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ORIGINAL RESEARCH

Long-Term Follow-up of STAT5B Deficiency in Three Argentinian Patients: Clinical and Immunological Features

Liliana Bezrodnik · Daniela Di Giovanni · María Soledad Caldirola · María Esnaola Azcoiti · Troy Torgerson · María Isabel Gaillard

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Abstract
The signal transducer and activator of transcription (STAT) family of proteins regulate gene transcription in response to a variety of cytokines. STAT5B, in particular, plays an important role in T cells, where it is a key mediator of interleukin-2 (IL-2) induced responses. STAT5B deficiency causes a rare autosomal recessive disorder reported in only a handful of individuals. There are currently ten published cases of STAT5B deficiency, four of which are Argentinians.
Aim This is a report of more than 10 years follow up of the clinical and immunological features of three Argentinian STAT5B deficient patients.
Conclusion More than a decade of follow-up demonstrates that STAT5B deficiency is associated with various clinical pathologies that cause significant morbidity. Early diagnosis is critical for the prevention and improvement of clinical outcomes for STAT5B deficient patients.

Keywords Stat5B · primary immunodeficiency · growth hormone · lymphocytic interstitial pneumonitis · dysregulatory syndrome · regulatory T cells

L. Bezrodnik · D. Di Giovanni · M. S. Caldirola · M. E. Azcoiti (✉) · M. I. Gaillard
"Dr. Ricardo Gutiérrez" Children's Hospital, Gallo 1330, Capital Federal, Argentina
e-mail: mesnaolaazcoiti@gmail.com

T. Torgerson
Seattle Children's Research Institute, 1900 9th Ave, Seattle, USA

T. Torgerson
Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA

T. Torgerson
Immunology Diagnostic Laboratory within the Center for Immunity and Immunotherapies at Seattle Childrens Research Institute, 1900 9th Ave, Seattle, USA

STAT5B is a member of the signal transducer and activator of transcription family of proteins which regulate gene transcription in response to a variety of cytokines (IL-2, IL-15, IL-3, IL-5, IL-7, granulocyte-macrophage colony-stimulating factor) and growth factors [1].

STAT5B, in particular, plays an important role in T cells, where it is a key mediator of interleukin-2 (IL-2) induced responses. Like other STAT proteins, STAT5B is present as a monomer in the cytoplasm of quiescent cells. It is recruited to the activated interleukin-2 receptor (IL-2R), where it is phosphorylated by the receptor-associated tyrosine kinase JAK3. The phosphorylated subunits dimerize through their SH2 domains and translocate to the nucleus where they regulate gene transcription by binding to DNA [2]. Both human and murine models have demonstrated that Stat5b is a critical transducer of the IL-2 mediated signals required to promote T cell growth, to sustain FOXP3 expression in regulatory T cells (T_{REG}) and to maintain T_{REG} cells themselves [3]. Mice lacking Stat5b have a significant reduction in the number of $FOXP3^+$ T_{REG} cells in the thymus and spleen and as a consequence develop splenomegaly and a marked increase of activated T cells in the periphery [4, 5].

STAT5B deficiency causes a rare autosomal recessive disorder reported in only a handful of individuals. The most remarkable clinical feature of the syndrome is dwarfism associated with normal serum growth hormone (GH) levels, but very low insulin-like growth factor-1 (IGF-1) levels. Other physical features include a prominent forehead, saddle nose and high-pitched voice. Most patients who lack functional STAT5B also have symptoms suggestive of immune dysregulation, including early-onset chronic diarrhea, eczema and lymphocytic interstitial pneumonitis [6–13]. Some patients exhibit unusually high susceptibility to varicella virus and herpes virus. There are currently ten published cases of STAT5B deficiency, four of which are Argentinians [14] (See Table 1).

Between 1990 and 2000, the endocrinology unit of our hospital identified three patients with severe growth failure (<5th percentile). Because of their clinic of severe varicella, eczema and pulmonary impairment they were referred to the Immunology service. During follow-up, they were diagnosed with STAT5B deficiency. Each of the three patients presented had a distinct mutation: homozygous missense (p.A630P), homozygous nonsense (p.R152X) and a homozygous missense mutation (p.F646S), respectively [6, 10, 12]. Here we report the long-term follow-up of these patients including their clinical and immunological features.

