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Novel Acid-Labile Subunit (*IGFALS*) Mutation p.T145K (c.434C>A) in a Patient with ALS Deficiency, Normal Stature and Immunological Dysfunction

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Key Words
Insulin-like growth factor · Acid-labile subunit · *IGFALS* mutation · Immunological dysfunction

Abstract
We report a novel missense mutation p.T145K in the insulin-like growth factor (IGF) acid-labile subunit (*IGFALS*) gene identified in a Turkish patient with normal growth, transient pancytopenic episodes and signs of immunological dysfunction. Because of recurrent cutaneous mycoses and absence of pubertal development until the age of 14.75 years we determined several endocrine parameters in order to rule out autoimmune-polyendocrine syndromes. Despite a normal height between the 25th and 50th percentile we found severely decreased IGF-1 and undetectably low IGFBP-3 levels. Laboratory signs of immunological dysfunction included reduced total lymphocyte count with diminished B and T helper cell fractions, decreased serum concentrations of IgM and IgG subclass 4, and elevated antinuclear antibody and anti-dsDNA titers as well as persistently high interleukin-2-receptor levels. Further endocrine work-up revealed elevated fasting insulin and undetectably low ALS serum levels, leading to the diagnosis of ALS deficiency. Sequencing of the coding region of the *IGFALS* gene showed a novel homozygous missense mutation (c.434C>A; p.T145K). Since immunological abnormalities have not been reported in more than 20 ALS-deficient patients so far and our patient was born to consanguineous parents, a second autosomal recessive defect is likely to underlie the immunological phenotype, although a causative role of *IGFALS* p.T145K cannot be entirely ruled out.

Introduction

The growth hormone (GH) insulin-like growth factor I (IGF-1) axis plays a fundamental role in human postnatal growth. GH exerts its proliferation-stimulating effects mainly via circulating IGF-1, 80% of which is expressed in the liver. The majority of circulating IGF-1 is bound to IGF-binding proteins, mainly IGFBP-3, and the acid-labile subunit (ALS). As part of this ternary complex, the half-life of serum IGF-1 is increased from a few minutes to several hours [1]. Until now, more than 20 patients

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with ALS deficiency have been reported in the literature [2–4]. Patients typically present with markedly decreased IGF-1 and extremely low IGFBP-3 levels, but only moderate short stature. In addition, delay of pubertal development was reported in about 50% of male patients, and some degree of insulin resistance seems to be another common finding in ALS-deficient patients [3, 5].

Here, we report a novel *IGFALS* gene mutation p.T145K (c.434C>A) found in a patient with apparently unaffected growth, delayed puberty and signs of immune dysfunction. Since no immunological abnormalities have been reported in more than 20 patients with ALS deficiency so far, and our patient was born to consanguineous parents, a second autosomal recessive defect seems likely to underlie his immunological phenotype.

Case Report

A 14-year-old Turkish boy was referred to the Pediatric Oncology Department with pancytopenia, weight loss of 10 kg (in part due to religious fasting) and leg pain for about 2 months. During this time, he had no fever or night sweats. One week before admission, he complained about a nonitching rash on the right lower leg and mild respiratory symptoms including runny nose and sneezing, so that the family doctor prescribed cetirizine. Shortly after starting cetirizine treatment, the patient noted red-colored urine, which normalized a few days after discontinuation of cetirizine treatment. However, detection of splenomegaly, petechiae and a blood count revealing pancytopenia (Hb 8.3 g/dl; leukocytes 2,900/μl; platelets 11,000/μl) led to immediate hospitalization. Hematological work-up including bone marrow biopsy ruled out a leukemic disease, whereas elevated hemolysis parameters and high reticulocyte count pointed to an autoimmune or infection-related acute hemolytic event. Hematological analyses for hereditary erythrocytopathies were negative, as was screening for triggering acute viral infections (parvovirus B19, HSV, EBV, HIV, etc.) in blood samples. Further serological analyses revealed high soluble interleukin-2 receptor (IL-2R) levels (1,311 U/ml; norm < 710 U/ml), elevated anti-double strand DNA (1:56; norm < 1) and antinuclear antibodies (ANA 1:640; norm < 1:10), with homogenous plus nucleolar pattern (nucleosome-, PM-Scl and PM-Scl100 immunoblot positive, AMA-M2 weak positive, SS-A, Scl-70 and Sm negative), whereas ANCA and anti-phospholipid antibodies were negative. In addition to the hematological findings leading to hospitalization, the patient initially presented with several nummular scaling eczemas (<2 cm) on his right hand and right lower leg, of which one larger lesion (4 cm) was necrotic and superinfected by *Staphylococcus aureus*. Skin biopsy and histological examination did not show signs of florid inflammation or lupus-associated vasculitis, and with antimycotic and antibiotic treatment most lesions healed without scarring. Red blood cell and platelet count normalized within 2 weeks, but lymphocytes remained subnormal (leukocytes 1,800–4,200/μl; norm 4,200–10,800/μl; lymphocytes 360–780/μl; norm 1,200–5,000/μl), with persistently diminished B lymphocytes (CD19+ 73/μl; norm 200–600/μl) and T helper cell counts (CD3+/CD4+ 162/μl; norm 400–2,100/μl) in FACS subpopulation analysis and selectively decreased IgM (0.12–0.20 g/l; norm 0.4–2.0 g/dl) and IgG subclass 4 (0.01–0.02 g/l; norm 0.05–1.98 g/l) serum levels until today (follow-up >2 years).

