CASE REPORT

A novel *STAT5B* mutation causing GH insensitivity syndrome associated with hyperprolactinemia and immune dysfunction in two male siblings

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Abstract

Background: GH insensitivity (GHI) syndrome caused by *STAT5B* mutations was recently reported, and it is characterized by extreme short stature and immune dysfunction. Treatment with recombinant human IGF1 (rhIGF1) is approved for patients with GHI, but the growth response to this therapy in patients with *STAT5B* mutations has not been reported.

Objectives: To report the clinical features, molecular findings, and the short-term growth response to rhIGF1 therapy in patients with *STAT5B* mutation.

Subjects and methods: Hormonal and immunological evaluations were performed in two male siblings with GHI associated with atopic eczema, interstitial lung disease, and thrombocytopenic purpura. *STAT5B* genes were directly sequenced. The younger sibling was treated with rhIGF1 at a dose of 110 µg/kg BID.

Results: Both siblings had laboratory findings compatible with GHI associated with hyperprolactinemia. Lymphopenia and reduced number of natural killer cells without immunoglobulin abnormalities were observed. *STAT5B* sequence revealed a homozygous frameshift mutation (p.L142fsX161) in both siblings. The younger sibling (9.9 years of age) was treated with rhIGF1 at appropriate dosage, and he did not present any significant change in his growth velocity (from 2.3 to 3.0 cm/year after 1.5 years of therapy). The presence of a chronic illness could possibly be responsible for the poor result of rhIGF1 treatment. Further studies in patients with *STAT5B* defects are necessary to define the response to rhIGF1 treatment in this disorder.

Conclusion: GHI associated with immune dysfunction, especially interstitial lung disease, and hyperprolactinemia is strongly suggestive of a mutation in *STAT5B* in both sexes.

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Introduction

GH insensitivity (GHI; OMIM: #262500) is a genetic disorder characterized by severe postnatal growth failure, craniofacial disproportion, elevated serum GH, low insulin-like growth factor 1 (IGF1), and the inability to respond to endogenous or exogenous GH with appropriate growth and metabolic effects (1). It is classically associated with mutations in the GH receptor (*GHR*; OMIM: *600946), and is transmitted in an autosomal recessive manner (1, 2).

Recently, homozygous mutations in the signal transducer and activator of transcription 5B gene (*STAT5B*; OMIM: *604260) were described in patients

with GHI (3–9). Besides the clinical and hormonal phenotype of GHI, the majority of these patients also had severe immune dysfunction and elevated prolactin (PRL) levels (3–9). *STAT5B* is a critical molecule involved in GHR signal transduction, mediating the growth-promoting actions of the GHR. In addition to its role in GHR signal transduction, *STAT5B* is also involved in the immune system (10–12). *STAT5B* is an important mediator of interleukin-2 actions (13), and disruption of this signal transduction is responsible for T-cell function defects (14).

Of the six cases with *STAT5B* mutations reported to date, five were female. Although lymphoid interstitial pneumonia, chronic diarrhea, severe eczema, herpes

keratitis, herpes zoster, severe varicella, and juvenile arthritis were described in female patients with *STAT5B* mutations (15), the only male patient reported did not have a clear evidence of immunological defects (7, 8). It was conjectured that the immune-dysfunction phenotype associated with *STAT5B* deficiency could be sex dependent (8). In the present study, we describe a novel frameshift mutation in *STAT5B* in two male siblings with GHI, immunological defects, and elevated PRL levels. Moreover, the first year growth response to recombinant human IGF1 (rhIGF1) therapy is reported.

Case reports

Two male Brazilian Caucasian siblings born from non-consanguineous parents of normal stature (mother's height = 156 cm (−1 SDS) and father's height = 165 cm (−1.5 SDS)) were studied. Both were born premature and with adequate weight and length for gestational age (Table 1). The older sibling (sibling 1) was first evaluated at 6 years of age because of severe short stature, atopic eczema, and interstitial lung disease that manifested in the first year of life. At that time, he was 86 cm tall (height SDS = −5.6), weighed 10 kg (body mass index (BMI) SDS = −1.9), and had delayed bone age (BA = 3 years). He presented mild

Table 1 Clinical characteristics of two male siblings with the novel p.L142fsX161 *STAT5B* mutation.

