Genetic disorders in the growth hormone-IGF-I axis

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Growth hormone secretion and immunological function of a male patient with a homozygous STAT5b mutation

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Abstract

Objective: STAT5b is a component of the GH signaling pathway. Recently, we described a 31-year old male patient (height – 5.9 SDS) with a novel homozygous inactivating mutation in the STAT5b gene. The purpose of this study is to describe the phenotype in detail, including GH secretion and immunological function. In addition, we report four family members of this patient, all heterozygous carriers of the mutation.

Design and methods: Twenty-four hour GH and prolactin secretion characteristics were assessed by blood sampling at 10-min intervals. An IGF-I generation test was performed. Monocyte function was tested by stimulation of whole blood with lipo-polysacharide (LPS) in the presence or absence of Interferon-γ (IFN-γ). In addition, T cell function was determined by measuring proliferative responses of peripheral blood mononuclear cells (PBMC) after stimulation by various polyclonal activators and Interleukin-2 (IL-2). Clinical and biochemical characteristics were determined in the carriers of the mutation.

Results: GH secretory parameters were comparable to that of healthy male controls (mean fat percentage 25), but likely increased in relation to the patient's 40% body fat. The regularity of GH secretion was diminished. Prolactin secretion was increased by sixfold. The IGF-I generation test showed a small increase of IGF-I and IGFBP-3 on lower GH doses and an increase of IGF-I to –2.4 SDS on the highest dose of GH. *In vitro*, IL-12p40, IL 10 and TNF-α production rates by PBMC increased to values within the normal range upon stimulation of LPS. Heterozygous carriers of the mutation did not show abnormalities, although the height of the males was below the normal range.

Conclusions: This report shows that GH and prolactin secretion was increased is this patient homozygous for a new STAT5b mutation. Although STAT5b plays a role in signaling within immune cells, clinical immunodeficiency is not an obligatory phenomenon of STAT5b deficiency *per se*. Heterozygous carriers of a STAT5b mutation show no signs of GH insensitiviy.

Introduction

The growth hormone receptor (GHR) uses the JAK-STAT proteins as signal transduction pathway. Out of the seven STAT proteins, STAT5b is preferred. Upon phosphorylation, STAT5b dissociates from the GHR, dimerizes and translocates to the cell nucleus, where it transcriptionally regulates the expression of a variety of target genes, including the gene coding for insulin-like growth factor-I (IGF-I) (1). In addition to the role of STAT5b in GHR signal transduction, clinical observations and studies in knock out mice have suggested that STAT5b is involved in the immune system (2, 3).

To date, two immunodeficient females homozygous for a missense mutation and a frameshift mutation respectively, in the STAT5b gene, have been described (4, 5). Recently, we reported the first male with a homozygous frameshift mutation, due to insertion of a C-residue at nucleotide position 1102-1103, that caused a premature truncation of the STAT5b protein (6). The patient presented with severe short stature (Fig. 1), low IGF-I levels and elevated prolactin levels. However, in contrast to the two previously reported females, he showed no overall signs of immunodeficiency.

In this report of our male patient, we present the GH and prolactin secretion patterns. We have evaluated his immunological status in more detail and, in addition, we investigated the clinical and biochemical features of four family members who appeared to carry the mutation.

Methods

All studies were performed after obtaining written informed consent from all subjects.

Clinical measurements and auxology
We measured height with a Harpenden stadiometer, and head circumference with a tape measure. Height and head circumference were expressed as standard deviation score (SDS) for the Dutch population (7).

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). The detection limit was 0.03 mU/l. Plasma IGF-I, IGF-II, IGF-binding-protein (IGFBP)-1, IGFBP-2, IGFBP-3, and IGFBP-6 were determined by specific RIAs (8). Acid-labile subunit (ALS) was measured by an ELISA (Diagnostics Systems Laboratories, Inc., Webster, TX, USA) (9). With the exception of free IGF-I and IGFBP-1, smoothed references were available for all parameters, based on the LMS method (10), allowing conversion of the data of the patient to SDS values. Plasma IGFBP-1 concentration after an overnight fast was compared with a reference group of six healthy adult controls. Prolactin was measured by a sensitive time-resolved immunofluorometric assay (Wallac, Turku, Finland). The standards were calibrated against the WHO third International Standard for prolactin 84/500. The detection limit of the assay was 0.04 μg/l.

Twenty-four hours plasma hormone profiles

Blood sampling protocol

Patients and controls were admitted to the hospital on the day of study. An indwelling i.v. cannula was inserted into a vein of the forearm 60 min before sampling began and blood samples were withdrawn at 10-min intervals starting at 0900 h and for the next 24 h. A slow infusion of 0.9% NaCl and heparin (1 U/ml) was used to keep the line open. The subjects were free to ambulate, but not to sleep during daytime. Meals were served at 0800, 1230, and 1730 h and lights were turned off between 2200 and 2400 h. Plasma samples were collected on ice in heparinized tubes. The samples were centrifuged at 4°C for 15 minutes; the plasma was then separated, frozen and stored at -20°C until the assays were performed.