Several months after the first admission, he developed elevated systolic blood pressure and significant proteinuria (>3.0 g/24 h). However, the kidney biopsy revealed only a slight increase of mesangial cellularity and discrete signs of podocyte degeneration, but no immunohistochemical or electron microscopical signs of lupus-associated or minimal change glomerulonephritis. Blood pressure and proteinuria normalized after initiation of enalapril treatment and remained within normal ranges after discontinuation of enalapril intake 6 months later.

Recently, the patient presented at the local hospital with a second episode of pancytopenia and hemolysis (total leukocytes 4,450/μl; lymphocytes 400/μl; platelets 18,000/μl; Hb 8.6 g/dl) following an upper airway infection (influenza A/B negative). Hemoglobin and platelet count again normalized within 2 weeks, whereas lymphopenia persisted. He also developed significant proteinuria, requiring reinitiation of nephroprotective treatment.

Although the patient showed persistently diminished B and T helper cell counts together with selective antibody deficiency (very low IgM and IgG subclass 4) and recurrent skin lesions, adequate vaccination titers (except rubella) and the absence of severe invasive infections indicated a mild and not severe combined immunodeficiency. Thus, he has not received any prophylactic antimicrobial treatment so far. The recurrent pancytopenic episodes may either be a consequence of transiently increasing autoimmune activity or they may be directly caused by infections with hemotropic pathogens facilitated by the presence of immunological dysfunction. Several clinical and biochemical aspects are reminiscent of systemic lupus erythematodes. However, the lack of lupus-characteristic histological findings in kidney and skin biopsy specimens is inconsistent with the diagnosis of systemic lupus erythematodes.

Endocrine work-up was initiated because of the absence of pubertal development until the age of 14.75 years. With respect to recurrent cutaneous mycoses we also considered autoimmune-polyendocrine syndromes and determined several endocrine parameters. Surprisingly, we found severely decreased IGF-1 and undetectably low IGFBP-3 levels despite normal height and growth velocity (table 1; fig. 1). Unstimulated GH concentrations varied between 0.6 and 19.6 ng/ml (no stimulation tests done). Brain MRI did not show any abnormalities. Nonsomatotropic pituitary function, including gonadotropin response to LHRH, as well as parathyroid, thyroid (isolated anti-TG elevation, anti-TPO normal, euthyroid function) and adrenal function appeared to be unaffected. Sequencing of genes *MEFV* and *AIRE* revealed no abnormalities. Further biochemical analyses during follow-up showed elevated fasting insulin levels (28.3 mU/l; norm < 24.0 mU/l) which, similar to delayed puberty, represent a frequently reported symptom in ALS-deficient patients. Indeed, ALS serum concentration in our patient was undetectably low, leading to the diagnosis of ALS deficiency.