	Sibling 1		Sibling 2	
	Values	SDS	Values	SDS
At birth				
Gestational age (weeks)	28		33	
Birth length (cm)	39	0.9	49	2.3
Birth weight (kg)	1.7	3.0	2.4	2.0
At the start of rhGH therapy ^a				
Age (years)	10.0		3.0	
Height (cm)	106	−4.9	78	−5.0
Weight (kg)	19	−4.0	9.5	−4.2
Growth velocity (cm/year)	5.5	+0.5	2.0	−4.3
At the end of rhGH therapy				
Age (years)	14		7.3	
Height (cm)	118	−5.1	99	−4.6
Weight (kg)	20	−6.7	15	−4.2
Growth velocity (cm/year)	5.4	−3.4	3.6	−2.5
At the start of rhIGF1 therapy ^b				
Age (years)			9.9	
Bone age (years)			3.5	
Height (cm)			107.5	−5.0
Weight (kg)			15.9	−6.0
Growth velocity (cm/year)			2.3	−4.0
At the end of rhIGF1 therapy ^b				
Age (years)			11.4	
Bone age (years)			6.0	
Height (cm)			112.0	−5.0
Weight (kg)			20.0	−4.5
Growth velocity (cm/year)			3.0	−2.4

^aThe mean dose of rhGH was 50 µg/kg per day for sibling 1 and 43 µg/kg per day for sibling 2.

^bThe mean dose of rhIGF1 was 110 µg/kg per day BID.

Figure 1 Clinical features of the patients with *STAT5B* mutation. Sibling 1 is shown on the left and sibling 2 is shown on the right. The patients have typical characteristics of GHI, such as prominent forehead, depressed nasal bridge, and centripetal fat distribution, especially in sibling 2. (The patients' parents provided written informed consent to use these images).

facial dysmorphic features observed in patients with GHI (Fig. 1). He was under corticosteroids and oxygen therapy due to severe lymphocytic interstitial pneumonitis confirmed by biopsy. Puberty started late at 16.8 years. Owing to impairment in his pulmonary function, at 17.5 years, a living-donor lung transplantation was performed successfully. The surgical pathology report revealed parenchymal fibrosis, bronchiectasis, and emphysema. Currently, 9 months after transplantation, the patient is treated with immunosuppressant agents and no longer needs oxygen therapy.

His younger brother (sibling 2) was first evaluated at 2.0 years of age with a history of atopic eczema and interstitial lung disease. At this age, his height was 76 cm (height SDS = −3.0), his weight was 7.5 kg (BMI SDS = −3.9), and his BA was 6 months. He had a marked GHI phenotype, with a prominent forehead, depressed nasal bridge, and centripetal fat distribution (Fig. 1). At the age of 4, he developed thrombocytopenic purpura requiring chronic treatment with glucocorticoids, which were replaced by hydroxychloroquine at 8 years of age, but at the age of 11.1 years, 1.2 mg/kg

Figure 2 Axial chest computerized tomography (CT) scan of sibling 2 showing areas of ground glass attenuation affecting mainly the posterior aspects of the lungs.

prednisone was reintroduced due to worsening of pulmonary function (Fig. 2). Patients have an older healthy half-sister with normal adult stature (height SDS = −1.5).

Materials and methods

This study was approved by the local Ethics Committee, and the patients' parents provided written informed consent.

