 5. ALS serum levels are lower in neonates, reach a peak in late puberty, and then slowly decrease throughout adulthood. ALS deficiency has consequences on growth, hydrocarbon and bone metabolism, and, in some cases, it affects pubertal development. To date, 25 patients with complete ALS deficiency due to *IGFALS* gene mutations have been found.

**Key words:** acid-labile subunit, insulin-like growth factor-1, short stature.

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### GLOSSARY

ALS:	acid-labile subunit
ALS-D:	complete acid-labile subunit deficiency
BMD:	bone mineral density
GH:	growth hormone
IGF:	insulin-like growth factor
IGFBP:	insulin-like growth factor-binding protein
kDa:	kilodalton
LRR:	leucine-rich repeats
SDS:	standard deviation score

### INTRODUCTION

The acid-labile subunit (ALS) is an 85 kilodalton (kDa) glycoprotein that belongs to the leucine-rich repeat (LRR) superfamily, characterized for its involvement in protein-protein binding. The gene responsible for encoding it is located in chromosome 16 and comprises 2 exons and 1 intron.<sup>1</sup>

This glycoprotein is secreted by the liver in response to growth hormone (GH) and mainly circulates

in serum bound to a high molecular weight (150 kDa) ternary complex. In such complex, ALS is associated with some of the proteins of the insulin-like growth factor (IGF) family and one of the insulin-like growth factor-binding proteins (IGFBP): IGFBP3 or IGFBP5.<sup>2,3</sup>

The IGF family is comprised of 2 peptide hormones synthesized by the liver: IGF1 and IGF2. These share approximately 50% of their amino acid sequence and a large portion of their sequence displays homology to proinsulin but, unlike the latter, they retain C-peptide and are therefore longer. IGF actions and bioavailability are regulated by a family of 6 IGF-binding proteins, known as IGFBP1, 2, 3, 4, 5, and 6,<sup>4</sup> together with ALS. Of these, only IGFBP3 and IGFBP5 can be part of the ternary complex.<sup>5</sup>

### Molecular description

For human ALS, the *IGFALS* gene encodes approximately 3300 base pairs, located in chromosome region 16p13.3, and is comprised of 2 exons separated by 1 intron. Exon 1 encodes the first 5 amino acids of the signal peptide and the first codon base corresponding to the 6<sup>th</sup> amino acid; exon 2 encodes the last 2 codon bases of the 6<sup>th</sup> codon and the remaining 599 amino acids.<sup>1</sup> The signal peptide comprises the first 27 amino acids. The mature protein is made up of 578 amino acids organized into 3 domains: 1 N-terminal domain, 1 central domain containing 21 LRR of 24 amino acids each (comprising 75% of ALS), and 1 C-terminal domain; both terminal domains contain cysteine-rich residues. The protein folds into a doughnut-shaped structure with LRR-1 aligned closely to LRR-20.<sup>3</sup>

The first structural model was

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described in 1999. More recently, David et al.<sup>2</sup> have introduced a new model analyzing ALS glycosylation, charge distribution, and the mechanisms by which missense mutations affected the protein structure.<sup>2</sup> This structural model contains 6 disulphide bridges, which are mainly clustered towards the N-terminus (LRR-1) and the C-terminus (LRR-20). It also proposes 7 potential N-glycosylation sites; 6 of these are located towards the N- and C-terminal ends whereas 1 site is located in the central region of the ALS (LRR-14). Fourteen potential O-glycosylation sites are predicted: 8 are located towards the N- and C-terminal ends; 12 of these 14 sites are located on the outer convex surface and 2 on the inner concave surface. N- and C-terminal regions are rich in proline. In relation to the electrostatic potential, this model shows positively charged regions on the outer surface and a predominance of negatively charged regions on the inner concave surface.<sup>2</sup> Such structure may facilitate the interaction with the C-terminal region of IGFBP3, which is rich in basic residues and is therefore positively-charged.<sup>3</sup>

