

Human ALS Deficiency: Clinical, Endocrine and Metabolic Consequences

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Abstract

The majority of IGF-I and IGF-II circulate in the serum as a complex with IGFBP-3 or IGFBP-5, and an acid-labile subunit (ALS). The function of ALS is to prolong the half-life of the IGF-I-IGFBP-3/IGFBP-5 binary complexes.

Fourteen different mutations of the human *IGFALS* gene have been identified in seventeen patients, suggesting that ALS deficiency may be prevalent in a subset of patients with extraordinarily low serum levels of IGF-I and IGFBP-3 that remain abnormally low upon GH stimulation.

Postnatal growth was clearly affected. Commonly, height SDS before puberty was between -2 and -3, and were approximately 1.4 SD shorter than the midparental height SDS. Pubertal delay was found in 50%.

Circulating IGF-II, IGFBP-1, -2, and -3 levels were reduced, with the greatest reduction observed for IGFBP-3. Insulin resistance was a common finding, and some patients presented low bone mineral density.

Human ALS deficiency represents a unique condition in which the lack of ALS protein results in the disruption of the entire IGF circulating system. Despite a profound circulating IGF-I deficiency, there is only a mild impact on postnatal growth. The preserved expression of locally produced IGF-I might be responsible for the preservation of linear growth near normal limits.

Background

The insulin-like growth factor (IGF) system, consisting of two ligands (IGF-I and IGF-II), two receptors (IGF-I receptor, IGFIIR; and IGF-II receptor, IGFIIR), and six high-affinity IGF binding proteins (IGFBPs), plays a critical role in mammalian growth, as repeatedly demonstrated in human case studies and in rodent models. The IGFs are highly homologous growth factors that are structurally similar to insulin. Interactions of the IGFs with the IGFIIR result in a cascade of signaling events leading to critical endocrine, paracrine and autocrine effects on growth, differentiation and metabolism of normal, as well as malignant cells (1). IGF-II can also bind to IGFIIR, a mannose-6-phosphate receptor, which serves predominantly as a scavenger receptor.

In humans, the IGFs are ubiquitously expressed and produced throughout prenatal and postnatal life. Targeted disruption of the *Igf1* gene in rodent models (2), as well as mutations identified in the *IGF1* gene in two well characterized human cases, have been associated with intrauterine growth retardation (IUGR) and severe postnatal growth failure (3, 4). The severe or total lack of functional IGF-I from the time of conception also results in sensorineural deafness and mental retardation, suggesting that IGF-I is also essential for several critical embryonic developmental processes. The biological significance of IGF-II is less well characterized in humans, although it is of note that IGF-II concentrations are higher than those for IGF-I *in utero* and in sera from children (5, 6).

IGF-I (and IGF-II) circulates in the serum in complex with IGFBP-3 or IGFBP-5, and an acid labile subunit (ALS) (7). All three components are dramatically reduced in clinical conditions of GH deficiency (GHD) and GH insensitivity (GHI), indicating the critical role of the GH-GHR-JAK-STAT pathway in the production of each component of the ternary complex (8). When the first case of an inactivating mutation in the gene encoding the ALS protein (the *IGFALS* gene) was found, associated with short stature, insensitivity to GH and abnormally low serum IGF-I and IGFBP-3 levels, it provided direct support for the importance of ALS in the maintenance of normal serum IGF-I and IGFBP-3 levels (9). The number of mutations identified in the *IGFALS* gene has rapidly increased since the first described case, suggesting that ALS deficiency may be prevalent in a subset of

patients that have extraordinarily low serum levels of IGF-I and IGFBP-3 even upon GH stimulation. This review will summarize insights gained in human growth and metabolism as a consequence of ALS deficiency.