Laboratory evaluation

GH, IGF1, and IGF binding protein 3 (IGFBP3) were measured using commercially available chemiluminescence assays (Immulite-DPC 2000, Diagnostic Products Corp., Los Angeles, CA, USA), whereas PRL was determined using the electrochemiluminescence method (Roche E170, Roche Diagnostics GmbH). To estimate the presence of macroPRL, serum was assayed for PRL before and after treatment with polyethylene glycol, as previously described (16). To assess GH secretion, clonidine was given orally in a dose of 0.10 mg/sq. m., and samples for serum GH were taken at 0, 30, 60, 90, and 120 min after its administration. The IGF1 generation test involved daily injections of rhGH (0.1 U/kg per day or 33 µg/kg per day s.c.) for 4 days. Blood samples were drawn in the morning before the first injection and 12 h after the last dose. IGF1 increase <15 µg/l was considered for the diagnosis of GHI (17). Immunophenotyping was performed by flow cytometry standard methods. The levels of immunoglobulins IgA, IgG, and IgM were determined by nephelometry (BN2, Behring, GmbH, Marburg, Germany), and that of IgE was determined by chemiluminescence (Immulite-DPC 2000, Diagnostic Products Corp).

Molecular studies

Genomic DNA was isolated from peripheral blood leukocytes from the two patients and their parents. *GHR* exons 2–10 and *STAT5B* exons 2–19 were amplified using specific intronic primers to cover the entire coding region (primer sequences and amplification protocols will be sent upon request). PCR products from these exons were directly sequenced with dideoxy chain-termination method using a kit ABI Prism™ BigDye Terminator (Perkin Elmer, Foster City, CA, USA) and analyzed by an autosequencer ABI Prism Genetic Analyser 3100 automatic DNA sequencer (Perkin Elmer). *GHR* exon 5 of 50 control samples (100 alleles) was amplified by PCR and directly sequenced.

In silico prediction of mutation effects

Missense variants identified by sequencing were classified based on their potential impact on protein function or structure using a new version of the PolyPhen method (18). These predictions are based on the analysis of multiple sequence alignments of homologous proteins, functional annotation, and structural information. PolyPhen defines the predictions of the mutations as follows: i) probably damaging, it is with high confidence that it is supposed to affect protein function or structure; ii) possibly damaging, it is supposed to affect protein function or structure; and iii) benign, it is most likely that it lacks any phenotypic effect (<http://genetics.bwh.harvard.edu/pph>).

Results

Hormonal results

Hormonal evaluations are shown in Table 2. Patients had normal basal and stimulated GH serum levels. IGF1 and IGFBP3 serum levels were extremely low (< −3 SD). IGF1 levels did not increase significantly during the generation test or during rhGH treatment in both patients. Interestingly, serum PRL levels were elevated in both siblings, and the presence of macroPRL was ruled out. Hypothalamic–pituitary magnetic resonance imaging of both patients was normal.

Immunological studies

Moderate lymphopenia was observed in both siblings, with reduced number of all evaluated cell lines (T cells CD4, CD3, and CD8; B cells CD19; and NK cells CD56; Table 2). The levels of immunoglobulins were within the normal range on several occasions during the follow-up period.

Table 2 Hormonal and immunological evaluation of two male siblings with *STAT5B* mutation (p.L142fsX161).

	Sibling 1	Sibling 2	Reference value
Hormonal evaluation			
Basal GH ($\mu\text{g/l}$)	1.7	1.0	0.06–5.0
GH peak after clonidine ($\mu\text{g/l}$)	20.6	14.2	> 5
Basal IGF1 (ng/ml)	34	<25	95–400
IGF1 after generation test	48	<25	Increase > 15
IGF1 range during rhGH therapy ^a	<25–47	<25–43	
IGF1 range during rhIGF1 therapy ^b		90–125	
Basal IGFBP3 (ng/ml)	520	754	1250–7060
PRL (ng/ml)	61	77	4.6–21
MacroPRL	Absent	Absent	
Immunological evaluation			
Leukocytes (per μl)	8200	6300	4000–11 000
Lymphocytes (per μl)	820 (10%)	662 (10.5%)	900–3400
T-lymphocytes (per μl)			
CD3	462 (56.4%)	404 (61%)	1545–2489
CD4	242 (29.5%)	285 (43%)	771–1180
CD8	185 (22.5%)	96 (14.5%)	629–1128
B-lymphocytes (per μl)			
CD19	82 (10%)	93 (14%)	278–481
NK-lymphocytes (per μl)			
CD56	90 (11%)	70 (10.5%)	113–237
Serum immunoglobulins (mg/dl)			
IgG	1630	1210	690–1400
IgM	131	58	34–210
IgA	431	118	88–410
IgE	15.5	35.4	<52