It was also shown that the removal of all negatively charged sialic acids on the glycosylated chains reduced the affinity of ALS for the binary complex but did not disrupt the formation of the ternary complex.<sup>6</sup>

### Synthesis and regulation

ALS synthesis is mainly restricted to hepatocytes.<sup>7</sup> Serum is probably the greatest source of extravascular ALS although in certain tissues synthesis also occurs locally. Using sensitive techniques, extrahepatic *IGFALS* gene expression has been found in the kidneys, developing bone, mammary glands during breastfeeding, thymus, and lungs.<sup>3,7</sup>

Regardless of its source, extravascular ALS may regulate local IGF action by binding to the ternary complex.<sup>8</sup>

The initiation of ALS synthesis is one of the last events in the development of the IGF system; ALS is not detectable in humans at 27 weeks of gestation but it is present in infants born at term.<sup>9</sup> Free ALS accounts for 30% of the total glycoprotein throughout fetal life. This percentage increases during childhood until reaching a peak of approximately 70% during puberty.<sup>10</sup>

GH is the most potent inducer of ALS mRNA in the liver and, therefore, of ALS plasma circulating levels.<sup>4</sup> This is evidenced by the marked reduction or even the absence of ALS

levels among patients with GH deficiency.<sup>11</sup> Studies in rats have shown that such GH effects on the liver are direct and occur at the transcriptional level of the *IGFALS* gene.<sup>12,13</sup>

There are also several conditions that reduce ALS serum levels, e.g., malnutrition, fasting, catabolic diseases, diabetes, brain injury, and cirrhosis.<sup>14-20</sup>

### Functions

The main and best-known function of ALS is to prolong the half-life of the binary complex IGF1-IGFBP3/IGFBP5. The half-life of free IGF1 is 12 minutes; however, the IGFBP-3/IGF-I complex with ALS prolongs IGF half-life to more than 12 hours. Thus, the ternary complex becomes the main IGF reservoir and regulates IGF bioavailability and bioactivity.<sup>21</sup> ALS also acts by regulating IGF action in certain extravascular tissues, forming the complex at a local level. The inhibitory role of ALS on IGF actions is consistent with the observation that generalized overexpression of ALS causes growth retardation in 4- and 8-week-old mice.<sup>22</sup>

It is worth noting that ALS has no affinity for free proteins, so the IGF1-IGFBP3/IGFBP5 binary complex needs to be formed before the ternary complex.<sup>8</sup>

ALS also plays an important role in the prevention of non-specific metabolic effects caused by IGFs, such as hypoglycemia. This is because IGFs in the ternary complex cannot cross the capillary endothelial barrier and activate the insulin receptor, thus restricting IGFs' insulin-like intrinsic effects.<sup>23</sup>

### Complete acid-labile subunit deficiency Effect on insulin-like growth factor system

The absence of circulating ALS causes a generalized deficiency of the so-called "IGF system," with reduced IGF1 and IGF2 levels; IGFBP3 levels are markedly reduced, and both IGFBP1 and IGFBP2 are also low.<sup>24</sup> A characteristic of such deficiency is a disproportionate reduction in IGF1 and IGFBP3 levels relative to the effect on postnatal growth because, in most cases, height before and after puberty ranges between -2 and -3 standard deviation score (SDS).<sup>25</sup>

GH in these patients is normal or sometimes even high when the sample is collected spontaneously or using a stimulation test; such effect may be described as a result of IGF1 negative feedback deficiency on GH secretion.<sup>26</sup> In children with complete ALS deficiency (ALS-D) it has been observed a birth weight below the mean -2.23 to -0.08 SDS; this suggests a potential effect

on prenatal development, although its mechanism is still uncertain.<sup>25</sup>

### Metabolic effects

Among patients with ALS-D for whom data were collected, fasting glucose was normal. Insulin resistance was suggested in 10 out of 11 patients because they met 1 or more lab test criteria as follows: fasting blood insulin level > 15 mU/L, insulin peak > 150 mU/L post-glucose (oral glucose tolerance test), blood insulin level at 120 minutes > 75 mU/mL or a glucose homeostasis index > 3.<sup>25</sup>