Methods

Immunoglobulin measurement Total serum immunoglobulin (IgG, IgM and IgA) were measured by kinetic nephelometry using commercially available kits (Array 360, Beckman Coulter Inc). IgE was measured by MEIA.

Response to protein antigens: Specific tetanus toxoid antibody (Abs) concentration was measured by an "in-house" ELISA (details available upon request. **Response to polysaccharide antigens:** Pneumococcal antibody concentration (IgG) was measured by commercial ELISA (The Binding Site ®) post 23-valent vaccination (response criteria: consecutive titer after stimulus ≥113 mg/L as the IDP Working Group consensus of the Argentina Society of Pediatrics).

Autoantibody assays Anti-nuclear (ANA), anti-smooth muscle (SMA), anti-mitochondrial (AMA), anti-liver kidney microsomal (LKM), anti-gastric parietal cell (GPCA), anti-mitotic apparatus (NUMA), anti-endomysium (EMA) IgA/IgG and anti-neutrophil cytoplasmic antibodies (ANCA) were analyzed by Indirect Immunofluorescence. Anti-centromere B protein (Cenp B) was analyzed by immunoblot. Anti-deaminated gliadin peptide DGP IgA/IgG, anti-transglutaminase (TG) IgA/IgG, anti Sm, anti RNP, anti SS-A/Ro, anti SS-B/La, anti scl70, anti Jo1 and anti histone were analysed by ELISA.

Flow cytometric immunophenotyping: Cells were stained and analyzed on a FACSCalibur flow cytometer using CellQuest software (Becton Dickinson, San Jose, CA, USA). ***Lymphocyte subsets***: Both percentage and absolute counts were performed for: CD19 B cells, CD3, CD4 and CD8 T cells and $CD3^{-}CD16^{+}CD56^{+}$ natural killer cells (NK). ***CD4 T cell subpopulations*** were analyzed on CD4 gating: Naïve $CD45RA^{+}$ $CD27^{+}$; central memory $CD45RA^{-}$ $CD27^{+}$, effector memory $CD45RA^{-}$ $CD27^{-}$ and T_{REG} cells defined as $CD25^{++}CD127^{low}FOXP3^{+}$. ***B-cell subsets*** were used to delineate distinct stages of peripheral B-cell maturation and differentiation gating on CD19: Naïve B cells ($CD27^{-}IgD/IgM^{+}$), non-switched IgM memory (marginal zone) B cells ($IgM^{+}CD27^{+}IgD^{+}$), switched memory B cells ($CD27^{+}IgD^{-}$ and $CD27^{-}IgD^{-}$), plasma cells ($CD38^{++}CD27^{++}$).

In vitro proliferative response Peripheral blood mononuclear cell (PBMC) response to phytohaemaglutinin (PHA), PHA+IL-2r, aCD3 (Beckman Coulter), aCD3+IL2r, ionomycin and phorbol myristate acetate (I+PMA) (Sigma-Aldrich, St Louis, MO, USA) was measured by ^{3}H-thymidine incorporation.

Flow cytometry NK-cell cytotoxicity assay K562 cells (a human erytroleukemia cell line) were used as target cells. The target cell concentration was adjusted to 1×10^{6} cells/ml. PBMC were isolated by centrifugation with Ficoll-Hypaque to a final concentration of 5×10^{6} cell/ml. Various concentrations of effector cells were incubated with target cells for 5 hs at 37 °C under 5 % CO_2 with propidium iodide (Sigma-Aldrich). Analysis was performed using CellQuest software (Becton Dickinson, San Jose, California).

The values of different subpopulations and functional studies were compared to the normal ranges for age-matched healthy donors.