^aThe mean dose of rhGH was 50 $\mu\text{g/kg}$ per day for sibling 1 and 43 $\mu\text{g/kg}$ per day for sibling 2.

^bSamples collected in the morning, 10–12 h after the last rhIGF1 injection (110 $\mu\text{g/kg}$).

Growth response to rhGH and rhIGF1 treatment

Both patients had long-term treatment (for 4 and 5 years) with high dose of rhGH (43–50 $\mu\text{g/kg}$ per day) with no clear improvement in growth rate (Table 1 and Fig. 3) or increase in IGF1 levels (Table 2). At the age of 9.9 years, a trial with rhIGF1 (Mecasermin, Increlex, Tercica Inc., Brisbane, CA, USA) was started with 110 $\mu\text{g/kg}$ BID in sibling 2. During 1.5 years of rhIGF1 therapy, no clear increase in growth velocity in relation to baseline was observed (Table 1 and Fig. 3B), despite documented increase in IGF1 levels during therapy (Table 2). During rhIGF1 treatment, the patient remained prepubertal and had mild symptoms compatible with of hypoglycemia, but no severe side effects.

Molecular results

Direct sequencing of *GHR* (cDNA reference sequence: NM_000163) of sibling 1 revealed a heterozygous missense mutation at exon 5 located in the extracellular portion of the receptor: a nucleotide substitution (c.409 G>A), which replaces aspartic acid by asparagine in codon 137 (p.D137N) (Supplementary Figure 1, see section on supplementary data given at the end of this article). The unaffected patient's father was also heterozygous for this mutation, whereas his mother and sibling 2 are homozygous for *GHR* wild-type allele. This allelic variant was not found in 100 alleles from

the control group. *In silico* analysis by PolyPhen suggested that p.D137N is benign.

Sequencing of the *STAT5B* (cDNA reference sequence: NM_012448.3) revealed a deletion of four nucleotides (CTCC) in the positions 424–427 in exon 5 (c.424_427 del), which results in a frameshift and premature truncation of the protein in codon 161 (p.L142fsX161) (Supplementary Figure 1). This mutation was found in the homozygous state in both siblings and in the heterozygous state in their parents.

Discussion

GHI is a rare disorder characterized by marked short stature, which results from failure to generate IGF1 in response to GH. The majority of the cases are caused by *GHR* defects, being named Laron syndrome (1, 2). Recently, a Laron syndrome-like phenotype associated with immune dysregulation has been described, which is caused by mutations in the *STAT5B* gene leading to a *GHR* signaling defect at post-receptor level.

In this study, two male siblings with classical GHI phenotype associated with immunological defects were studied. The patients were first evaluated for *GHR* mutations. To date, ~60 *GHR* defects in homozygous or compound heterozygous states have been described (reviewed in Ref. (2)). An allelic variant in *GHR* exon 5 (p.D137N) was identified in sibling 1 and in his non-affected father, but not in sibling 2. Considering that this

Figure 3 Height for age chart of sibling 1 (A) and sibling 2 (B). Growth charts were drawn using Growth analyser 3.5 (Ed. Dutch Growth Foundation, PO Box 23068, 3001 KB, Rotterdam, The Netherlands). rhGH treatment is shown as solid line, whereas rhIGF1 treatment is shown as a dashed line. The moment of lung transplantation is shown by the solid arrow.

mutation was found in heterozygous state and that it did not segregate with the disease in this family, along with the fact that *in silico* analysis predicted this variant to be benign, we considered that p.D137N is not the cause of the GHI in our patients.