Deconvolution analysis

Multiparameter deconvolution analysis was used to determine kinetic and secretory parameters of 24-h spontaneous GH secretion, calculated from GH plasma concentrations. Initial parameters were created with Pulse 2, an automated pulse-detection program. Subsequent waveform-dependent analyses were performed as described previously (11). This technique estimates the rate of basal release, the number and mass of secretory bursts, and the subject-specific half-life. Daily pulsatile GH secretion is the product of secretory burst-frequency and mean mass

of GH released per event. Total GH secretion is the sum of basal and pulsatile secretion.

Approximate entropy

Approximate entropy (ApEn) is a scale- and model-independent statistic, applicable to a wide variety of physiological and clinical time-series data. ApEn quantitates the orderliness or regularity of serial GH concentrations over 24 h. Normalized ApEn parameters of $m = 1$ (test range) and $r = 20\%$ (threshold) of the intraseries standard deviation were used, as described previously (12). Hence, this member of the ApEn family is designated ApEn (1, 20%). The ApEn metric evaluates the consistency of recurrent subordinate (nonpulsatile) patterns in a time series and thus yields information distinct from and complementary to cosinor and deconvolution (pulse) analyses (13). Higher absolute ApEn values denote greater relative randomness of hormone patterns. Data are presented as absolute ApEn values and normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1000 randomly shuffled versions of the same series (14).

Cluster Analysis

For the detection of discrete prolactin peaks, cluster analysis was used. This computerized pulse algorithm is largely model-free, and identifies statistically significant pulses in relation to dose-dependent measurement error in the hormone time series (15). A concentration peak is defined as a significant increase in the test peak cluster vs. the test nadir cluster. We used a 2 x 1 cluster configuration (2 samples in the test nadir and 1 in the test peak) and t-statistics of 2.0 for significant up- and down-strokes in prolactin levels to constrain the false positive rate of peak identification to less than 5% of signal-free noise. The locations and widths of all significant concentration peaks were identified, the total number of peaks was counted, and the mean inter-peak interval was calculated in minutes. In addition, the following pulse parameters were determined: peak height (highest value attained within the peak), incremental peak amplitude (the difference between peak height and prepeak nadir), and area under the peak. Interpulse valleys were identified as regions embracing nadirs with no intervening upstrokes. The total area under the curve was also calculated, as well as the summed pulse areas.

Immunologic investigations

Whole blood was collected in endotoxin-free tubes (Endotube ET; Chromogenix, Milan, Italy), diluted in the ratio 1:5 and stimulated overnight with 0.1–100 ng/ml lipopolysacharide (LPS)(*Escherichia coli* 0111 LPS) in the presence or absence of 100 IU IFN-γ/ml. In several experiments either growth hormone or prolactin was added to a final concentration of 50–500 ng/ml, and 10 ng, respectively. After 18 h, supernatants were collected and tested by ELISA for the presence of IL-12p40 (R&D Systems), IL-10 and TNF-γ (Sanquin Research, Amsterdam)(16). PBMCs were incubated overnight with increasing amounts of IFN-gamma (0–100 IU/ml) in hydron-coated wells to prevent adherence of the monocytes. Cells were washed and stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD64 and PE-conjugated anti-CD14 to analyze CD64 expression on CD14-positive cells using a FACSCalibur (16).

PBMCs were phenotyped by four color immunostaining using FITC-, PE-, PerCPCy5.5-, and activated protein C (APC)-conjugated antibodies, and analyzed by flow cytometry (FACSCalibur; Becton Dickinson, Baltimore, MD, USA). Data were analyzed using CELLQuest software (Becton Dickinson).

Results

Phenotype

The index patient was born in the Dutch Antilles. 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) (7). He was born after a full-term uncomplicated pregnancy with a birth weight of 3270 g (-0.7 SDS) and birth length of 50 cm (-0.4 SDS)(17). During childhood severe postnatal growth retardation was noticed (Fig. 1), although evaluation did not take place until the age of 16 years, when he emigrated to The Netherlands.

Physical examination at 16 years revealed normal body proportions, although his pubertal development was delayed (Tanner stage G1P1), testicular volume being only 1 ml. His bone age was 9 years. He was treated with recombinant human GH (rhGH, Genotropin, Pharmacia) 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 during an additional 3 months. However, this treatment did not significantly improve growth rate, except for a slight growth acceleration probably due to pubertal development (Fig. 1).
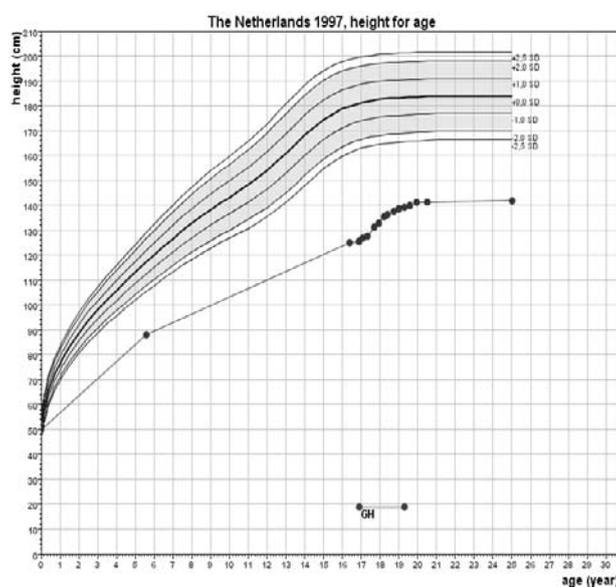
Figure 1. Height for age chart of index patient, based on Dutch references (7). In the absence of local growth standards, we have assumed that inhabitants of the Dutch Antilles of African descent have a similar height as the Dutch population.

