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.

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 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...
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 mg/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 PrismTM 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.
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 μg/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 μg/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. 3 B), 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

| Table 2 Hormonal and immunological evaluation of two male siblings with STAT5B mutation (p.L142fsX161). |
|--------------------------------------------------------|-----------------|------------------|
| **Hormonal evaluation**                                  | **Sibling 1**   | **Sibling 2**    |
| Basal GH (μg/l)                                         | 1.7             | 1.0              |
| GH peak after clonidine (μg/l)                          | 20.6            | 14.2             |
| Basal IGF1 (ng/ml)                                      | 34              | <25              |
| IGF1 after generation test                              | 48              | <25              |
| IGF1 range during rhGH therapy                          | <25–47          | <25–47           |
| IGF1 range during rhIGF1 therapy                        | 90–125          | Increase >15     |
| Basal IGFBP3 (ng/ml)                                    | 520             | 754              |
| PRL (ng/ml)                                             | 61              | 77               |
| MacropRPL                                               | Absent          | Absent           |
| **Immunological evaluation**                            |                 |                  |
| Leukocytes (per μl)                                     | 8200            | 6300             |
| Lymphocytes (per μl)                                    | 820 (10%)       | 662 (10.5%)      |
| T-lymphocytes (per μl)                                  | 462 (56.4%)     | 404 (61%)        |
| CD4                                                     | 242 (29.5%)     | 285 (43%)        |
| CD8                                                     | 185 (22.5%)     | 96 (14.5%)       |
| B-lymphocytes (per μl)                                  | 82 (10%)        | 93 (14%)         |
| CD19                                                    | 242 (29.5%)     | 285 (43%)        |
| NK-lymphocytes (per μl)                                 | 70 (10.5%)      | 113–237          |
| Serum immunoglobulins (mg/dl)                           |                 |                  |
| IgG                                                     | 1630            | 1210             |
| IgM                                                     | 131             | 58               |
| IgA                                                     | 431             | 118              |
| IgE                                                     | 15.5            | 35.4             |
| aThe mean dose of rhGH was 50 μg/kg per day for sibling 1 and 43 μg/kg per day for sibling 2. |
| bSamples collected in the morning, 10–12 h after the last rhIGF1 injection (110 μg/kg). |
A novel STAT5B mutation in male siblings

A novel STAT5B mutation in male siblings

GHI due to STAT5B only the NH2-terminal domain and lacks the DNA mutation leads to a truncated protein, which retains (p.L142fsX161) was identified in both siblings. This 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.I142fsX161) 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 (Criciuma, 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.

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References

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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