Role of the Acid Labile Subunit

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 family of proteins characterized by their ability to participate in protein-protein interactions (10). Molecular modeling of the ALS protein predicted 20 LRR motifs spanning 75% of the mature protein, arranged in a donut-like shape and flanked by N- and C-terminal regions that contain short cysteine-rich segments (10). The protein surface contains charged residues, and while charged residues are relatively evenly distributed on the outer regions of the domain, the center hole of the donut is notably lined with large regions of electronegative surfaces. This, together with the negatively charged N-linked carbohydrates on the ALS protein, may provide the necessary electrostatic potential to interact with the IGF-I-IGFBP-3 binary complex (10, 11).

The well-established function of ALS is 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 (reviewed in 7). There is 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 (12). In rodents, genetic targeted ablation of the *Igfals* gene appeared to increase sensitivity to insulin (13). Hence, it is of note that some of the subjects severely deficient in ALS presented with phenotypes suggestive of abnormal carbohydrate metabolism (see below). The mechanism(s) for this association remains unknown.

The *Igfals*^{-/-} (ALS-knock-out) mouse model

Inactivation of the *Igfals* gene in mice, which results in the complete absence of circulating ALS, produced mice that were only modestly growth deficient (13-20% smaller at 10 weeks) despite a 62% and 88% reduction of IGF-I and IGFBP-3 plasma levels, respectively (14, 15). The IGF-I and IGFBP-3 deficiencies were likely

a result of increased turnover, as their synthesis in liver and kidney remained normal. Interestingly, analysis of glucose metabolism in the ALS-KO mice revealed that although fasting plasma levels of glucose, insulin, and nonesterified fatty-acids were normal (14), a faster glucose clearance during intraperitoneal glucose tolerance tests was observed, as was a trend towards higher whole body utilization and muscle uptake of glucose during hyperinsulinemic-euglycemic clamping experiments (13). Altogether, these results suggested that the ALS-KO mice had a higher sensitivity to insulin compared to controls (13). In addition, 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 noted, with but a 24% reduction in femoral cortical bone volume. The ALS-KO mice also showed a 37% reduction in trabecular bone (15). It is not clear, at present, if these physiological alterations are a direct or indirect result of a total ALS deficiency.

Human ALS deficiency

Auxological characteristics

The ALS mutations identified to date have been described in short children and adults. It is possible that the association of ALS deficiency with growth retardation represents, at least in part, some degree of ascertainment bias, as serum concentrations of the various components of the IGF system are not measured routinely in normal-statured individuals. It is uncertain whether prenatal growth is affected by an *IGFALS* gene defect, as birth weights together with data on gestational age were reported in only 8 cases, but the low mean birth weight (-1.0 SDS, range -2.23 to -0.08 SDS) suggests that it may be low (Table 1). Birth length could be analyzed in only 4 cases, and appears normal.

However, postnatal growth was clearly affected by mutations in the *IGFALS* gene (summarized in Tables 2 and 3). In the majority of cases, height SDS before and during puberty was between -2 and -3, and approximately 1.4 SD shorter than the midparental target height SDS (Table 3). Of the 12 male patients for whom data were available, 6 clearly had a delayed pubertal onset (>14 years), and in the remaining male patients puberty started around 13 years. Although these results are not definitive, it would appear that an ALS defect in males may be associated with delayed puberty, with an average onset of approximately 14 years of age. This may

explain why, in most of the cases, adult height SDS was higher than prepubertal height, but still 1.0 SD lower than the midparental target height SDS. The only affected female for whom pubertal data were available, had her menarche at 13 years.

No discernable aberrance in the pubertal growth spurt pattern has emerged from the reported cases (Table 2). In the first reported case, the growth spurt was well documented and considered normal (20), as were the two boys with delayed puberty described by Domené et al (21) and two of the Spanish cases (22). In contrast, the three Kurdish males appeared to have a poor pubertal growth spurt (23).