The involvement of *STAT5B* in GHI patients without mutations in *GHR* has been recently described in six patients (3–9). A major characteristic of patients with GHI due to *STAT5B* defects is the presence of immunological dysfunction. Therefore, *STAT5B* was screened in our family, and one homozygous frameshift mutation (p.L142fsX161) was identified in both siblings. This mutation leads to a truncated protein, which retains only the NH₂-terminal domain and lacks the DNA binding, the tyrosine activation, and the C-terminal SH2 domains, making this protein biologically inactive. The parents carry this mutation in heterozygous state, and although consanguinity was denied, both parents' families come from the same South Brazilian city (Criciúma, 28°40'39"S 49°22'11"W).

Although immunological dysfunction was described in female patients with *STAT5B* mutations (15), the only male patient previously reported in the literature had normal immunological evaluation (7, 8), suggesting that immunological dysfunction was female sex limited. However, both our male patients had atopic eczema and interstitial lung disease; and sibling 2 also had thrombocytopenic purpura. Furthermore, their laboratory evaluation showed T- and B-cell lymphopenia, as well as reduced NK cell number. These results are in line with another study that also characterized the immune system abnormalities in a female patient with *STAT5B* mutation (6), characterizing the importance of *STAT5B* in immune regulation in both sexes.

Interstitial lung disease is a life-threatening condition observed in the majority of *STAT5B*-mutated patients (3, 4, 7–9). It is noteworthy that sibling 2, who started glucocorticoid therapy at the age of 4 due to

thrombocytopenic purpura, presented less severe lymphocytic interstitial pneumonitis than his brother. Furthermore, the pulmonary functional test worsened during the period when the patient was treated with hydroxychloroquine. Another therapeutic option to correct the immunological dysfunction would be a bone marrow transplantation to prevent worsening and end-stage pulmonary insufficiency.

Elevated PRL levels were also described in patients with *STAT5B* mutations (3, 6, 7). Owing to the immunological dysregulation associated with *STAT5B* mutations, this hyperprolactinemia could reflect the presence of macroPRL, a high molecular weight IgG–PRL complex which has reduced bioactivity. Our patients had PRL levels three times higher than the upper limit of the normal range, and the presence of macroPRL or pituitary tumor was excluded. These results corroborate the hypothesis that there might be a PRL resistance state in *STAT5B* defects, since *STAT5B* plays a role in mediating the negative feedback action of PRL in mice (19). The adult male patient with *STAT5B* mutation previously described had normal LH, FSH, and testosterone despite elevation of PRL levels (7, 8), suggesting a lack of action of circulating PRL. The pubertal delay observed in patients with *STAT5B* defects can be explained by the state of chronic illness or related to the low levels of circulating IGF1 (20–22). For this reason, at this moment, we did not recommend any specific treatment for the mild hyperprolactinemia observed in these patients.

It is noteworthy that basal and stimulated GH levels were in the normal range in contrast to elevated levels usually observed in patients with *GHR* defects (23, 24). The same was observed in other patients with *STAT5B* defects (4, 7). In addition to the heterogeneity between the adopted assays in each study, the chronic illness state and glucocorticoid use could contribute to this finding.

Patients with GHI due to *GHR* defects are typically unable to respond to exogenous rhGH with appropriate growth and metabolic effects. As *STAT5B* is crucial for the growth-promoting actions of the *GHR*, it is expected that patients with *STAT5B* defects will also be unresponsive to GH treatment. Indeed, similar to other cases reported in the literature, our patients did not significantly improve growth rate or increase IGF1 levels during rhGH treatment. Therapy with rhIGF1 is approved for the treatment of children with severe primary IGF1 deficiency, a condition that includes patients with mutations in *GHR* and post-*GHR* signaling pathway (25). The use of rhIGF1 has been described in patients with GHI due to *GHR* defects. The largest cohort comprehends 76 children treated with rhIGF1 and the median increment in first-year height velocity was 5.3 cm/year (from 2.8 cm/year at baseline to 8.0 cm/year on average) (26). To date, there are no reports of patients with GHI due to *STAT5B* mutations treated with rhIGF1. Sibling 2 has been treated with rhIGF1 at a regular dose for the past year, without a significant improvement in growth velocity. Possibly, the presence of a chronic illness could be responsible for the poor result of rhIGF1 treatment in our *STAT5B*-mutated patient. Further studies in patients with *STAT5B* defects are necessary to define the long-term response to rhIGF1 treatment in this disorder.