These data suggest that ALS-D has implications on hydrocarbon metabolism. The pathophysiological mechanisms involved in such alteration are still unknown. The slightly elevated GH levels reported in these patients may contribute to this deficiency in insulin action due to multiple mechanisms, such as an increase of free fatty acids because of the lipolytic effect, which is a signal intertwining effect between GH receptor and insulin receptor signaling pathways, among other factors.

Anyhow, this slight increase in GH levels does not seem to be the only cause of insulin resistance in these patients. It has been shown that IGF1 improves glucose absorption, especially in the skeletal muscle,<sup>27</sup> and it has been suggested that a marked reduction in IGF1 may contribute to the insulin resistance observed in these patients. A slight increase in GH secretion, a reduction in IGF1 levels or the interaction of these two factors may be the potential cause of the disorder in hydrocarbon metabolism.<sup>21</sup>

### Bone effects

IGF1 plays a key role in bone tissue acquisition and maintenance, and ALS is responsible for the increase in IGF1 half-life; therefore, a reduced half-life of circulating IGF1 may be the cause of the effects on bone mineral density (BMD). A reduction in BMD has been described in 3 patients with ALS-D.<sup>28,29</sup> This finding may be consistent with those reported in mice with ALS gene inactivating deletions, which showed significant differences compared to controls in terms of BMD, femur length, and cortical thickness.<sup>30</sup>

### Other effects

One half of patients with ALS-D have also shown delayed puberty, predominately males, although the mechanism of this effect has not been studied yet.<sup>25</sup>

### Effect of mutation on simple heterozygosity

To assess the effect of ALS gene mutations on simple heterozygosity, the International ALS Society conducted a study on family members of patients with ALS-D. They used data collected for 21 patients who were homozygotes or compound heterozygotes, and for 44 family members who were heterozygous carriers<sup>36</sup> or who lacked ALS gene mutations<sup>8</sup>. This study demonstrated a potential dose effect because homozygotes and compound heterozygotes were approximately 1.5 SDS shorter than heterozygous carriers, and, in turn, these were an average of 1 SDS shorter than individuals without mutations.<sup>21</sup>

However, in 2014, a family was described to have 3 members with simple heterozygosity for an *IGFALS* gene mutation that resulted in a premature stop codon, and their height ranged between -2 and -3.2 SDS.<sup>31</sup> These data emphasize the need for more extensive studies to investigate the dose effect in detail and considering the severity of molecular damage.

### When should complete acid-labile subunit deficiency be suspected?

ALS-D should be suspected in patients with the following:

- Normal or exaggerated response to GH stimulation test.

- Low IGF1 serum levels with profoundly reduced IGFBP3 serum levels.

- Mild growth retardation relative to the degree of IGF1 and IGFBP3 deficiency.

- Lack of response to a IGF1 generation test.

The following flow chart (*Figure 1*) is proposed for the assessment of children with short stature (< -2.5 SDS) and suspected ALS-D.

### Clinical laboratory assessment

The first step when ALS-D is suspected is to check blood ALS levels. An enzyme immunoassay is the most commonly used tool to this effect. There are tests in the market to measure ALS levels that offer adequate specificity. This is a sandwich test that uses 2 monoclonal antibodies with high affinity against ALS.<sup>32</sup>

In relation to the expected values for children and adolescents without somatotrophic axis disorders, several studies have reported that ALS serum levels are lower in neonates, increase continuously until reaching a peak during late puberty, and then slowly decrease throughout adulthood.<sup>4,10,33-35</sup> In relation to preterm infants, their ALS levels are lower than those of term

infants.<sup>10</sup> Several publications have reported differences in terms of sex, indicating that girls have higher ALS levels than boys<sup>33-35</sup> whereas at least one report found no significant differences between male and female individuals.<sup>10</sup>

