Genetic disorders in the growth hormone-IGF-I axis

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Clinical and biochemical characteristics of a male patient with a novel homozygous STAT5b mutation

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Abstract

Context: GH insensitivity can be caused by defects in the GH receptor (GHR) or in the postreceptor signaling pathway. Recently, two female patients with severe growth retardation and pulmonary and immunological problems were described with a defect in STAT5b, a critical intermediary of downstream GHR signaling.

Objective: The objective was to determine the functional characteristics of a novel STAT5b mutation and describe the phenotype.

Patients: We describe an adult male patient with short stature (-5.9 SDS), delayed puberty, and no history of pulmonary or immunological problems. GH-binding protein level as well as GH secretion characteristics were normal. Plasma prolactin level was elevated. Extremely low levels of IGF-I (-6.9 SDS), IGF-binding protein-3 (-12 SDS), and acid-labile subunit (-7.5 SDS) were found.

Results: We found a homozygous frameshift mutation in the STAT5b gene (nucleotide 1102-3insC, Q368fsX376), resulting in an inactive truncated protein, lacking most of the DNA binding domain and the SH2-domain.

Conclusions: This report confirms the essential role of STAT5b in GH signaling in the human. We show for the first time that immunological or pulmonary problems or elevated GH secretion are not obligatory signs of STAT5b deficiency, whereas hyperprolactinemia appears to be part of the syndrome. Therefore, in patients with severe short stature, signs of GH insensitivity, and a normal GHR, analysis of the STAT5b gene is recommended.

Introduction

Primary GH insensitivity is characterized by severe postnatal growth failure, elevated levels of GH and decreased levels of IGF-I. The majority of patients with GH insensitivity have mutations in the extracellular domain of the GH receptor (GHR), resulting in reduced GH binding. The first report of a mutation concerning the GH signaling pathway was published by Kofoed *et al.* (1) describing a female patient with a homozygous missense mutation in the STAT5b gene. Recently, Hwa *et al.* (2) described another female patient with a homozygous frameshift mutation of the STAT5b gene. In both patients short stature is associated with pulmonary problems and severe immunodeficiency.

STAT5b is a component of the Janus kinase-STAT signal transduction pathway. Of the seven STAT proteins, the GHR preferentially uses STAT5b for signal transduction. In the absence of STAT5b, the ability of GH to induce the expression of IGF-I mRNA is almost completely abrogated both in mice and man (1-3).

We now describe the clinical and biochemical characteristics of the first male with a homozygous frameshift mutation in the STAT5b gene, in whom short stature was not accompanied by immunodeficiency.

Patients and Methods

Clinical and molecular studies were performed after obtaining written informed consent.

Biochemical assays

Plasma GH was measured with time-resolved immunofluorometric assay (Wallac/PE, Turku, Finland), using the WHO 80/505 as a standard (1 mg=2.6 IU). Plasma IGF-I, IGF-II, IGF-binding-protein (IGFBP)-1, IGFBP-2, IGFBP-3, and IGFBP-6 were determined by specific RIAs (4).

Acid-labile subunit (ALS) was measured by an ELISA (Diagnostics Systems Laboratories, Inc., Webster, TX) (5). With the exception of IGFBP-1, smoothed references were available for all parameters, based on the LMS method (6), allowing

conversion of the data of the patient to SD score (SDS) values. Plasma IGFBP-1 concentration after an overnight fast was compared with a reference group of 6 healthy adult controls. Prolactin was measured by electrochemical luminescence immunoassay (Roche Diagnostics, Almere, Netherlands). GH-binding protein was measured with the ligand immunofluorometric method (7).

Analysis of STAT5b

A skin biopsy was taken, and a culture of dermal fibroblasts was established. Genomic DNA was isolated from whole blood. STAT5b mRNA isolated from fibroblasts and STAT5b coding exons were amplified by PCR and subjected to direct sequencing. Primer combinations are available on request.