At the age of 30 years, he was referred to our clinic for evaluation of his short stature. Auxological parameters are summarized in Table 3. Basal endocrinological parameters were within the normal range: free T4 10.8 pmol/l (10-24 pmol/l), TSH 3.8 mU/l (0.3-4.8 mU/l), LH 6.5 U/l (2-8 U/l), FSH 5.6 mU/l (2-10 U/l) and testosterone 13.1 nmol/l (8-50 nmol/l).

Twenty-four hour profiles of plasma GH and prolactin
The plasma GH concentration profile of the patient and that of a representative control subject are illustrated in Fig. 2. The results of the deconvolution analyis are shown in Table 1. Basal secretory rate and GH burst frequency were increased in the patient, but the other deconvolution parameters were comparable with that of the controls. It should be noted that although the body mass index (BMI) of the patient was within the range of that of the controls, his fat percentage was much higher. ApEn and ApEn ratio were increased in the patient, denoting decreased GH secretory regularity (Table 1).
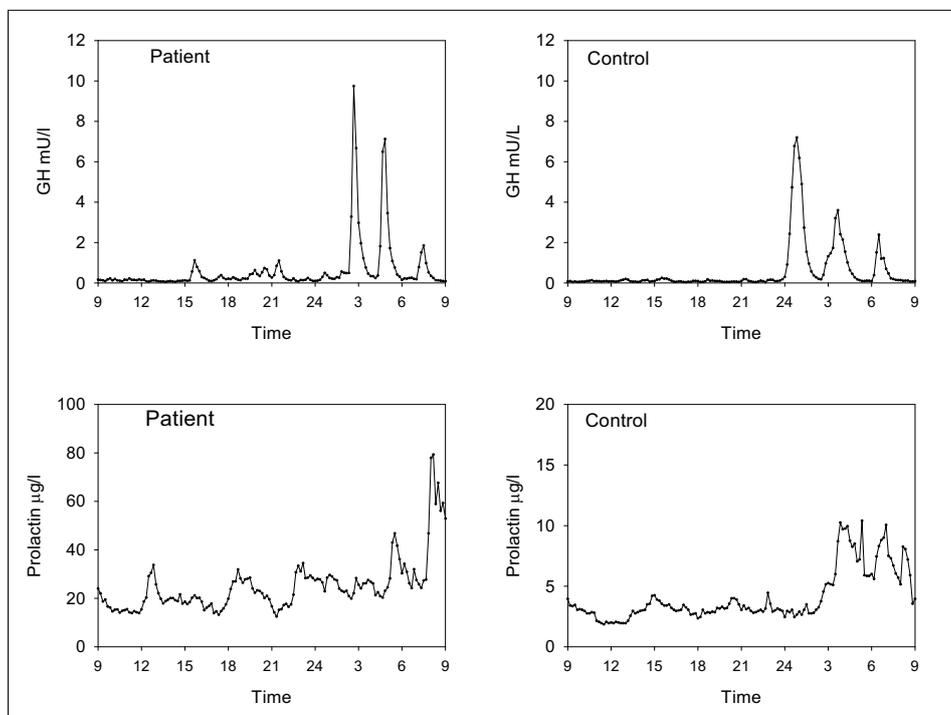
Figure 2. Plasma GH and prolactin profiles obtained by blood sampling at 10 min intervals in the patient and a representative healthy control subject.

The plasma prolactin profiles of the patient and a healthy control are displayed in Fig. 2. Cluster analysis revealed a five- to six fold increase in mean pulse height, mean pulse amplitude, mean pulse mass and pulsatile production. The prolactin secretory regularity was not decreased in the patient.

IGF-I generation test

At the age of 30 years an IGF-I generation test was performed with 12.5, 25.0 and 50.0 µg GH/kg per day for 7 consecutive days for each dose, interrupted by washout periods of 2 weeks and 9 months respectively. The results of the IGF-I generation test are shown in Fig. 3. On the low and intermediate dose, a slight increase of IGF-I and IGFBP-3 was noted. However on the highest dose IGF-I level increased from -6.6 SDS to -2.4 SDS, IGFBP-3 from -11.6 SDS to -6.4 SDS and ALS from –7.2 SDS to –5.3 SDS.

Table 1. Deconvolution analysis and approximate entropy of 24-h GH profiles in a male patient with STAT 5b mutation and eight male healthy controls.