The patient was born in Germany to consanguineous (first-degree cousins) Turkish parents. He was born at a gestational age of 39 weeks with a birth weight of 2,870 g (–1.37 SDS; reference data for Turkish newborns [9]) and a birth length of 49 cm (–0.56 SDS). Except for bilateral orchidopexy due to undescended testis at the age of 1 year, idiopathic facial nerve palsy, mycosis pedis and

Table 1. IGFALS genotype, height and biochemical parameters of the family

	Patient[1]	Mother	Father[4]	Uncle (maternal)
IGFALS p.T145K	homozygous	heterozygous	n.a.	control (Thr/Thr)
Height, cm/SDS	173/–0.19[2]	164/+1.0[3]	170/–0.44[3]	174/+0.17[3]
IGF-1, ng/ml	43 (227–964)	97 (113–297)	n.a.	106 (106–277)
IGFBP-3, μg/ml	<0.5 (3.3–9.6)	2.4 (3.4–6.9)	n.a.	3.7 (3.4–6.6)
ALS, mU/ml	<100 (986–1,678)	318 (692–1,252)	n.a.	1,177 (770–1,457)

[1] Auxological and biochemical data at age 16 years, 11 months.
[2] SDS based on reference height measuremets in Turkish boys born in Germany [6, 7].
[3] SDS based on reference height measurements in Turkish adults [8].
[4] The father lives in Turkey, height as reported by the mother.
n.a. = Not analyzed.

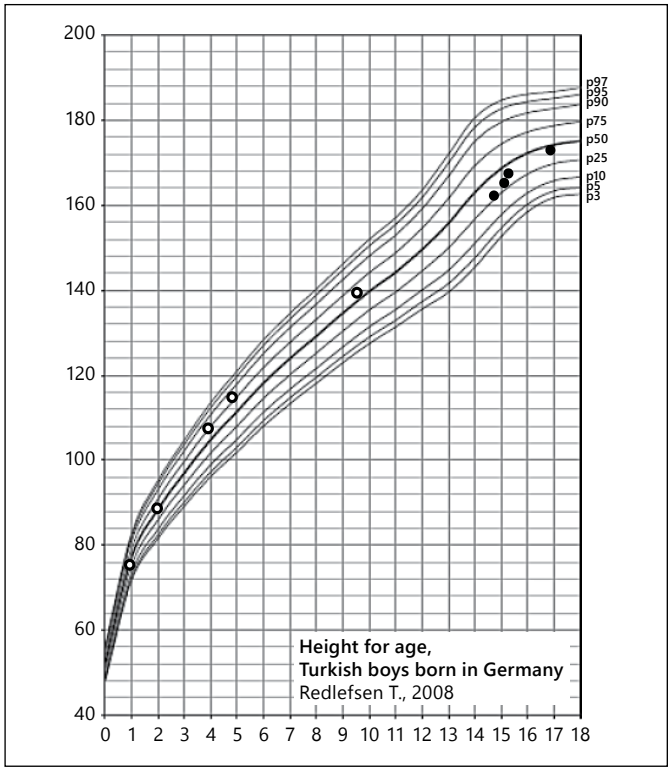

Fig. 1. Growth curve including height measurements at local routine pediatric check-up visits (open circles) and during hospitalization/follow-up visits at our hospital (filled circles). Puberty started spontaneously around the 15th birthday and proceeded normally; at the same time, body weight increased gradually from the 75th to the 97th percentile, as did the BMI (currently 29.8 kg/m^2; 97th percentile).

pollinosis, early childhood development was unremarkable. He had no siblings and lived together with his mother and his uncle in Germany, while his father had separated from the family and had returned to Turkey. Family history regarding short stature, delayed puberty and known immunological disorders was unremarkable (mother's height 164 cm, +1.0 SDS, age at menarche 12–13 years; father's height approx. 170 cm, –0.44 SDS, no information on puberty).

Methods

Hormone Measurements

Serum concentrations of IGF-1 and IGFBP-3 were measured by an automated immunochemiluminescence assay (Immulite 2000; Siemens Healthcare Diagnostics). Concentrations of circulating ALS were determined by Dr. Martin Bidlingmaier, LMU Munich, Germany, using an in-house immunofluorometric assay, with gender- and age-specific reference values adjusted to those reported by Juul et al. [10].

DNA Sequencing

Exons and splice sites of *IGFBP3* and *IGFALS* were analyzed by direct sequencing. Reaction conditions and primer sequences are available on request.