In summary, the presence of extreme short stature in a child with immune dysfunction and hyperprolactinemia suggests GHI due to *STAT5* inactivating mutations in both sexes. Owing to the important pulmonary/immunological dysfunction associated with *STAT5B* defects, these patients are probably first evaluated by pulmonologists or immunologists. Clinical and laboratory features of GHI associated with immunological defects and elevated PRL levels are strongly suggestive of a mutation in *STAT5B* gene, and should trigger investigation for defects in this gene.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-10-0272>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- 1 Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958–2003. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1031–1044.
- 2 Diniz ET, Jorge AA, Arnhold IJ, Rosenbloom AL & Bandeira F. Novel nonsense mutation (p.Y113X) in the human growth hormone receptor gene in a Brazilian patient with Laron syndrome. *Arquivos Brasileiros de Endocrinologia e Metabologia* 2008 **52** 1264–1271.
- 3 Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, Pratt KL, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ & Rosenfeld RG. Growth hormone insensitivity associated with a *STAT5b* mutation. *New England Journal of Medicine* 2003 **349** 1139–1147.
- 4 Hwa V, Little B, Adiyaman P, Kofoed EM, Pratt KL, Ocal G, Berberoglu M & Rosenfeld RG. Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4260–4266.
- 5 Fang P, Kofoed EM, Little BM, Wang X, Ross RJ, Frank SJ, Hwa V & Rosenfeld RG. A mutant signal transducer and activator of transcription 5b, associated with growth hormone insensitivity and insulin-like growth factor-I deficiency, cannot function as a signal transducer or transcription factor. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1526–1534.
- 6 Bernasconi A, Marino R, Ribas A, Rossi J, Ciaccio M, Oleastro M, Ornani A, Paz R, Rivarola MA, Zelazko M & Belgorosky A. Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel *STAT5b* gene mutation. *Pediatrics* 2006 **118** e1584–e1592.
- 7 Vidarsdottir S, Walenkamp MJ, Pereira AM, Karperien M, van Doorn J, van Duyvenvoorde HA, White S, Breuning MH, Roelfsema F, Kruithof ME, van Dissel J, Janssen R, Wit JM & Romijn JA. Clinical and biochemical characteristics of a male patient with a novel homozygous *STAT5b* mutation. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 3482–3485.
- 8 Walenkamp MJ, Vidarsdottir S, Pereira AM, Karperien M, van Doorn J, van Duyvenvoorde HA, Breuning MH, Roelfsema F, Kruithof ME, van Dissel J, Janssen R, Wit JM & Romijn JA. Growth hormone secretion and immunological function of a male patient with a homozygous *STAT5b* mutation. *European Journal of Endocrinology* 2007 **156** 155–165.
- 9 Hwa V, Camacho-Hubner C, Little BM, David A, Metherell LA, El-Khatib N, Savage MO & Rosenfeld RG. Growth hormone insensitivity and severe short stature in siblings: a novel mutation at the exon 13–intron 13 junction of the *STAT5b* gene. *Hormone Research* 2007 **68** 218–224.
- 10 Antov A, Yang L, Vig M, Baltimore D & Van Parijs L. Essential role for *STAT5* signaling in CD25⁺CD4⁺ regulatory T cell homeostasis and the maintenance of self-tolerance. *Journal of Immunology* 2003 **171** 3435–3441.
- 11 Imada K, Bloom ET, Nakajima H, Horvath-Arcidiacono JA, Udy GB, Davey HW & Leonard WJ. *Stat5b* is essential for natural killer cell-mediated proliferation and cytolytic activity. *Journal of Experimental Medicine* 1998 **188** 2067–2074.
- 12 Snow JW, Abraham N, Ma MC, Herndier BG, Pastuszak AW & Goldsmith MA. Loss of tolerance and autoimmunity affecting multiple organs in *STAT5A/5B*-deficient mice. *Journal of Immunology* 2003 **171** 5042–5050.