### Molecular testing assessment

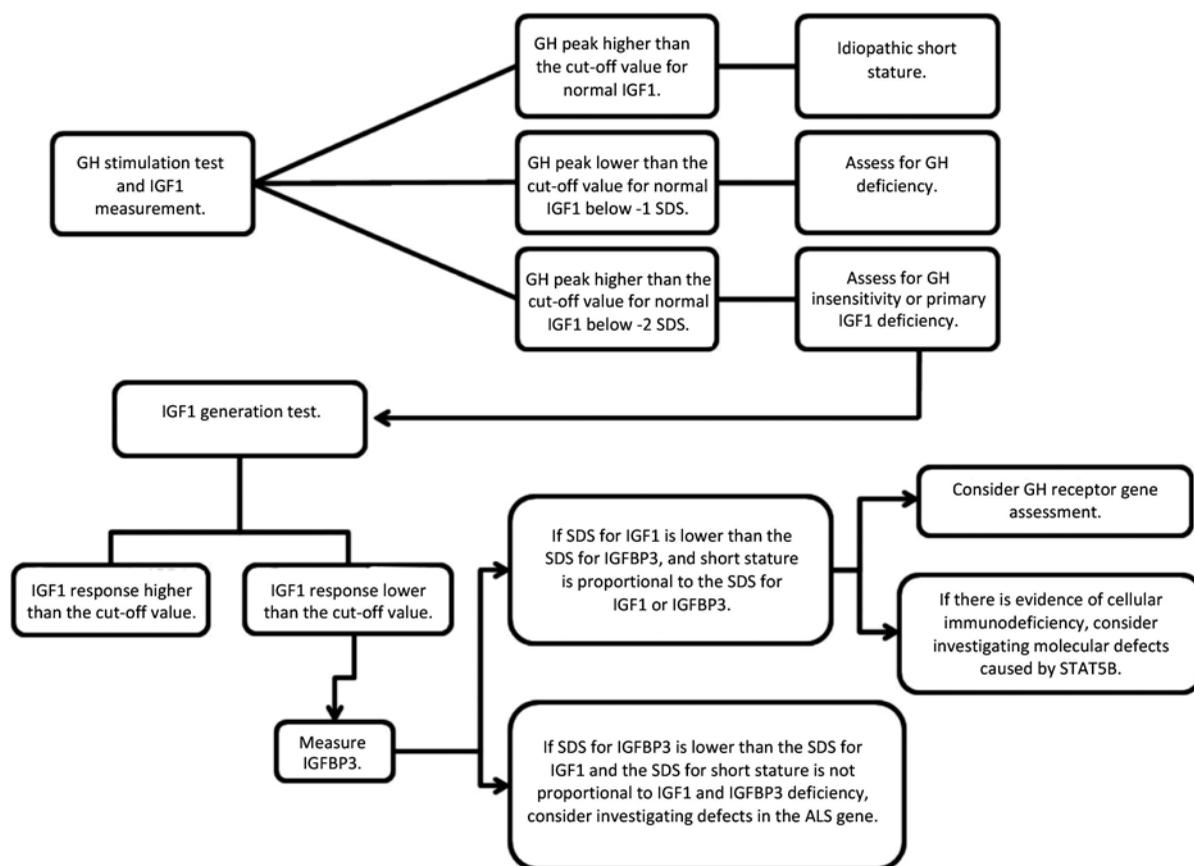
Once it has been checked that ALS serum levels are reduced or undetectable, the next step is to find the molecular origin of this condition. Tests to look for potential mutations are done using genomic DNA, which may be collected from peripheral blood leukocytes. Specific primers are applied to amplify the *IGFALS* gene using a polymerase chain reaction assay. DNA fragments are first visualized in agarose gel and subsequently sequenced. Such DNA sequencing is compared to normal *IGFALS* gene sequencing

to look for discrepancies.<sup>21,36</sup> Any genomic variant observed should be tested using the databases available from the 1000 Genomes Project to assess its frequency in the reference population and classify it as a potential deleterious variant. *In silico* prediction studies serve as a strategy to guide the potential pathogenicity of the variant found. Recent studies have shown results on this type of testing.<sup>37</sup>