Western blotting

Western blotting to detect phospho-Erk1, Erk2 and PKB/Akt was performed using fibroblast cultures from the patient and an age- and sex-matched normal subject as described in detail previously (8). Antibodies directed to inact STAT5b were obtained by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Results

Phenotype

The index patient was born in the Dutch Antilles. His parents were not aware of any consanguinity. Paternal and maternal heights were 164.3 cm (-2.8 SDS) and 165.6 cm (-0.8 SDS), target height was 176 cm (-1.1 SDS) (9). He was born after a full-term uncomplicated pregnancy. At birth, his weight was 3270 g (-0.7 SDS) and his length was 50 cm (-0.4 SDS) (10). Shortly after birth, congenital ichthyosis was diagnosed.

At the age of 16 yr, he emigrated to The Netherlands. Shortly after arrival, he was admitted to the hospital because of hemorrhagic chickenpox. He made a full recovery after treatment with acyclovir. He was referred to a paediatric endocrinologist, for evaluation of growth retardation. His body proportions were normal, although his pubertal development was delayed (Tanner stage G1P1), testicular volume being only 1 ml. Biochemical evaluation revealed a low IGF-I level (3.8 nmol/liter, -5.6 SDS) and

a slightly elevated prolactin level 1.4 U/liter (normal value < 0.3 U/liter). His maximal GH response to 50 μg GHRH iv was 25 μg/liter, which is considered to be a normal response. A computed tomography scan of the pituitary and hypothalamus revealed no abnormalities. His bone age was 9 yr. A 24-h plasma GH profile was normal. Nevertheless, a presumptive diagnosis of GH secretory dysfunction was made and a trial with recombinant human GH treatment (Genotropin) was started in a dose of 1.5 IU (0.5 mg)/day for 25 months, followed by a dose of 3.0 IU (1.0 mg)/day for an additional 3 months. However, this treatment did not significantly improve growth rate, except for a slight growth acceleration probably due to pubertal development. IGF-I levels increased only slightly during treatment with recombinant human GH (5.8 nmol/liter, -4.7 SDS). At the age of 19.8 yr, bromocriptin was started (2.5 mg/day) and serum prolactin decreased (<0.05 U/liter). Eight months later, he returned to the Dutch Antilles, and he was lost to follow-up for 10 yr.

At the age of 30 yr, he was referred to our clinic for evaluation of his short stature (Table 1). He did not have any complaints and reported normal libido, erections, and ejaculations. He used to shave every four days. There was no history of infectious diseases during the previous 16 yr. There was striking central obesity and pseudogynecomastia. He did not have galactorrhea. Secondary sexual characteristics were typical for a male, facial hair was present, and body hair distribution showed a male pattern, although scarce. The ichthyosis was not active. There were no dysmorphic features. Testicular volume was 12 ml. The stretched penile length was 8 cm (P10-P90, 10.2-16.4 cm) (11). Cardiovascular, respiratory, and abdominal examinations were normal.

Endocrine features at 30 yr of age

Circulating levels of IGF-I, IGF-II, IGFBP-3, and ALS were markedly reduced (Table 1). The concentration of prolactin was markedly elevated (*i.e.* five times the upper limit of normal). Basal GH level was normal (0.33 mU/liter). The maximal GH response after administration of insulin was 37.0 mU/liter (14.2 ng/ml), and after GHRH-arginine, 29.4 mU/liter (11.3 ng/ml), which are considered to be normal responses (12).

Mutational analysis

Sequence analysis of STAT5b mRNA derived from dermal fibroblasts revealed the

Table 1. Clinical and biochemical features of the patient at the age of 30 yr.

Parameter	Value
Age (yr)	30
Height [cm (SDS)]	141.8 (-5.9)
Weight (kg)	56
BMI (kg/m^2)	28.2
Head circumference [cm (SDS)]	54 (-1.4)
Glucose (<110 mg/dl)	74
Insulin (0-20 mU/liter)	11
Prolactin (0-22 μg/liter)	110
TSH (0.3-4.8 mU/liter)	3.8
Free T_4 (0.8-1.8 ng/dl)	0.84
LH (2-8 U/liter)	6.5
FSH (2-10 U/liter)	5.6
Testosterone (>230 ng/dl)	373
GH (mU/liter)	0.33
GHBP[a] (pmol/liter)	1524
IGF-I [ng/ml (SDS)]	8 (-8.2)
IGF-II [ng/ml (SDS)]	83 (-6.2)
IGFBP-1[b] (ng/liter)	31
IGFBP-2 [ng/ml (SDS)]	142 (-0.6)
IGFBP-3 [mg/liter (SDS)]	0.18 (-12.4)
IGFBP-6 [ng/ml (SDS)]	230 (1.3)
ALS [mg/liter (SDS)]	0.7 (-7.0)