In an effort to improve growth, GH therapy was provided for some of the patients. While the length of treatment ranged from 6 months to more than 2 years, growth response, on the whole, was poor. For the patient who was given GH for 6 months, the change in growth velocity (6.2 vs 4.6 cm/yr, in mid-puberty) was considered low (9). Case 3 was treated with GH for a total period of 17 months, beginning at the age of 15.5 years, and the response was considered insufficient. It should be noted that in the last half of this treatment, testosterone enanthate was added (21). Case 8 was treated with GH from 15.5 to 18.8 years of age, but as this period coincided with the pubertal growth spurt, it was difficult to judge the efficacy of therapy (22). In case 12, GH was administered from 8.5 to 9.5 years and from 10-12 years. Between 10.5 and 12 years a GnRH analogue was added to preserve growth potential during GH therapy. In both periods the growth response was considered insufficient, and from 12 years of age IGF-I therapy (Increlex, Tercica, California) was started (24); results are pending. Case 13 was treated with GH from 4.4 years onward and the growth response appeared small (24). Of particular note in all these cases, was that IGF-I and IGFBP-3 concentrations remained strikingly low, despite GH therapy. In summary, the efficacy of GH appears poor in patients carrying mutations in the *IGFALS* gene. In the few cases where testosterone was administered for delayed puberty, the effect was as expected in boys with constitutional delay of growth and puberty.

With respect to other growth parameters, weight-for-height (expressed as body mass index) is generally normal. Head circumference was only reported in the three Kurdish males, where it was decreased in comparison to heterozygous and wildtype relatives (23).

It is noteworthy that the mean parental height is approximately 1.3 SDS lower than the population's mean (Table 3). This suggests that heterozygosity for an ALS mutation may have a mild growth phenotype. Interestingly, in the family where the height of 3 homozygous cases could be compared with 5 heterozygous carriers and 5 wild-type relatives, height SDS was -4.1, -2.3 and -1.3 respectively, although the differences between heterozygous carriers and wild-type did not reach statistical significance (23). Further studies are necessary to evaluate the effect(s) of *IGFALS* heterozygosity on growth.

Spectrum of *IGFALS* gene mutations

The human *IGFALS* gene maps to chromosome 16, location 16p13.3. The gene contains two exons, 97 and 1987 bp in size, separated by a 1235-bp intron. Whereas exon 1 encodes only the first 5 amino acids 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 aminoacids (25). 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, flanked by two amino- and carboxyterminal regions containing 13 cysteine residues (10). The protein contains 7 asparagine residues that are potential glycosylation sites.

To date, at least seventeen patients with complete acid-labile subunit deficiency have been characterized at the molecular level (Table 4). These include one patient from Argentina (9), one from Turkey (27), five from the USA (21, 24), three from Spain (22), three from Kurdistan (23), three from England (24, 26), and one from Canada (24) (Table 4). Only two are female, probably the result of a more frequent concern of parents for the height of their male offspring.

Fourteen different mutations of the human *IGFALS* gene have been identified in the seventeen patients studied (Table 5, Fig 1). Eleven patients were found to be homozygous and six were compound heterozygous. In two separate families, three siblings were affected with the same mutations (21, 23). In all cases where the parents and siblings of the affected subjects were available for the characterization of the *IGFALS* gene, they presented the same mutations as their affected children, demonstrating an autosomal recessive pattern of inheritance. Consanguinity was present in three families including five patients (three of them siblings), absent in six families including eight patients (three of them siblings), and unknown in the other four families.

Different types of mutations in the *IGFALS* gene have been reported, including missense, nonsense, deletion, duplication and insertion resulting in frameshift and premature stop codons, and two different in-frame duplication mutations leading to insertions of three extra amino acid residues (Table 5, Figure 1). The majority of *IGFALS* gene mutations produce defects in the leucine-rich repeat region of the protein: 7 missense, 1 nonsense, 2 frameshift, and 2 in-frame duplication mutations. One frameshift mutation was detected in the region encoding the amino terminal flanking domain, and one missense mutation in the region encoding the carboxyl-terminal flanking domain.