### Mutations

The first case of an inactivating mutation in the human *IGFALS* gene associated with short stature, GH insensitivity, and abnormally low IGF1 and IGFBP3 levels, causing a frameshift with a premature stop codon was detected in Argentina by Domené et al. in 2004.<sup>38</sup> Since that, 20 mutations have been identified (Figure 2).

FIGURE 1. Flow chart of the differential diagnosis of patients suspected of acid-labile subunit deficiency



SDS: standard deviation score; GH: growth hormone; IGF1: insulin-like growth factor-1; IGFBP3: insulin-like growth factor-binding protein; ALS: acid-labile subunit; STAT5B: signal transducer and activator of transcription 5B.  
Flow chart translated and modified by Domené et al., 2011.<sup>21</sup>

Twenty-four patients from 19 families have been reported to have one of these different 20 mutations described to date in the *IGFALS* gene together with congenital ALS-D. The 19 families come from various ethnic origins and countries of residence. Sixteen patients had a homozygous mutation and 9 had a compound heterozygous mutation<sup>21,24,29,36,38-40</sup> (Table 1). Only 5 patients were girls, probably because mild short stature does not call the attention of parents, and this may result in an underestimation of the proportion of girls with this condition.

An autosomal recessive inheritance pattern has been demonstrated.<sup>21</sup> In addition, simple heterozygous mutations have been identified.<sup>31,37,47</sup>

The variety of *IGFALS* gene mutations, either homozygous, simple or compound heterozygous ones, covers most of the gene and are all located in the area encoded by exon 2 (Figure 2).

#### Clinical usefulness

Patients diagnosed with ALS-D fail to adequately respond to treatment with GH. Therefore, the main use for the assessment of children with clinical suspicion of ALS-D is trying to prevent metabolic, bone, and pubertal

development consequences that may affect their quality of life and avoid ineffective treatments.

#### DISCUSSION

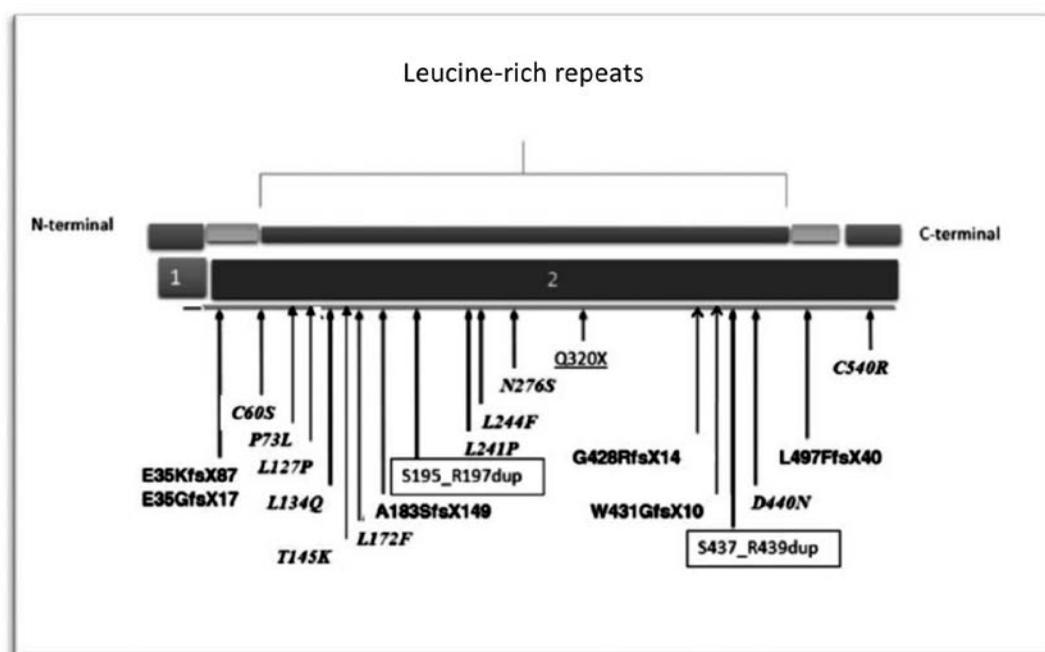
Over the past 12 years, since the description of the first patient with ALS-D, a comprehensive study of both the molecule and potential new mutations has been conducted, but there is still much to be learned.