	Patient	Controls (n=8)
Basal secretory rate (mU/l per min x 10^3)	3.25	1.6 (0.7-2.4)
Endogenous GH half-life (min)	13.0	16 (13.6-18)
No. of secretory bursts/ 24 h	16	10 (7-13)
Mean mass GH secreted/burst (mU/l)	2.3	4.4 (2.2-6.5)
Pulsatile GH secretion (mU/l per day)	37	36 (25-47)
Total GH secretion (mU/l per day)	41	38 (27-49)
Age (years)	30	33 (30-36)
ApEn (1, 20%)	0.383	0.241 (0.189-0.335)
ApEn ratio	0.46	0.32 (0.27-0.36)
BMI (kg/m^2)	28	26.0 (23.4-29.3)
Fat percentage	40	25 (21-28)

Data of the controls are shown as mean and (95% confidence interval). To convert mU/l into µg/l divide by 2.6. The fat percentage in the patient was measured by dual-energy X-ray absorptiometry (DEXA), and in the controls by BIA.

Table 2. Cluster analysis and approximate entropy of 24-h prolactin profiles in a male patient with STAT 5b mutation and eight male healthy controls.

	Patient	Male adult controls (n=8)
Pulse frequency (no./24 h)	17	15.8 (12-19.5)
Mean pulse height (µg/l)	20.5	4.4 (3.5-5.3)
Mean pulse amplitude (µg/l)	7.2	1.4 (1.0-1.7)
Mean pulse mass GH (µg/l)	340	67 (46-87)
Mean nadir concentration (µg/l)	19.1	2.9 (2.2-3.7)
Pulsatile production (µg/l per 24 h)	5750	1000 (700-1300)
AUC (µg/l/24 h)	36250	26.0 (23.4-29.3)
ApEn (1, 20%)	0.746	0.700 (0.590-0.810)
ApEn ratio	0.47	0.44 (0.38-0.50)
Age (years)	30	33 (30-36)

Data of the controls are shown as mean and (95% confidence interval).
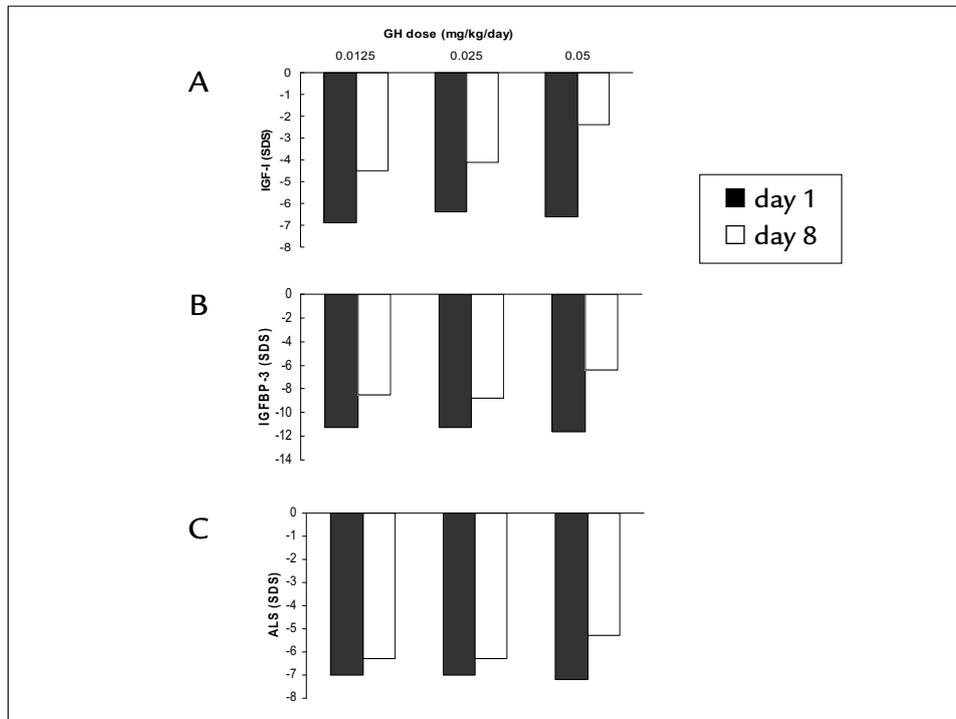
Figure 3. Results of the IGF-I generation test, performed in the index patient. (A) IGF-I (SDS), (B) IGFBP-3 (SDS), and (C) ALS (SDS).

Immunological evaluation

At least one of the first reported female patients with a STAT5b mutation was severely immunocompromised. The first suffered lymphoid interstitial pneumonia and had a *Pneumocystis Jiroveci* (previously, *Pneumocystis Carinii*) lung infection, and presented with severe hemorrhagic varicella and recurrent episodes of herpes zoster (4). The second female patient had primary idiopathic pulmonary fibrosis, a condition in which an infectious etiology cannot be ruled out (5).

Our male patient has had neither pulmonary complaints nor signs of immunodeficiency. Remarkably, he had a hemorrhagic varicella infection at the age of 16 years, but this could well be explained by lack of a history of chickenpox in the past and the fact that he developed the varicella secondary to his brother, who suffered a less severe case. Thus, we do not consider the severe case of chickenpox in this patient as sufficient evidence for immunodeficiency. Otherwise, the medical history of our

patient was unremarkable for childhood infections, and response to vaccinations. Since the two previously reported patients with a homozygous STAT5b mutation appeared to be immunocompromized, we investigated various immunological functions of PBMCs.