A single base deletion (c.103delG), a single base duplication (c.1490dup) and a single base insertion (c.546_547insA) all result in frameshift mutations leading to premature stop codons and truncated proteins (p.Glu35LysfsX87, p.Leu497PhefsX40, p.Ala183SerfsX149) that are likely to be completely inactive.

Four leucine residues at positions 1, 4, 6, and 11, in the highly conserved segment LxxLxLxxN/CxL of ribonuclease inhibitor (RI)-like subfamily of LRR proteins, participate in the hydrophobic core of these proteins (29). Interestingly, the p.Leu134Gln and p.Leu244Phe mutations affect leucine residues at position 11 in the 4th LRR and at position 1 the 9th LRR, respectively. These two mutations may influence the hydrophobic core and thereby the affinity of ALS for the binary IGF-1/IGFBP-3 complexes. Another missense mutation, p.Leu241Pro, also involves a leucine residue. Similar substitutions of conserved leucine residues have been

previously reported in the nyctalopin gene (NYX) associated with congenital stationary night blindness type 1 (30). Two other missense mutations predict changes in two conserved cysteine residues. The p.Cys60Ser mutation, in the amino-terminal domain, and the p.Cys450Arg mutation, in the carboxy-terminal domain, involves the loss of two highly conserved residues in LRR proteins, most likely involved in disulfide bonds. Thus, it is very likely that these mutations preclude formation of disulfide 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 for presenting 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 two amino and carboxy terminal domains in close proximity, able to form disulfide bridges (10). Inactivating mutations in the two highly conserved Cys residues at the carboxy terminus have been described in other LRR proteins, such as nyctalopin (30), LH receptor (31), TSH receptor (32), and glycoprotein I α (33). Another missense mutation in ALS (p.Asn276Ser) affects a phylogenetically highly conserved Asn residue located within a consensus LRR β -strand motif (LxxLxLxxN/CxL), in the 10th LRR domain of the ALS protein (11).

An interesting aspect of *IGFALS* mutations is the finding of two different nine base in-frame duplications (c.583_591dup and c.1308_1316dup) at 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 for nyctalopin (30). 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.

A nonsense mutation (p.Gln320X) introduces a premature termination codon, which is predicted to generate a truncated and functionally inactive protein lacking 284 amino acids of the carboxy terminus.

Only the *in vitro* expression and determination of ternary complex formation by the mutant proteins will allow the characterization of the biological impact of these mutations. However, at present all described *IGFALS* gene mutations have resulted in either completely absent or barely detectable ALS levels, as determined by ELISA, RIA, or Western immunoblot using specific monoclonal antibodies directed against either the amino or the carboxy terminus of the ALS protein. Undetectable ALS levels are even observed in those cases where the mutations predict the synthesis of truncated proteins, the eight mutations that predict a single amino acid change, and the two leading to insertion of three extra amino acids. The lack of detectable ALS levels in sera from patients with these distinct mutations, suggests that the mutated proteins are unable to be normally secreted, or that they are unstable and rapidly degraded after secretion. The *in vitro* expression of the p.Asp440Asn (mature mutant peptide designated p.Asp413Asn-ALS; patient reported in reference 27) indicated that this mutant ALS was inefficiently secreted, remaining trapped inside the cells (34). 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 and the disruption of the acidic internal surface, a proposed IGFBP-3 binding site (27, 34). In cases where *in-vitro* ternary complex formation was investigated, serum samples from ALS-deficient patients could not form ternary complexes, indicating either the absence of ALS protein or the lack of a bioactive form. The complete absence of ALS protein in the circulation in patients homozygous or compound heterozygous for inactivating *IGFALS* mutations, and the low to low-normal ALS levels in heterozygous carriers, suggest that, in normal conditions, both alleles of *IGFALS* genes are expressed.