It is important to understand how ALS-D affects hydrocarbon metabolism, bone metabolism, and pubertal development, as well as the gene dosage effect in relation to the severity of molecular damage.

On the contrary, advances have been made regarding the knowledge of the molecule, its structure, and how it is affected by missense mutations, and also on the differential diagnosis and alertness necessary to make a final diagnosis of these patients.

The importance of an adequate diagnosis in these children with short stature lies on the development of useful treatment strategies to prevent consequences and avoid ineffective hormonal replacement therapies, such as GH treatment and hormonal replacement to induce puberty. ■

FIGURE 2. Schematic of the 20 mutations found to date in the acid-labile subunit gene including their location



Mutations: underlined, nonsense; boxed, duplication; italics, missense; bold, frameshift.

Schematic developed based on Domené et al., 2011,<sup>21</sup> and updated with the mutations found subsequently.

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TABLE 1. Homozygous or compound heterozygous mutations found in 24 patients with complete acid-labile subunit deficiency

Year of publication	Mutation	Mutation type	Homozygous/heterozygous	Ethnic origin	Patient number	Reference
2004	p.E35KfsX87	FS, PSC	Homozygous	Argentine	1	(38)
2006	P.D440N	MS	Homozygous	Turkish	2	(45)
2007	P.C540R p.S195_R197dup	MS DWRF	Compound heterozygous	Norwegian/German	3, 4, 5	(24)
2008	p.N276S	MS	Homozygous	Spanish	6,7	(40)
	p.Q320X	NS	Homozygous	Spanish	8	(40)
	p.L497FfsX40	FS, PSC	Homozygous	Kurdish	9, 10, 11	(41)
2009	p.L437 L439dup	DWRF	Homozygous	Mayan	12	(29)
	p.C60S P.L244F	MS MS	Compound heterozygous	Jewish/Eastern European/Icelandic/European	13	(29)
	p.L134Q	MS	Homozygous	Hindu/Pakistani	14	(29)
	p.P73L P.L241P	MS MS	Compound heterozygous	Ashkenazi Jew	15	(29)
	p.L172F	MS	Homozygous	Swedish	16	(44)
	P.L172F p.S195_R197dup	MS DWRF	Compound heterozygous	Swedish	17	(44)
	p.N276S p.E35GfsX17	MS FS, PSC	Compound heterozygous	Spanish	18,19	(43)
2010	P.L134Q p.A183SfsX149	MS FS, PSC	Compound heterozygous	British Asian	20	(42)
	p.P73L	MS	Homozygous	British Asian	21	(42)
2013	P.T145K	MS	Homozygous	Turkish	22	(46)
2014	P.L127P	MS	Homozygous	Arab-Muslim	23	(39)
	p.W431GfsX10	FS, PSC	Homozygous	Moroccan	24	(36)

FS: frameshift; PSC: premature stop codon; MS: missense; NS: nonsense; DWRF: duplication of 3 amino acids within the reading frame.

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