Case presentations

Patients Demographics and history (Table 2)

Case 1 Figure 1a

Thirty-year-old female (P1) was the first child of consanguineous parents. She was born prematurely at 33 weeks of gestation and weighed 1400 g. There was no family history of growth failure and her younger sisters are of normal stature. At birth, she required care in a neonatal unit due to respiratory difficulties. Poor weight gain and growth failure were noted during her first 3 years of life. She

Table 1 Comparative table of all STAT5B deficient patients described in literature

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Reference Values
Mutation	A630P Homozygous	R152X	F646S Homozygous	119*ins*G Homozygous	R152X	1102*ins*C Homozygous	1680*delG* Homozygous	1680*delG* Homozygous	424_427*del* Homozygous	424_427*del* Homozygous	
Country	Argentina	Argentina	Argentina	Turkey	Argentina	Dutch Antilles	Kuwait	Kuwait	Brazil	Brazil	
Sex	Female	Female	Female	Female	Female	Male	Female	Female	Male	Male	
Consanguinity	Yes	No	Adopted	Yes	Adopted	NA	Yes	Yes	NA	NA	
Age (yr)	16.5	16	18	16.4	12	31	2	4	6	2	
Height (SDS)	−7.5	−9.91	−5.9	−7.8	−4.0	−5.9	−5.8	−5.6	−5.6	−3	
Chronic Pulmonary Disease	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	
Skin Pathology	Eczema	Eczema Severe Infections	Seborrheic dermatitis	Eczema	Eczema	Ichtyosis	No	No	Atopic	Atopic	
Autoimmunity	+Abs to BEC		Thyroiditis Psoriasis Alopecia	ITP + Abs to platelets	Hipothyroidism	NA	SJIA	No	ITP	No	
Varicella Infection	Yes (severe hemorrhagic) and recurrent Herpes Zoster	Yes (Complicated) and recurrent Herpes Zoster	Yes (Severe)	NA	Yes (Severe) and recurrent Herpes Zoster	Yes (Hemorrhagic)	NA	NA	NA	NA	
Birth Weight (g) Length (cm)	1.400 NA	2500 NA	2.250 44	2.350 49	NA NA	3.270 50	2.400 NA	3.600 NA	1.700 39	2.400 49	
Puberty	Delayed	Delayed	Normal	Delayed	Delayed	Delayed	NA	Delayed	NA	NA	
GH Axis											
GH Basal	9.4	6.6	1.7	14.2	1.8	0.13	17.7	5.7	1.7	1	
GH Stimulated	53.8	NA	27.1	NA	12.5	14.2	NA	NA	20.6	14	>6.0
IGF-1 Stimulated	38	ND	16	7.0	0.8	14	<5	<5	34	<25	119–483
IGFBP-3	874	ND	840	543	500	180	700	800	520	750	210–740

GH, IGF-1 and IGFBP-3 expressed in ng/ml

NA not available, *ND* no detectable, *BEC* bronchial epithelial cells, *SJIA* systemic juvenile idiopathic arthritis, *ITP*, idiopathic thrombocytopenic purpura

Table 2 Clinical features of 3 patients with STAT5B deficiency: Description of the background of patients during follow-up

Patient	1	2	3
Family	Consanguineous parents Two sisters	Non consanguineous parents One brother	Adopted
Age at first consultation	7 years	6 years	11 years
Clinical presentation	*Intrauterine growth retardation* *Severe growth failure* *Eczema* *Failure to thrive* *Chronic diarrhea*	*Severe growth failure* *Eczema* *Failure to thrive* *Chronic diarrhea* *Recurrent severe skin infections* *Pneumonia* *Chronic lung disease*	*Severe growth failure* *Eczema* *Failure to thrive* *Upper and lower respiratory tract recurrent infections*
	***7 yrs**: LIP* - Chronic lung disease	***4 yrs:*** Varicella complicated with bacterial infection	***4.5 yrs:*** Autoimmune thyroiditis, psoriasis, alopecia
	8 yrs: Severe hemorrhagic varicella and several episodes of Herpes zoster	***10 yrs:*** Recurrent Herpes zoster keratitis, uveitis	***11 yrs:*** Severe varicella with cutaneous infection. *S. pyogenes* (blood cultures)
	10 yrs: Lung infection: P*neumocystis jirovecii and Rhodococcuss spp* ***20 yrs:*** Whooping cough		***20 yrs:*** Celiac disease
Neurological development	Normal	Mildly retarded	Mildly retarded
Age at diagnosis	16 yrs	16 yrs	15 yrs
Age: Height	***16 yrs:*** 117 cm (<7.5 SD)	***6 yrs:*** 83 cm (<6 SD)	***11 yrs:*** 106.8 cm (<4.3 SD)
Deceased	Yes (at 30 yrs due to respiratory insufficiency)	Unknown	No