Effect on the GH-IGF system

Human ALS deficiency results in a peculiar form of IGF-I deficiency. Whereas circulating levels of IGF-I decrease dramatically (9, 20-24, 26, 27), local production, and presumably action, appears to be preserved. In addition to IGF-I, other members of the circulating IGF system are also affected. Circulating IGF-II levels are reduced (20, 22-24), while IGFBP-3 levels are even more profoundly diminished. Levels of IGFBP-1 and -2 are reduced, although to a lesser extent (20, 22-24) (Table 6).

The complete absence of ALS represents a unique situation leading to generalized deficiency of the circulating, so-called “endocrine IGF system”, with the expression and action of the peripheral, or “autocrine/paracrine IGF system” being preserved or even up-regulated due to normal or increased GH levels, present in this alteration. Assuming that the various GH assays and standards are comparable, and assuming a conversion rate of 1 mg = 2 IU, the average GH peak in stimulation tests was 46 ng/ml (range 10-98 ng/ml, n=12). When the nocturnal 12-hours spontaneous GH profile was investigated in the first described ALS-deficient patient, a moderate elevation of GH levels was found (18.9 ± 12.6 ng/ml; reference values 4.5 ± 1.4 ng/ml) (9). However, the elevation of GH levels in ALS deficiency appears to be less marked than that observed in patients with GH insensitivity, although in both cases they show a comparable reduction in IGF-I levels. These findings suggest that in ALS deficiency the local production of IGF-I is able to partially control GH secretion.

As indicated above, the main role of ALS protein is to maintain the circulating IGF-I pool by the formation of a 150 kDa ternary complex with IGF-I and IGFBP-3 or -5 (35, 36), extending the IGF-I half-life up to more than 12 hours (37, 38) by preventing its passage to the extravascular compartment (39) and preserving both IGF-I and IGFBP-3 from proteolysis. Given its abundant molar excess in the sera, its completely different primary and three-dimensional spatial structure, and its relatively low affinity for binary IGF-IGFBP complexes (300 to 1000 fold lower than that of IGFBP-3 for either IGF-I or IGF-II) (40), ALS has received much less attention in comparison with the other members of the circulating IGF system (7), as it was considered a redundant circulating protein with low binding affinity properties. However, while the targeted inactivation of individual IGFBPs in rodent models has little if any effect on the phenotype and the circulating IGF system (41), probably related to some redundancy of function among the different IGFBPs, the ALS-KO mouse exhibits a profound impact on the whole IGF system, with marked reduction in IGF-I and IGFBP-3 levels (see above; 14). It is also remarkable that up to now no other single IGFBP deficiency has been characterized in the human, perhaps because the compensatory presence of the remaining IGFBPs partially compensates for any selective IGFBP deficiency or absence. In contrast, the irreplaceable function of ALS protein for the stabilization of the ternary complexes makes it impossible for

these to be formed in its absence, which leads to the rapid clearance of IGF-I, IGF-II, and IGFBP-3 from the circulation and their permanently reduced levels. Consequently, the reduction in IGF-I, IGF-II, and IGFBP-3 found in ALS deficiency seems to result in the instability of these proteins and their increased turn-over, rather than a defect in their synthesis. In support of this hypothesis, a normal IGFBP-3 concentration was detected in media conditioned by primary dermal fibroblast cells established from an ALS-deficient patient (27). Moreover, null ALS mice have normal abundance of IGF-I mRNA in liver and IGFBP-3 mRNA in liver and kidney (14).