Conversion factors: glucose x 0.0551 (millimoles per liter), free T_4 x 2.87 (picomoles per liter), and testosterone x 0.035 (nanomoles per liter)

[a] Normal range, 330-2437 pmol/liter

[b] Normal range for non-fasting subjects, 24-58 ng IGFBP-1/ml. After overnight fasting, there was an average 5-fold rise in normal individuals

insertion of an extra C between nucleotide positions 1102 and 1103 (Fig. 1A). The parents, brother, and sister were carriers of the mutation (Fig. 1A). This resulted in a frame shift and premature truncation of the protein (Q368fsX376). The presence of the frame shift mutation was confirmed in genomic DNA. No other mutations were detected in the open reading frame of the STAT5b gene. The truncated protein lacked a large part of the DNA binding domain and completely lacked the

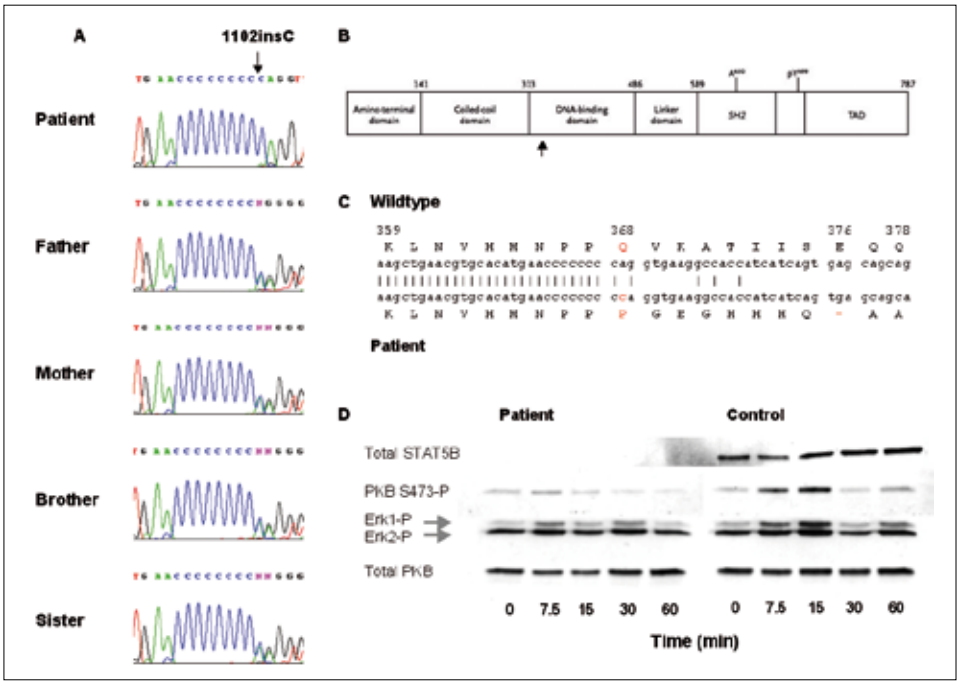

Figure 1: Identification of a homozygous frame shift mutation in STAT5b.

A. Electropherogram of the sequence analysis of the patient and his family members. The position of the extra C is indicated by the *arrow*.
B. Insertion results in a premature stopcodon in the N-terminal half of the protein. The truncated protein lacks the majority of the functional domains of STAT5b. The position of the mutation is depicted schematically by the *arrow*.
C. Alignment of part of the cDNA sequence corresponding to amino acids 359 to 378 of wild type (*upper part*) and patient's STAT5b (*lower part*) showing the consequence of the frame shift mutation for the open reading frame.
D. Western blot analysis using protein extracts isolated from fibroblasts from the patient and an age- and sex-matched control after a challenge with 500 ng/ml human GH. No STAT5b protein could be detected in the patient's cells. In addition, the activation of PKB/Akt and Erk1/2 was impaired. Total PKB was used to check loading efficiencies.