Western Blot

SDS-PAGE electrophoresis of 3-μl serum samples and Western blotting were performed according to standard protocols. After blocking with 1% BSA (6 h) and immunodetection using a goat anti-mouse ALS antibody (R&D systems) in a final concentration of 0.15 μg/ml (O/N), results were visualized by incubation with 0.2 μg/ml (1 h) anti-goat peroxidase-conjugated IgG antibody (Santa Cruz) and chemiluminescence analysis.

In vitro Ternary Complex Formation

In vitro ternary complex formation (TCF) was performed as previously described by van Duyvenvoorde et al. [11]: IGF-IGFBP-complexes were determined by neutral gel filtration through a 16 × 60 cm HiLoad Superdex-200 column. Each serum sample (250 μl) was incubated with 100 μl of 80,000 cpm ^{125}I-IGF-1 in 50 mM sodium phosphate buffer (pH 7.4), containing 0.2% BSA, 10 mM EDTA and 0.05% (w/v) Tween-20, for 17 h at 4°C. The various molecular size classes of complexes were eluted from the column at a rate of 0.5 ml/min using 50 mM sodium phosphate buffer (pH 7.4). The ^{125}I content of each 1.0-ml fraction was measured in a γ-counter.

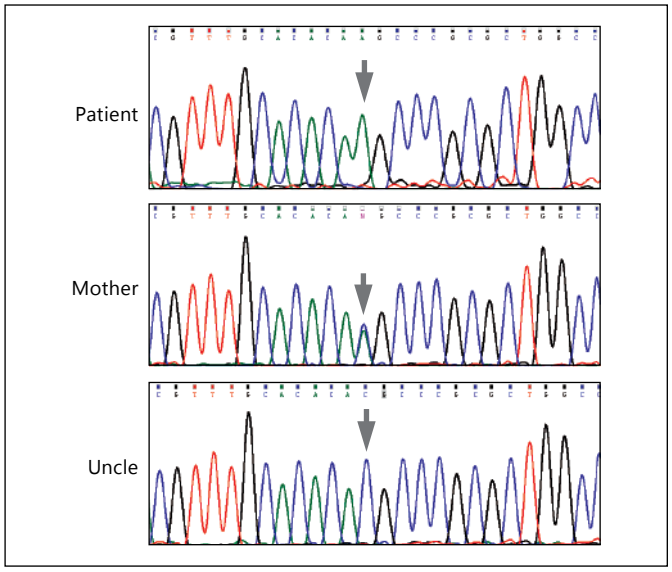

Fig. 2. Sequencing of the patient's *IGFALS* gene revealed a novel homozygous C to A substitution at nucleotide 434 (NM_004970.2: c.434C>A), leading to a Thr to Lys amino acid change at codon 145 (p.Thr145Lys).

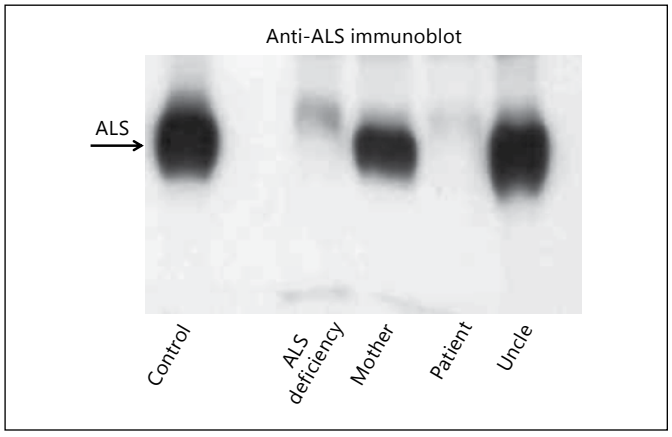

Fig. 3. Anti-ALS immunoblot of serum samples showing ALS protein levels comparable to those determined by the immunofluorometric assay.