The mechanism involved in the moderate IGFBP-1 and -2 deficiencies is less obvious. High levels of insulin, a common finding in ALS deficient patients, are probably the result of some degree of insulin resistance, and could be responsible for the repression of IGFBP-1 expression. Perhaps, the same mechanism might explain the reduction in IGFBP-2 levels. Insulin is an important regulator of the hepatic synthesis of both IGFBP-1 and IGFBP-2 (42), but while the suppression of liver IGFBP-1 expression by insulin occurs at the transcriptional level (43), the effect on IGFBP-2 occurs at the translational level, and the changes observed are not as rapid as those observed for IGFBP-1 (44). The increased GH levels might also play a role, since in GH-deficient patients IGFBP-2 levels are usually increased (45), and administration of GH decreases plasma IGFBP-2 in adult subjects with a normal caloric intake (46).

A remarkable effect of the absence of ALS is the predominant reduction of circulating IGFBP-3 levels compared with those of IGF-I. In GH-deficient and GH-insensitive patients circulating IGF-I is more reduced than IGFBP-3, as IGFBP-3 is less dependent on GH than IGF-I. The predominant deficit of IGFBP-3 over IGF-I could therefore be a useful marker to direct the investigation of the etiology of short stature toward the diagnosis of ALS deficiency.

Carbohydrate metabolism

Carbohydrate metabolism data were available in 11 of the 17 patients presented in this review. Fasting blood glucose levels were normal in these 11 patients (87.7 ± 7.3 mg/dL; range 79-100 mg/dL). In one of the 4 patients that underwent an OGTT, slight glucose intolerance was suggested (glycemia at 120' =

149 mg/dL). In all patients except one (HOMA index = 2.9; basal insulin: 14 mU/mL), insulin-resistance was suggested (46-49) by one or more of the criteria analyzed: fasting insulin levels >15 mU/mL (10 of 11 patients; mean \pm SD: 21.7 \pm 7.8 mU/mL; range: 10.9-32.4 mU/mL); insulin peak post-OGTT >150 mU/mL (2 of 4; mean \pm SD: 191.2 \pm 96.0 mU/mL; range: 78.5-281 mU/mL); insulinemia at 120' >75 mU/mL (3 of 4; mean \pm SD: 135.2 \pm 103.8; range: 44-279); or HOMA index >3 (9 of 11; mean \pm SD: 4.3 \pm 1.9; range: 2.1-7.8). Serum IGFBP-1 levels, an indirect indicator of insulin sensitivity (50), were very low or undetectable in all patients analyzed. Together, these data suggest that patients with congenital ALS deficiency demonstrate some degree of insulin resistance; however, the pathophysiological mechanisms involved are not clear. 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. 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 (51). Subsequent studies demonstrated that increased circulating GH levels result in hyperinsulinemia, insulin resistance and possibly carbohydrate intolerance or diabetes mellitus. In contrast, GH deficiency is associated with hypoinsulinemia, increased insulin sensitivity and decreased fasting glucose levels (52). Although the metabolic consequences of altered GH levels on insulin sensitivity and action are well known, the underlying mechanisms involved are not. Multiple factors are most likely involved including alterations in insulin sensitivity due to increased free fatty acids resulting from the lipolytic effects of excess GH ("lipotoxic effect") (53, 54), post-receptor crosstalk between the insulin receptor and GH receptor signaling pathways (55), decreased expression of the insulin-sensitizing adipo-cytokines adiponectin and visfatin (56, 57) or the hyperinsulinemia induced by the excess GH that reduces the number of insulin receptors and alters their kinase activity (58). Moreover, GH has stimulatory effects on insulin directly at the level of the β cell, but prolonged exposure to excess GH results in β cell failure, followed by decreased insulin secretion and glucose tolerance, a feature resembling the long-term sequelae of altered glucose homeostasis in untreated acromegalic patients (59).