- 13 Kisseleva T, Bhattacharya S, Braunstein J & Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* 2002 **285** 1–24.
- 14 Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosfeld G & Ihle JN. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 1998 **93** 841–850.
- 15 Rosenfeld RG, Belgorosky A, Camacho-Hubner C, Savage MO, Wit JM & Hwa V. Defects in growth hormone receptor signaling. *Trends in Endocrinology and Metabolism* 2007 **18** 134–141.
- 16 Hattori N, Ikekubo K, Ishihara T, Moridera K, Hino M & Kurahachi H. Effects of anti-prolactin autoantibodies on serum prolactin measurements. *European Journal of Endocrinology* 1994 **130** 434–437.
- 17 Blum WF, Cotterill AM, Postel-Vinay MC, Ranke MB, Savage MO & Wilton P. Improvement of diagnostic criteria in growth hormone insensitivity syndrome: solutions and pitfalls. Pharmacia Study Group on insulin-like growth factor I treatment in growth hormone insensitivity syndromes. *Acta Paediatric. Supplementum* 1994 **399** 117–124.
- 18 Ramensky V, Bork P & Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Research* 2002 **30** 3894–3900.
- 19 Grattan DR, Xu J, McLachlan MJ, Kokay IC, Bunn SJ, Hovey RC & Davey HW. Feedback regulation of PRL secretion is mediated by the transcription factor, signal transducer, and activator of transcription 5b. *Endocrinology* 2001 **142** 3935–3940.
- 20 Chandrasekar V, Zaczek D & Bartke A. The consequences of altered somatotrophic system on reproduction. *Biology of Reproduction* 2004 **71** 17–27.
- 21 Domene HM, Scaglia PA, Lteif A, Mahmud FH, Kirmani S, Frystyk J, Bedecarras P, Gutierrez M & Jasper HG. Phenotypic effects of null and haploinsufficiency of acid-labile subunit in a family with two novel IGFALS gene mutations. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 4444–4450.
- 22 Laron Z & Klinger B. Effect of insulin-like growth factor-I treatment on serum androgens and testicular and penile size in males with Laron syndrome (primary growth hormone resistance). *European Journal of Endocrinology* 1998 **138** 176–180.
- 23 Jorge AA, Menezes Filho HC, Lins TS, Guedes DR, Damiani D, Setian N, Arnhold IJ & Mendonca BB. Founder effect of E180splice mutation in growth hormone receptor gene (GHR) identified in Brazilian patients with GH insensitivity. *Arquivos Brasileiros de Endocrinologia e Metabologia* 2005 **49** 384–389.
- 24 Jorge AA, Souza SC, Arnhold IJ & Mendonca BB. The first homozygous mutation (S226I) in the highly-conserved WSXWS-like motif of the GH receptor causing Laron syndrome: suppression of GH secretion by GnRH analogue therapy not restored by dihydrotestosterone administration. *Clinical Endocrinology* 2004 **60** 36–40.
- 25 Collett-Solberg PF & Misra M. The role of recombinant human insulin-like growth factor-I in treating children with short stature. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 10–18.
- 26 Chernausek SD, Backeljauw PF, Frane J, Kuntze J & Underwood LE. Long-term treatment with recombinant insulin-like growth factor (IGF)-I in children with severe IGF-I deficiency due to growth hormone insensitivity. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 902–910.

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