IL-12p40, TNF-γ and IL-10 production in response to LPS

Monocyte function of the patient was determined by culturing whole blood in the presence of LPS. There was a LPS-dose dependent increase in IL-12p40 production that was similar to that in the control (Fig. 4A, grey and black bars). Similarly, IL-10 production increased in response to LPS, to a level somewhat higher than observed in the control, but the response was well within normal values (Fig. 4B). These findings indicated that IL-12p40 and IL-10 production in response to LPS was not impaired in the patient. Similar data were obtained for TNF-α production (Fig. 4C). To investigate whether TNF-α and IL-12p40 production could be up-regulated and IL-10 production down-regulated by IFN-γ, the combination of LPS and a high dose of IFN-γ (100 IU/ml) was used to stimulate whole blood. In the patient, IL-12p40 and TNF-α production was increased, and IL-10 decreased, both in an LPS-dose-dependent fashion, and to a level similar to that observed in controls (Fig. 4A-C, hatched bars). Of note, incubation with neither GH nor prolactin influenced the IL-12p40, TNF-γ and IL-10 production after stimulation with LPS (data not shown).

IFN-γ responsiveness in PBMCs

To study the response to IFN-γ in more detail, monocytes were cultured in the presence of 0, 1, 10 and 100 U IFN-γ/ml and the increase in the expression of CD64 (FcγR1) was measured by FACS analysis. The CD64 promoter contains a STAT1 binding site and up-regulation of CD64, in response to IFN-γ stimulation, is a well-accepted marker of IFN-γ responsiveness of these cells (18). IFN-γ stimulation of the monocytes of the patient resulted in a dose-dependent increase in CD64 expression, and at the highest (100 IU IFN-γ/ml) concentration was about equal to the maximal increase in CD64 expression for the healthy control: the cell-surface expression increased upon increasing concentrations of IFN-γ 1.2-, 2.9- and 4.5-fold respectively, for monocytes of the patient and 1.3-, 2.9- and 5.3-fold respectively, for control monocytes, as compared to cell-surface expression without IFNγ. Neither GH nor prolactin influenced basal CD64 receptor expression (data not shown).
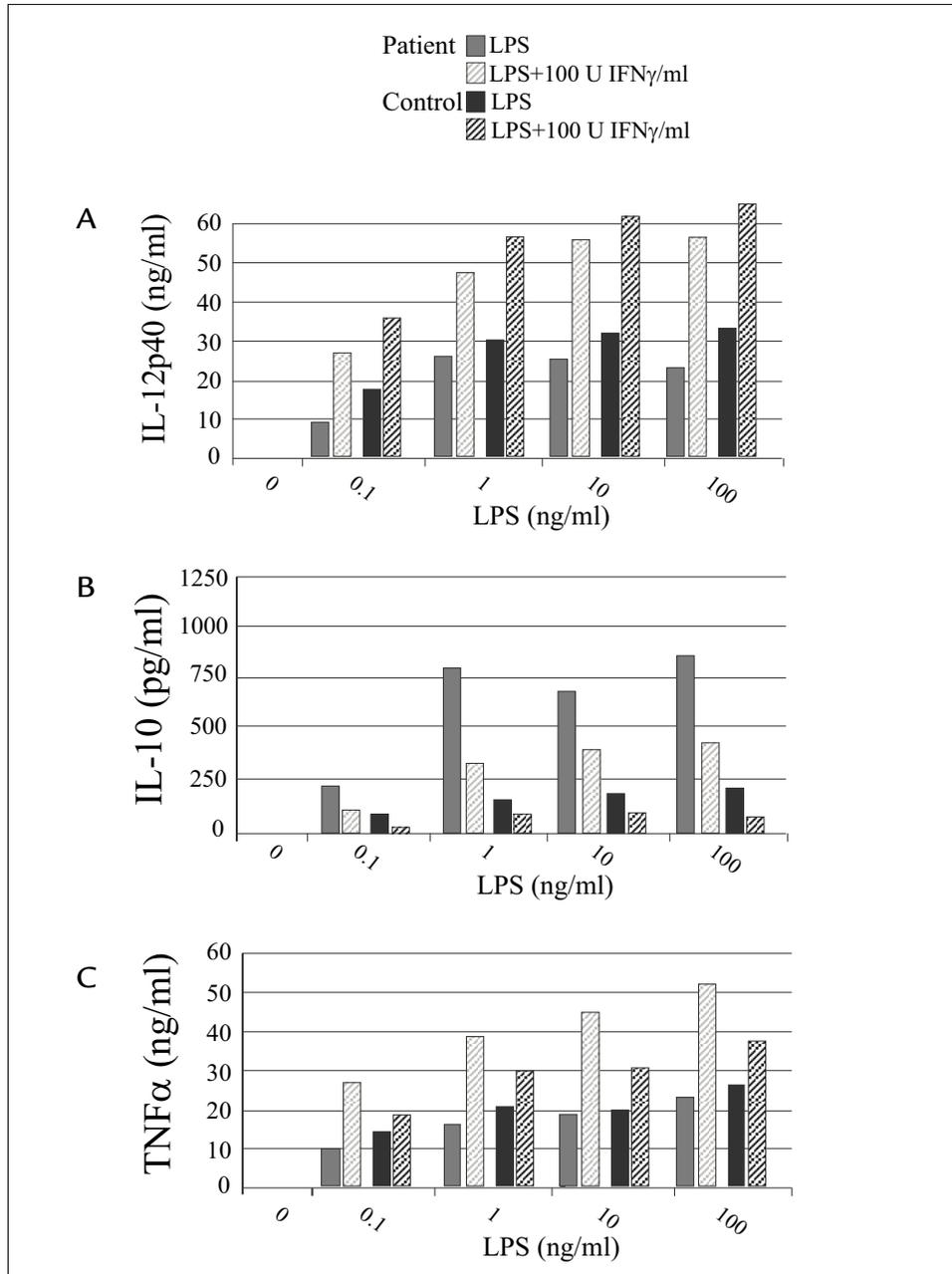
Figure 4. Production of (A) IL-12p40, (B) IL-10, and (C) TNF-α in whole blood of the patient and a healthy control. Whole blood was stimulated with lipopolysacharide (LPS) in the presence or absence of 100 U IFN-γ/ml. Supernatants were collected after 18 h and IL-12p40, IL-10 and TNF-α was measured by ELISA. Experiments were performed in duplicate.