The slight increase in GH secretion found in patients with congenital ALS deficiency suggests that this is unlikely to be the only mechanism involved in their

insulin-resistant states. Recent data suggest that the IGFs may play an important role in carbohydrate metabolism, maintaining a balance between insulin and GH actions (60). Important contributions to the clarification of the mechanisms of action of insulin and IGF-I action have been provided by transgenic and knockout mouse models. The marked insulin resistance observed in the liver-specific IGF-I knock-out mouse (61, 62), that suggests an important role for circulating IGF-I in glucose homeostasis, is blocked by deletion of the ALS gene (13). However, findings in humans and mice cannot always be directly extrapolated, since although the ALS knockout mouse has a slight reduction in postnatal growth, no alterations in glucose metabolism or insulin resistance are found (13); on the contrary, the ALS knockout mice appear to display an increased sensitivity to insulin (13).

Various studies have demonstrated that IGF-I can augment glucose uptake by peripheral tissues, decreasing blood glucose levels (60, 63), although to a much lesser degree than insulin. Given that the affinity of IGF-I for the insulin receptor is very low, it is thought that the majority of its metabolic effects are mediated through the IGF-I receptor. This receptor is not present in liver or adipose tissue (55), but is present in high concentrations in skeletal muscle, where IGF-I has been shown to induce glucose uptake (64). Furthermore, as skeletal muscle expresses both insulin and IGF-I receptors, hybrid receptors (heterodimers of one $\alpha\beta$ -subunit of the insulin receptor and one $\alpha\beta$ -subunit of the IGF-I receptor) with a higher affinity for IGF-I than insulin, could mediate IGF-I stimulated glucose uptake in this tissue (65). However, as IGF-I can also decrease GH secretion, it is difficult to dissect the direct actions of IGF-I on carbohydrate metabolism from those induced by decreased GH secretion. Finally, IGF-I has also been shown to play a crucial role in the maintenance of insulin secretion and the development and survival of the β cell population (66).

In conclusion, human subjects with ALS deficiency due to mutations in the *IGFALS* gene present low levels of IGF-I, IGFBP-3 and ALS, with moderate hyperinsulinemia and very low levels of IGFBP-1. The pathophysiological mechanisms explaining this hyperinsulinemia are still only partially understood.

Bone metabolism and mineralization

As ALS knockout mice have a low bone density (15, 67, 68), one might expect

patients with an ALS defect to have osteopenia. In fact, the first reported case had a marked reduction in bone mineral density (BMD) (-4.7 SDS) at 16 years of age, with a partial recovery at the time of completion of puberty (-2.1 SDS at 19 years of age) (69). When two of the Kurdish cases presented with severely low BMD, one of them presenting with two fractures after minor trauma, the probability of an effect of ALS deficiency seemed to increase further. However, in this family a low BMD was also noticed in heterozygous and wild-type carriers, so that the effect of the ALS defect could not be proven (23). In cases 2 (Dr. Haeusler, personal communication), 6 and 7 (21) BMD was normal. There is no information about the other cases. In summary, there is no proof that in the human an ALS defect causes a low bone mineral density.

Identification of patients with an ALS Mutation

Identification of the patient with an *ALS* mutation is problematic, largely because the natural history of this condition remains uncertain. To some extent, this is the result of the inevitable ascertainment bias that has impacted the initial reports; specifically, serum concentrations of IGF-I, IGFBP-3 and, in particular, ALS, are not commonly measured in children of normal height, so it is difficult to determine how prevalent this mutation may be in the normal population. It is already clear that some patients with homozygous or compound heterozygous mutations may have childhood and/or adult heights within the normal range. It is also likely that some children labeled as "GH deficient" or "idiopathic short stature", and characterized by low serum concentrations of IGF-I and IGFBP-3 may, in fact, be homozygous or heterozygous for *ALS* mutations.

In general, the phenotype may be described as modest short stature, typically in the -2 to -3 SD range, accompanied by low serum concentrations of IGF-I and IGFBP-3, in the face of normal GH stimulation tests. Often, the low serum concentrations of IGF-I and IGFBP-3 appear to be out of proportion to the relatively minor degree of growth retardation. In particular, the extremely low serum concentrations of IGFBP-3 provide a diagnostic clue to the possibility of an *ALS* defect as, in general, serum levels of IGFBP-3 are less impacted than those of IGF-I in GH deficiency and idiopathic short stature. Failure of the patient to respond to GH therapy, either by accelerated growth or by substantive increases in serum levels of

IGF-I and IGFBP-3 provides further support for a presumptive diagnosis of ALS deficiency. High levels of insulin, a common finding in ALS-deficient patients, may also be useful to characterize these patients.