Results

Sequencing of the coding regions of the *IGFBP3* gene revealed no abnormalities except for heterozygosity for the known polymorphism rs2854746. Sequence analysis of the *IGFALS* gene showed homozygosity for two known polymorphisms, rs3817902 and rs3751893, and a novel homozygous C to A substitution at nucleotide 434 (NM_004970.2: c.434C>A; fig. 2) leading to a Thr to Lys amino acid exchange at codon 145 (p.Thr145Lys/p.T145K). p.T145K is carried heterozygously by the patient's mother, who also presents significantly decreased serum concentrations of IGF-1, IGFBP-3 and ALS (table 1). The uncle (brother of the mother) does not carry the p.T145K mutation and has normal serum levels of circulating IGF-1, IGFBP-3 and ALS. Notably, all investigated family members are of normal stature with height SDS well within population-specific norms (table 1).

Anti-ALS Western blotting (fig. 3) of serum samples revealed ALS protein levels comparable to those determined by the immunofluorometric assay, and size exclusion chromatography (fig. 4) with ^{125}I-labeled IGF-1 molecules confirmed the inability of the patient's serum to form ternary complexes.

Discussion

Except for the lack of moderate short stature, the reported patient presented typical biochemical and clinical findings of ALS deficiency. Biochemically, ALS deficiency causes a severe reduction of circulating IGF-1 and, even more profoundly, IGFBP-3 [2, 3, 5, 12]. The extremely low IGFBP-3 level observed in our patient, in combination with normal growth and immunological abnormalities, led us first to consider primary IGFBP-3 deficiency. Accordingly, knockout mice lacking either IGFBP-3, -4, or -5 do not show major growth impairment [13]. However, sequencing of the coding regions of the *IGFBP3* gene in our patient revealed only heterozygosity for the known polymorphism rs2854746.

Elevated fasting insulin and undetectably low ALS serum levels then led to the diagnosis of ALS deficiency. Sequencing of the *IGFALS* gene revealed a novel homozygous missense mutation (c.434C>A; p.T145K). This mutation cosegregates with biochemical findings of ALS deficiency in the patient and his heterozygous mother, while his uncle does not carry p.T145K and has normal serum levels of ALS, IGF-1 and IGFBP-3. The functional relevance of this novel mutation is further illustrated by in vitro TCF tests, demonstrating the completely abolished ability of the patient's serum to form ternary complexes.

Compared to previously reported patients with ALS deficiency, our patient presents with a remarkably normal growth pattern. From age 2–9 years, height measurements were consistently above the population mean (fig. 1). Later on, a slight drop presumably reflects the transient reduction in growth velocity frequently seen in