Cell surface expression of IFN-γ-receptors and lymphocyte proliferation

The expression of IFN-γR1 and TNF-γR1 and TNF-γR2-receptors on peripheral blood mononuclear cells did not differ from that in cells of healthy controls, as evident by the relative cell-surface expression of these receptors of 1.14, 1.69, and 0.86 respectively, in the patient as compared with the control.

To determine T cell function, phytohemagglutinin (PHA) blasts were obtained after 2-week culture of PBMCs and proliferative responses were measured after stimulation with various polyclonal activators and Il-2. Stimulation by anti-CD2/28 mAbs or with phorbol-12-myristate-13-acetate (PMA) resulted in somewhat lower responses than in the controls, but this could be overcome by additional co-stimulation with IL-2 (Fig. 5). It should be noted that also between healthy controls, such reactivity can differ markedly. We hypothesize that the overall damping of
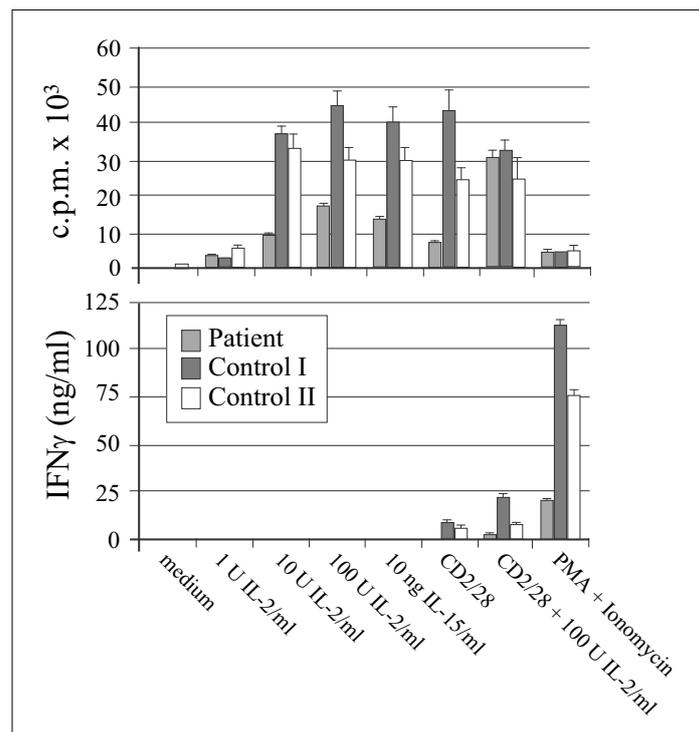
Figure 5. T-cell proliferative responses (top) and IFN-γ production (bottom). PHA blasts obtained after 14-day culture of PBMC were stimulated via TCR ligation by anti-CD2/28 mAbs or with PMA. The somewhat decreased response in the patient was well within normal range and could be overcome by additional co-stimulation with IL-2.

T cell reactivity may reflect the signaling defect due to the STAT5b mutation, but apparently the impaired intracellular signaling can be largely compensated by other mechanisms in the presence of a potent, combined stimulus like anti-CD2/28 with co-stimulation with IL-2. Although both the proliferative response and the IFN-γ production lagged behind the two controls used in this particular experiment, the patient's values are within normal range, in particular to the combined stimulation by CD2/28 and IL-2, or PMA plus Ionomycin.

Carriers of the STAT5b mutation

We investigated four family members of the index patient: his parents, brother and sister. They were all heterozygous carriers of the frameshift mutation in the STAT5b gene. All had a history of normal birth weight. Remarkably, his father and brother were short (height < -2 SDS; Table 3), while his mother and sister were not. They were all in good physical condition, except the father, who had type 2

Table 3. Clinical and biochemical features of the index patient and family members.