Conclusions

In summary, human ALS deficiency, the first monogenic defect involving an insulin-like growth factor binding protein represents a unique condition in which the lack of ALS protein results in the disruption of the entire IGF circulating system. In the cases of IGF-I and IGFBP-3, the inability for ternary complex formation seems to make these proteins unstable with a reduced half-life. Despite a profound circulating IGF-I deficiency, together with low levels of IGF-II, free IGF-I, bioactive IGF-I, and IGFBP-1, -2, and -3, ALS deficiency has only a mild impact on postnatal growth. Perhaps the preserved expression of locally produced IGF-I under the stimulation of normal or even increased GH levels, might be responsible for the preservation of linear growth near or within normal limits (Figure 2). Besides the effect on growth, the marked IGF-I deficiency observed in human ALS deficiency has other remarkable effects. The importance of IGF-I on the regulation of carbohydrate metabolism, is suggested by insulin-resistance present in almost all ALS-deficient patients in whom this has been investigated. Considering the opposite effects of GH and IGF-I on glucose internalization mediated by insulin, it is not obvious whether the insulin-resistance is the result of a GH increase, IGF-I reduction, or the combination of both. Another common clinical finding associated with ALS deficiency, is a delay in the onset of puberty, observed in half of the male patients. As delayed puberty is a common finding in IGF-I-deficient patients of different etiologies, it has been proposed that circulating IGF-I levels may represent a peripheral signal for the activation of the hypothalamic pulse-generator of gonadotrophic secretion (70). A delay in bone mineralization has been reported in some, but not all ALS-deficient patients, suggesting that chronic circulating IGF-I deficiency may be involved in a reduced apposition of calcium on the growing cartilage. However, the underlying mechanism responsible for this alteration is not fully understood.

Since the original report of human ALS deficiency in early 2004, a surprising number of patients have been diagnosed with this condition. In addition, the finding of homozygous and compound heterozygous mutations in the *IGFALS* gene in apparently non-consanguineous families suggests that mutations in this gene may

be relatively frequent. Considering that when a new pathological condition is described, usually the more severe cases are first reported, it is quite likely that less severe cases of ALS deficiency, resulting from less detrimental gene mutations affecting both *IGFALS* alleles, or even one severe gene mutation in heterozygosis, may in time be characterized in children with poor growth.

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Legends to the figures:

Figure 1

Schematic representation of ALS protein indicating the location the fourteen identified human mutations. Black: missense; red: frameshift, magenta: nonsense; blue: duplication.

Figure 2

Growth hormone (GH) acts on both liver and extrahepatic tissues. After associating with a homodimeric GHR complex, Janus-family tyrosine kinase 2 (JAK2) is recruited, with the resulting activation of the phosphoinositide-3 kinase (PI3K)/AKT, Ras/MAPK (mitogen-activated protein kinase) and the signal transducer and activator of transcription type 5b (STAT5b) pathways. STAT5b seems to be the crucial mediator of GH-induced *IGF1*, *IGFBP3* and *IGFALS* gene transcription, resulting in IGF-I, IGFBP-3 and ALS synthesis, associated in a 150 kDa circulating complex. When *IGFALS* gene is mutated (*IGFALS*^{-/-}), ternary complexes are not formed and levels of IGF-I and IGFBP-3 are reduced in the circulation. In extra-hepatic tissues, even in *IGFALS*^{-/-} cells, IGF-I and IGFBP-3 are normally expressed and they may act normally by paracrine or autocrine mechanisms.

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