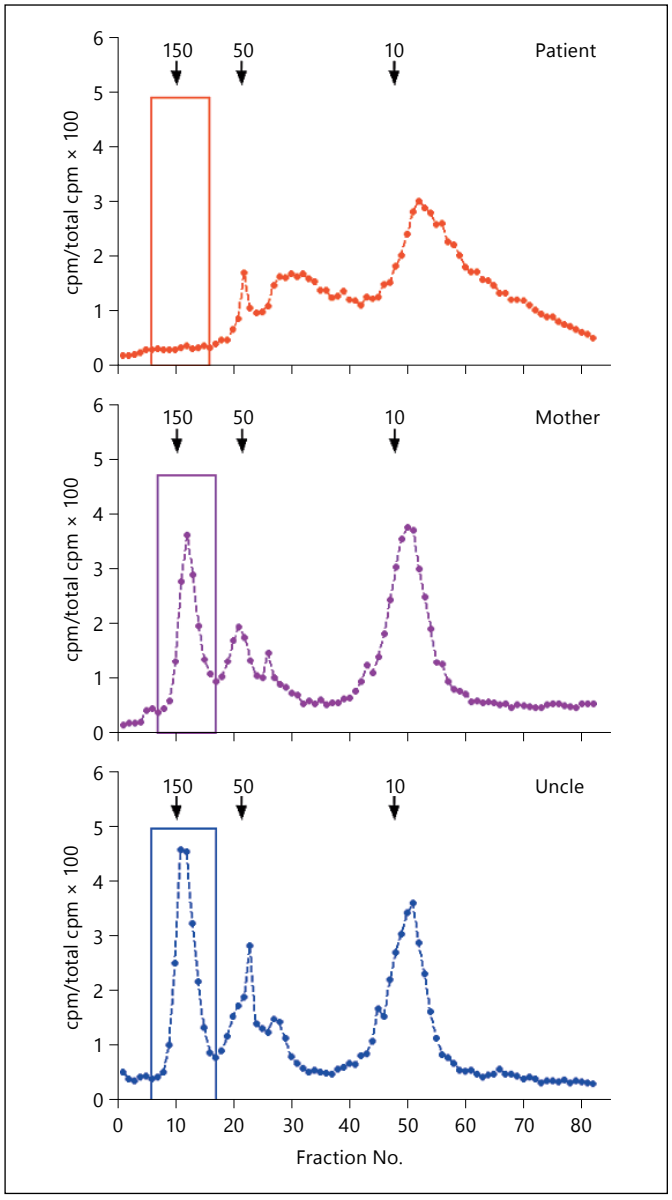

Fig. 4. In vitro TCF demonstrating the abolished ability of the patient's serum to form ternary complexes. As compared the normal in vitro TCF profile of the unaffected uncle, serum of the p.T145K heterozygous mother showed only low-to-normal TCF levels. Calculation of the area under the curve for the ternary complex peaks revealed percentages as follows: patient 1%, mother 21.7%, uncle 29.2%. Arrows indicate the position of the molecular weight markers 10, 50 and 150 kDa.

individuals with late puberty. With a current height of –0.19 SDS he still exceeds the upper range of height measurements in known ALS-deficient patients [3, 14]. Similarly, the difference of approximately 0.5 SDS between individual and midparental height is smaller than the expected difference of 1.48 ± 0.79 SDS (pooled data, International ALS Consortium: homozygosity or compound heterozygosity vs. heterozygosity [14]). However, only few patients with ALS deficiency have been identified so far, and owing to the generally mild growth impairment the prevalence of *IGFALS* mutations, especially in the near-to-normal phenotypic range, may be significantly underestimated.

In addition to late-normal to delayed puberty, which is reported in about 50% of male ALS-deficient patients [2–5], our patient presented several symptoms of a mild immune dysfunction. He suffers from recurrent pancytopenic episodes and cutaneous mycoses, and has diminished B and T helper lymphocytes, combined with low serum levels of IgM and IgG subclass 4 for at least 2 years. In addition, persistently elevated ANA titers and serum levels of IL-2 receptor may indicate a contribution of autoimmunity and chronic inflammation. Because none of the ALS-deficient patients described so far had overt immunological abnormalities ([2–5]; personal communication Dr. V. Hwa, Portland, Oreg., USA, Growth Genetics Consortium), and our patient was born to consanguineous parents, a second autosomal recessive defect rather than *IGFALS* p.T145K itself seems likely to underlie the immunological phenotype.

On the other hand, there is increasing evidence that IGFBP-3, which is typically profoundly diminished in ALS-deficient patients, modulates immunological processes via IGF-1-independent actions [reviewed in 15]. Although not always confirmed by recent meta-analyses, several epidemiological studies have linked IGFBP-3 to the individual risk of common malignancies such as prostate, breast, colorectal or lung cancer [15–19]. On a molecular level, IGFBP-3 has been shown to affect growth and other cell functions including apoptosis in an IGF-independent manner, possibly via interactions with members of the TGFβ and TNFα signaling pathways as well as by direct activation of proapoptotic molecules [20–22]. Recently, Ingermann et al. [23] identified and characterized a novel cell death receptor (IGFBP-3R) that is essential for IGFBP-3-mediated apoptosis and tumor suppression. Interestingly, IGFBP-3R signaling was subsequently shown to inhibit pivotal molecular aspects of airway inflammation and hyperresponsiveness in the pathogenesis of chronic inflammatory bronchial asthma in vitro and in vivo [24]. Thus, it would be interesting to reconsider the individual medical records of known patients with ALS deficiency in order to identify potential minor (auto)immunological abnormalities.