C-terminal SH2 domain required for dimerization with other STAT proteins and translocation to the nucleus, making this protein biologically inactive (Fig. 1, B and C). In line with this and in contrast to a normal control, total STAT5b could not be detected in protein extracts of the patient's fibroblast using Western blot even after stimulation with 500 ng/ml GH (Fig. 1D). Although no abnormalities were present in the GHR, absence of STAT5b also resulted in impaired activation of the

PKB/Akt pathway by decreased phosphorylation on serine 473 and Erk1 and -2 phosphorylation (Fig. 1D).
Because a stretch of eight consecutive Cs may be susceptible to replication errors, we carried out sequence analysis of this exon (PCR amplified from genomic DNA) of 71 individuals diagnosed with short stature. In four cases a heterozygous C>A transversion at the seventh C (nucleotide position 1101) was detected. This has no predicted effect on the coding sequence (Pro>Pro). No changes were found in any of the remaining 67 cases.

Discussion

This is the first report of a male patient, homozygous for a frame shift mutation in the STAT5b gene, which induces a premature stopcodon at position 376 (Q368fsX376). The resulting truncated protein lacks a functional DNA binding domain and misses the SH2 domain. The SH2 domain is critical for recruitment of STAT5b to the activated GHR complex and binding of STAT to the phosphorylated tyrosine residues. It also contains a conserved tyrosine residue at position 699, which is critical for STAT dimerization and its subsequent translocation to the nucleus, where it can act as a DNA binding transcription factor (13, 14). Based on this analysis, the mutation is presumed to result in inactivation of the protein. Indeed, we could not detect any STAT5b in fibroblasts of the patient, at least not with the antibody available to us.
The expression of IGF-I, IGF-II, IGFBP-3, and ALS is tightly controlled by GH. STAT5b appears to be the dominant transcription factor, mediating these effects of GH at the transcriptional level. In both the IGF-I and ALS genes, functional STAT5b response elements have been identified (15, 16). Fully in line with this, the serum levels of IGF-I, IGFBP-3, and ALS are extremely low in all patients with a STAT5b mutation (1, 2).

Our patient is the first known male with a STAT5b mutation. He shows the same degree of postnatal growth retardation as the two female patients (-7 and -7.8 SDS) (1, 2). Female STAT5b knockout mice are largely unaffected, whereas male knockouts, which are normally 30% larger than female mice, resemble females in size (17). Apparently, in humans, homozygous STAT5b mutations result in an equal pattern of growth retardation in males and females.

In line with the observations in STAT5b knockout mice (18), GHR gene-disrupted mice (19) and patients with Laron's syndrome (20), our patient showed markedly increased serum prolactin levels. In STAT5b-deficient mice the high serum prolactin concentrations could be suppressed by bromocriptine (dopamine D2 agonist), suggesting absence of endogenous dopaminergic inhibition. Hyperprolactinemia might also be the result of elevated hypothalamic GHRH secretion, under the diminished feedback restraint from IGF-I and GH. Indeed, the elevated prolactin levels in Laron's syndrome decrease upon IGF-I administration, in line with the inhibitory action of IGF-I on hypothalamic GHRH neurons (20).

The first reported patient with a STAT5b mutation had respiratory difficulties due to lymphoid interstitial pneumonia and a *Pneunocystis Carinii* infection (1). The second patient with a STAT5b mutation had primary idiopathic pulmonary fibrosis (2). In contrast, our patient has had neither pulmonary complaints nor signs of immunodeficiency. Our patient demonstrates that STAT5b deficiency is not obligatory resulting in a clinically immunodeficient state.

In conclusion, this report supports the essential role of STAT5b in GH signaling in the human and confirms that STAT5b deficiency leads to severe postnatal growth failure. In contrast with mice, the growth retardation in the human does not show a sexually dimorphic pattern. Hyperprolactinemia is apparently part of the syndrome, possibly by a deficient negative feedback loop in the hypothalamus. Although in earlier cases STAT5b deficiency was suggested to be associated with immunodeficiency, our patient shows that immunodeficiency is not an obligatory feature in patients with a STAT5b mutation. In cases with postnatal growth retardation, a low IGF-I, IGFBP-3, and ALS, high or normal GH secretion, and an absence of mutations or deletions in the GHR, genetic analysis of STAT5b is warranted. Our case illustrates that the heterogeneity of clinical manifestations of this syndrome is larger than suggested by the first two cases.

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