Parameter	Patient	Father	Mother	Brother	Sister
Age (years)	31	65	58	33	27
Height (cm) (SDS)	141.8 (-5.9)	164.3 (-2.8)	165.6 (-0.8)	167.4 (-2.3)	165.7 (-0.8)
Weight (kg)	56	87	71	78.5	56
BMI (kg/m^2)	28.2	32.2	25.9	28.0	20.4
Head circ (cm) (SDS)	54 (-1.4)	58 (0.1)	58 (1.6)	58.5 (0.4)	57 (1.0)
Hip/waist ratio[a]	0.94	1.05	0.86	0.94	0.68
GH (mU/l)	0.33	0.2	0.1	0.1	0.6
IGF-I (ng/ml) (SDS)	8 (-8.2)	102 (-0.3)	42 (-3.1)	147 (-0.3)	130 (-1.1)
IGF-II (ng/ml) (SDS)	83 (-6.2)	403 (-0.1)	396 (-0.3)	511 (0.9)	452 (0.2)
IGFBP-1[b] (ng/l)	31	68	18	4	26
IGFBP-2 (ng/ml) (SDS)	142 (-0.6)	247 (0.6)	193 (0.2)	102 [(1.3)	239 (1.0)
IGFBP-3 (mg/l) (SDS)	0.18 (-12.4)	1.58 (-0.8)	1.86 (-0.1)	1.72 (-1.1)	1.95 (-1.0)
IGFBP-6 (ng/ml) (SDS)	230 (1.3)	267 (1.2)	206 (0.8)	178 (0.3)	206 (1.5)
ALS (mg/l) (SDS)	0.7 (7.0)	9.5 (-1.9)	13.4 (-1.0)	16.3 (-0.2)	12.9 (-2.0)

[a] Normal value in men < 0.90, in women < 0.85 (WHO criteria).
[b] Normal range for non-fasting subjects: 24-58 ng IGFBP-1/ml. After overnight fasting there is an average fivefold rise in normal individuals.

diabetes mellitus and prostate carcinoma. None of the family members reported any infectious problems and their plasma prolactin levels and thyroid function were normal. Basal GH levels were within the normal range. Plasma IGF-I levels were also normal with exception of the mother of the index patient, who had a low IGF-I value (Table 3).

Discussion

The data described in this study indicate that total GH secretion is not obligatorily increased in patients with a mutation in the STAT5b gene when compared with healthy BMI-matched controls. In contrast, prolactin secretion was clearly enhanced. We also assessed immunological function in detail in the present patient, because the previous two patients with STAT5b mutations showed signs of immunodeficiencies. With the exception of chickenpox contracted in adolescence (see below), the patient did not suffer any infections, and the data obtained in our patient indicate that a homozygous deleterious STAT5b mutation is not necessarily accompanied by severe immunodeficiency. Finally, heterozygous carriers of an inactive STAT5b mutation are phenotypically normal.

All the three reported patients with STAT5b mutations (4-6) show the same growth pattern, resembling that of patients with classical GH insufficiency (GHI) (19). The degree of postnatal growth retardation is comparable to that of patients with an IGF-I deletion or mutation, who reached a final height of -6.9 and –8.5 SDS respectively (8, 20). The main difference is the intrauterine growth retardation that occurs in the patients with primary IGF-I deficiency, but not in those with STAT5b mutations, reflecting the GH-independent IGF-I gene expression *in utero*.

The pubertal growth spurt that occurred in our patient (15 cm) during GH therapy is about half the magnitude that would be expected in a boy (Fig. 1). We hypothesize, that this growth acceleration is mainly attributable to the rise in sex steroids during puberty, as GH treatment did not normalize IGF-I. This observation supports the hypothesis, that the full growth promoting action of testosterone can only be exerted in the presence of normal GH secretion (21).

The regulation of GH secretion is complex. Pulsatile secretion is important since most of the secretion occurs in bursts. The magnitude of GH bursts correlates with somatic growth and hepatic actions of the hormone. Decreased pulsatility may contribute to decreased IGF-I concentration as observed in aged subjects (22, 23). Pulses are generated by interactions among GHRH, ghrelin and somatostatin under negative feedback control by IGF-I and GH (24). A GH pulse autoregulates hypothalamic-pituitary outflow in a biphasic mode, imposing initial inhibition which is followed by disinhibition of GH secretion (25). In animal models, the increase in GH leads to prompt somatostatin release, which inhibits GHRH and sensitizes the somatotrope to the next GHRH stimulus. In this perspective, ghrelin further amplifies GH release (26).

The diminished negative feedback signaling by IGF-I and GH in GHI leads to amplified GH secretion in many, but not all patients with GHIS (27, 28). IGF-I administration in this syndrome diminishes GH secretion, indicating that feedback can be restored (28). The amplified GH secretion is likely mediated by increased hypothalamic GHRH output, while somatostatin release is not affected in view of the unchanged GH pulsatility (29-32).