In this context, it is noteworthy that patients carrying STAT5B (signal transducer and activator of transcription 5B) mutations typically suffer from short stature and immune dysfunction. They also have very low serum levels of IGF-1, IGFBP-3 and ALS. However, compared to ALS deficiency, growth failure is generally more pronounced, with reported height SDS ranging between –3.0 and –9.9 [reviewed in 25]. Furthermore, immune dysfunction substantially differs from that (presumably coincidentally) presented by our patient, whose first clinical presentation was in adolescence. The majority of patients with STAT5B mutations show significant symptoms of immune deficiency already in early childhood, including chronic progressive pulmonary disease (lymphoid interstitial pneumonia), severe eczema and T cell dysfunction. When analyzed, all except 1 patient presented reduced counts of both CD4 and CD8 T cells, whereas B cells and serum IgG levels appeared to be normal to elevated. Sporadically, patients also developed autoimmune thyroiditis (2/10), juvenile idiopathic arthritis (1/10) or thrombocytopenic purpura (1–2/10) [16]. Nonetheless, the fact that other molecular defects within the GH-IGF-1 axis, especially those located upstream of STAT5B (GH, GHR), do not associate with immune dysfunction may indicate a distinct role of STAT5B in immunological processes. Currently, immunological dysfunction in STAT5B-deficient patients is explained by disturbed intercellular signaling after activation of cytokine receptors, leading to impaired regulatory T cell (T*reg*) development, granulopoiesis, and thymic stromal lymphopoietin-dependent dendritic cell activation [25–27].

To conclude, we have reported a novel *IGFALS* mutation, p.T145K, identified in a patient with biochemical signs of ALS deficiency but normal growth. Although co-occurrence of immunological dysfunction is likely to result from a second autosomal recessive hit, this case supports the assumption that the prevalence and possibly also the phenotypic spectrum of ALS deficiency may be underestimated.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

References

1 Guler HP, Zapf J, Schmid C, Froesch ER: Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol (Copenh) 1989;121:753–758.

2 Domené HM, Bengolea SV, Martínez AS, Ropelato MG, Pennisi P, Scaglia P, Heinrich JJ, Jasper HG: Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. N Engl J Med 2004;350:570–577.

3 Domené HM, Hwa V, Jasper HG, Rosenfeld RG: Acid-labile subunit (ALS) deficiency. Best Pract Res Clin Endocrinol Metab 2011; 25:101–113.

4 Hess O, Khayat M, Hwa V, Heath KE, Teitler A, Hritan Y, Allon-Shalev S, Tenenbaum-Rakover Y: A novel mutation in IGFALS, c.380T>C (p.L127P), associated with short stature, delayed puberty, osteopenia and hyperinsulinaemia in two siblings: insights into the roles of insulin growth factor-1 (IGF1). Clin Endocrinol (Oxf), E-pub ahead of print.

5 Domené HM, Hwa V, Argente J, Wit JM, Camacho-Hübner C, Jasper HG, Pozo J, van Duyvenvoorde HA, Yakar S, Fofanova-Gambetti OV, Rosenfeld RG, International ALS Collaborative Group: Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. Horm Res 2009;72:129–141.

6 Redlefsen T, Commentz J, Meigen C, Hermanussen M: Reference values for height, weight and body mass index of German born Turkish children. Anthropol Anz 2007;65:263–274.

7 Redlefsen T: Perzentilenkurven für in Deutschland geborene Kinder türkischer Abstammung für Körperlänge, Körpergewicht und BMI. http://ediss.sub.uni-hamburg.de/volltexte/2008/3745/.

8 Özer BK: Secular trend in body height and weight of Turkish adults. Anthropol Sci 2008; 116:191–199.

9 Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu MM, Akın MA, Çoban D, Gökoğlu S, Baştuğ O: Body weight, length and head circumference at birth in a cohort of Turkish newborns. J Clin Res Pediatr Endocrinol 2012;4:132–139.

10 Juul A, Moller S, Mosfeldt-Laursen E, Rasmussen MH, Scheike T, Pedersen SA, Kastrup KW, Yu H, Mistry J, Rasmussen S, Müller J, Henriksen J, Skakkebaek NE: The acid-labile subunit of human ternary insulin-like growth factor binding protein complex in serum: hepatosplanchnic release, diurnal variation, circulating concentrations in healthy subjects, and diagnostic use in patients with growth hormone deficiency. J Clin Endocrinol Metab 1998;83:4408–4415.

11 Van Duyvenvoorde HA, Kempers MJ, Twickler TB, van Doorn J, Gerver WJ, Noordam C, Losekoot M, Karperien M, Wit JM, Hermus AR: Homozygous and heterozygous expression of a novel mutation of the acid-labile subunit. Eur J Endocrinol 2008;159:113–120.