Abbreviations: *SD* standard deviation, *yrs* years, *LIP* lymphocytic interstitial pneumonitis

presented chronic diarrhea and steatorrhea but subsequent evaluations for failure to thrive and malabsorption revealed no abnormalities (normal gut biopsy). She developed generalized eczema, which persisted with mild presentation until adulthood. At 7 years old, she was first referred to the endocrinology unit because her height and weight were far below the 5th percentile. She suffered from chronic respiratory difficulties and required oxygen beginning at 6 years of age. A lung biopsy revealed *lymphocytic interstitial pneumonitis (LIP)* (Fig. 2). Subsequent studies were negative for HIV, CMV and EBV. The patient was treated with corticosteroids and saw partial improvement but experienced multiple episodes of bronchial obstruction. At the age of eight, she presented with severe hemorragic varicella that was followed by several episodes of Herpes zoster. A progressive decline in pulmonary function resulted in a second lung biopsy at the age of ten that showed a return of LIP. *Pneumocystis jirovecii* and *Rhodococcuss spp*. were isolated from this sample.

Due to increased levels of GH, decreased levels of IGF-1, and height that was 7.5 SD below the mean at the age of sixteen, a defect in the GH signaling pathway was suspected. Molecular studies revealed a homozygous missense A630P mutation in *STAT5B* [6]. She is the first patient worldwide described with this mutation. During evolution she developed neither autoimmune disease, lymphadenopaty, nor hepatosplenomegaly. The chronic lung disease became progressively more severe, ultimately requiring oxygen therapy while at rest. She died from respiratory insufficiency at 30 years of age.

Case 2 Figure 1b

Twenty-four-year-old female (P2) was the first child of non-consanguineous parents. Her birth weight was 2500 g and she had severe growth failure from her first year of life. She suffered from chronic diarrhea from 2 months of age and had a history of complicated Varicella with bacterial superinfection, generalized eczema, and recurrent severe infections of the skin and respiratory tract that required multiple hospitalizations. She was evaluated by an immunology specialist at the age of six. In the following years she had recurrent Herpes zoster keratitis and uveitis of the left eye, with progressive loss of visual acuity. She exhibited evidence of chronic lung disease with signs of chronic hypoxemia. Serology was negative for HIV, CMV and EBV. At 16, a homozygous nonsense mutation p.R152X was identified in the *STAT5B* gene [10]. The patient was subsequently lost to follow-up.