The magnitude of GH secretion is determined by age, gender, sex hormones and adiposity (24). Especially, adiposity decreases spontaneous and stimulated GH secretion (33, 34). Detailed studies have established that particularly visceral fat mass is correlated with GH secretion (35, 36). It is therefore of interest that patients with Laron's syndrome have a greater total and regional fat mass than BMI- and gender-matched controls, showing that the normal relation between these two measures as found in the general population is lost (37-39). We hypothesize that the seemingly normal GH secretion in our patient was severely suppressed by his (visceral) adiposity. The relatively unchanged basal GH secretion and pulsatility argue against GH suppression by somatostatin. Detailed GH secretion studies in normal and adipose subjects point to decreased pituitary responsiveness to GHRH as a possible mechanistic explanation (36). Finally, the only moderately increased GH ApEn would fit with partly attenuated GHRH signaling.

It would seem that in our patient altered responsiveness only affected the somatotrophe but not the lactotrophe in view of the stimulated prolactin secretion. Increased prolactin secretion has also been found in other states of endogenous GHRH excess, such as ectopic GHRH-secreting tumors (unpublished data) and hGHRH-transgenic mice (40).

The IGF-I generation test in our patient showed a limited response of plasma IGF-I, IGFBP-3 and ALS levels on stimulation with the two lower doses of GH (Fig. 3). However, after administration of 0.05 mg GH/kg/day the concentration of IGF-I in plasma approached reference range values. Apparently, a high dose of GH can induce IGF-I transcription and secretion considerably, despite the absence of a functional STAT5b protein. Under this condition, possibly, other STAT proteins and/or alternative signal transduction pathways (e.g Microtubule-associated protein kinase (MAPK)/Erk or Protein Kinase B (PKB)/Akt) may play a compensatory role in the regulation of GH-dependent IGF-I expression. Indeed, we found some evidence for this *in vitro* (6). A high dose of GH could activate Erk1/2 and PKB/Akt although at a much lower level when compared with controls. Therefore, at high concentrations other mechanisms may partly compensate the loss of STAT5b, for example by recruitment of other STAT family members to the GHR (41). Since this effect is only seen at high GH concentrations, the affinity of other STAT proteins for the activated GHR appears much lower than of STAT5b.

The first reported female patient with a STAT5b mutation had respiratory difficulties due to lymphoid interstitial pneumonia and a *Pneunocystis Jiroveci* infection. At 8 years of age, she presented with severe hemorrhagic varicella and subsequently she had several episodes of herpes zoster (4). The second female patient with a STAT5b mutation also had pulmonary problems, characterized as primary idiopathic pulmonary fibrosis (5). In contrast, our male patient has had neither pulmonary complaints nor signs of immunodeficiency. Initially, we thought that the hemorrhagic varicella infection at the age of 16 years (6) could be a sign of immunodeficiency. However, the patient did not have a history of chickenpox in the past (a reliable surrogate of a lack of seroimmunity (42, 43)) and he developed the varicella secondary to his brother, who suffered a less severe case. Varicella secondary attack rate among susceptible household contacts is high and at higher age, e.g. above 10-14 years, the infection typically runs a more severe course than in infants (44, 45). For instance, adults with chickenpox, males in particular, have an about 25-fold higher risk of complications like pneumonitis or encephalitis, when compared with children with the disease (46). Thus, we do not consider the severe case of chickenpox in this patient as sufficient evidence for immunodeficiency, secondary to the STAT5b mutation. Moreover, with the exception of the chickenpox, the medical history of our patient was unremarkable for childhood

infections, and response to vaccinations. This was in line with additonal immuno-logical investigations.

The *in vitro* proliferative responses of T-cells to IL-2 and CD2/28 and the production of IFN-γ upon IL-2 stimulation were within the normal range, although the latter cytokine response lagged somewhat behind those in controls. Although the present findings certainly cannot exclude some sort of subtle immunodeficiency, our patient demonstrates that STAT5b deficiency is not obligatory resulting in a severe immunocompromised state, which was suggested by at least one of the other two cases with STAT5b mutations, and findings in STAT5b -/- mice. This suggests a role for other factors in the expression of immunodeficiency in humans with STAT5b deficiency. Since our case is the first male patient described with STAT5b deficiency, it might be possible that the presentation of the immunophe-notype of STAT5b deficiency is sex-dependent.

The heterozygous family members of the index patient did not exhibit signs of GH insensitivity. The short stature of the father and brother can not merely be explained by heterozygosity for the STAT5b mutation, as their IGF-I levels were normal. The mother had a low IGF-I level, but her normal IGFBP-3 and ALS levels argue against an effect of partial STAT5b deficiency.

In conclusion, STAT5b is essential for GH signaling and postnatal growth. GH secretion in our patient was likely attenuated by the visceral adiposity, but prolactin secretion was clearly amplified. Although STAT5b plays a role in signaling within the immune system, the clinical consequences of disrupting this signaling pathway appear limited, probably due to compensatory pathways, and immunodeficiency is not present in all patients with a STAT5b mutation. The heterozygous carriers show no signs of GH insensitivity. Other factors may contribute to the short stature of the male carriers.

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