12 Hwa V, Haeusler G, Pratt KL, Little BM, Frisch H, Koller D, Rosenfeld RG: Total absence of functional acid labile subunit, resulting in severe insulin-like growth factor deficiency and moderate growth failure. J Clin Endocrinol Metab 2006;91:1826–1831.

13 Ning Y, Schuller AG, Bradshaw S, Rotwein P, Ludwig T, Frystyk J, Pintar JE: Diminished growth and enhanced glucose metabolism in triple knockout mice containing mutations of insulin-like growth factor binding protein-3, -4, and -5. Mol Endocrinol 2006;20:2173–2186.

14 Fofanova-Gambetti OV, Hwa V, Wit JM, Domene HM, Argente J, Bang P, Högler W, Kirsch S, Pihoker C, Chiu HK, Cohen L, Jacobsen C, Jasper HG, Haeusler G, Campos-Barros A, Gallego-Gómez E, Gracia-Bouthelier R, van Duyvenvoorde HA, Pozo J, Rosenfeld RG: Impact of heterozygosity for acid-labile subunit (*IGFALS*) gene mutation on stature: results from the International Acid Labile Subunit Consortium. J Clin Endocrinol Metab 2010;95:4184–4191.
15 Jogie-Brahim S, Feldman D, Oh Y: Unraveling insulin-like growth factor binding protein-3 actions in human disease. Endocr Rev 2009;30:417–437.
16 Wang L, Habuchi T, Tsuchiya N, Mitsumori K, Ohyama C, Sato K, Kinoshita H, Kamoto T, Nakamura A, Ogawa O, Kato T: Insulin-like growth factor-binding protein-3 gene -202 *A/C* polymorphism is correlated with advanced disease status in prostate cancer. Cancer Res 2003;63:4407–4411.
17 Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M: Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393–1396.
18 Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ: Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor(IGF)-1 and IGF-binding protein-3. J Natl Cancer Inst 1999;91:620–625.
19 Yu H, Spitz MR, Mistry J, Gu J, Hong WK, Wu X: Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. J Natl Cancer Inst 1999;91:151–156.
20 Fanayan S, Firth SM, Butt AJ, Baxter RC: Growth inhibition by insulin-like growth factor binding protein-3 in T47D breast cancer cells requires transforming growth factor-β (TGF-β) and the type II TGF-β receptor. J Biol Chem 2000;275:39146–39151.
21 Rajah R, Lee KW, Cohen P: Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-α-induced apoptosis: role of Bcl-2 phosphorylation. Cell Growth Differ 2002;13:163–171.
22 Lee KW, Ma L, Yan X, Liu B, Zhang XK, Cohen P: Rapid apoptosis induction by IGFBP-3 involves an insulin-like growth factor-independent nucleomitochondrial translocation of RXRα/Nur77. J Biol Chem 2005;280:16942–16948.
23 Ingermann AR, Yang YF, Han J, Mikami A, Garza AE, Mohanraj L, Fan L, Idowu M, Ware JL, Kim HS, Lee DY, Oh Y: Identification of a novel cell death receptor mediating IGFBP-3-induced anti-tumor effects in breast and prostate cancer. J Biol Chem 2010;285:30233–30246.
24 Lee YC, Jogie-Brahim S, Lee DY, Han J, Harada A, Murphy LJ, Oh Y: Insulin-like growth factor-binding protein-3 (IGFBP-3) blocks the effects of asthma by negatively regulating NF-κB signaling through IGFBP-3R-mediated activation of caspases. J Biol Chem 2011;286:17898–17909.
25 Hwa V, Nadeau K, Wit JM, Rosenfeld RG: STAT5b deficiency: lessons from *STAT5b* gene mutations. Best Pract Res Clin Endocrinol Metab 2011;25:61–75.
26 Kimura A, Rieger MA, Simone JM, Chen W, Wickre MC, Zhu BM, Hoppe PS, O'Shea JJ, Schroeder T, Hennighausen L: The transcription factors STAT5A/B regulate GM-CSF-mediated granulopoiesis. Blood 2009;114:4721–4728.
27 Bell BD, Kitajima M, Larson RP, Stoklasek TA, Dang K, Sakamoto K, Wagner KU, Reizis B, Hennighausen L, Ziegler SF: The transcription factor STAT5 is critical in dendritic cells for the development of T_H2 but not T_H1 responses. Nat Immunol 2013;14:364–371.