Case 3 Figure 1c

Twenty-one-year-old female patient (P3) weighed

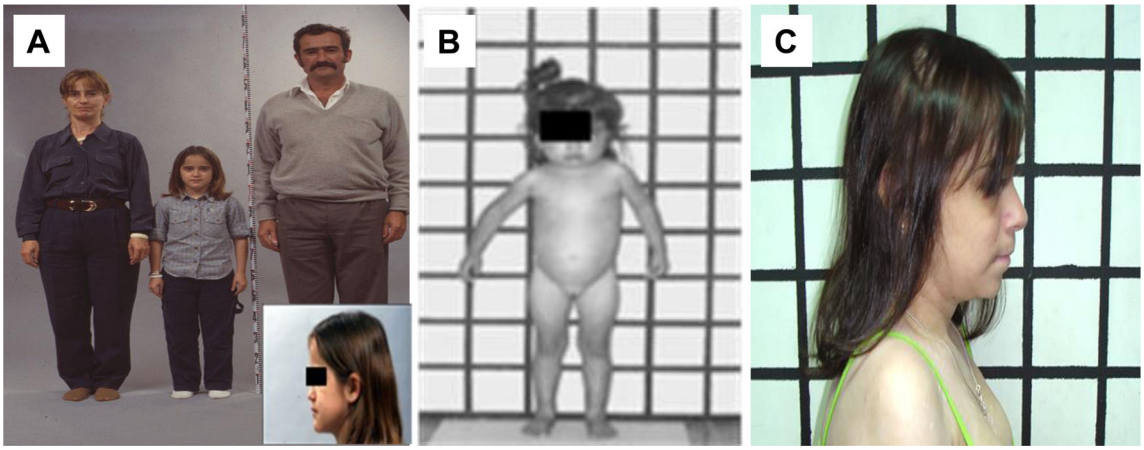

Fig. 1 **a**. Patient 1 at 11 years old. **b**. Patient 2 at 6 years old. **c**. Patient 3 alopecia

2250 g and was 44 cm long at birth. Data regarding family history, consanguinity, gestation and perinatal periods were unavailable because she was adopted at 4 days of age. Her early medical history was unremarkable, with adequate weight gain until the 3rd month of age when she had several episodes of infection (otitis media, cellulitis, and pneumonia) associated with failure to thrive. When she was 1 year old she developed a severe generalized seborrheic dermatitis. At two she was referred to a pediatric endocrinologist for growth evaluation. During follow up she developed autoimmune thyroiditis and began hormone replacement therapy. At the age of four she developed psoriasis and alopecia. She was later diagnosed with severe Varicella with cutaneous infection and *Streptococcus pyogenes* was isolated from blood cultures. Serology was negative for HIV.

At 11 years, still prepubescent, her height and weight were 4.30 SD and 3.65 SD below the mean, respectively. Studies revealed a novel homozygous missense p.F646S mutation in *STAT5B* [12]. At 20 years old she developed celiac disease. At present she has persistent secondary psoriasis dermatitis and hypothyroidism, but no signs or symptoms of lung disease.

Immunological evaluation (Table 3)

P1 Hypergammaglobulinemia (IgG and IgA isotypes) with normal IgM and IgE and positive autoantibodies (See Table 2). Slightly but consistently reduced CD4 and CD8 T cells counts, with the CD4 T cells being skewed to a memory phenotype. Low levels and reduced function (data not shown) of regulatory T cells with normal T cell proliferation to mitogens, specific antigens and anti-CD3 [15]. Low levels of IgM

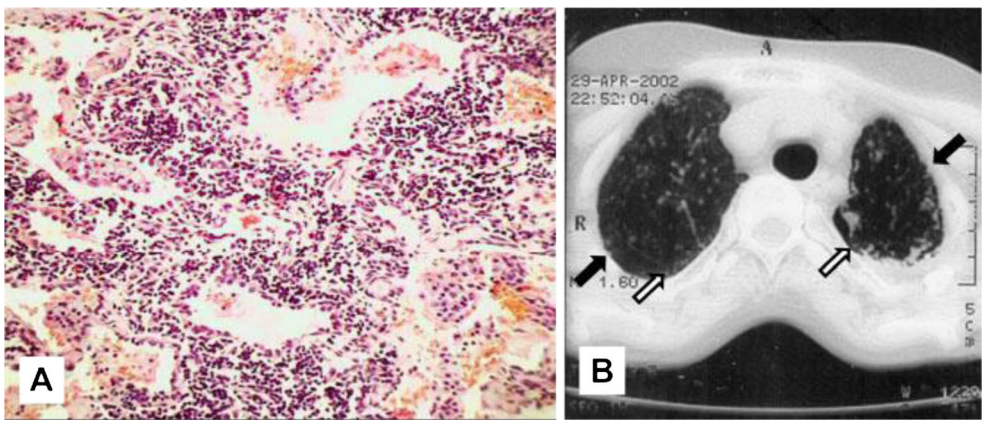

Fig. 2 **a** Histological analysis of lung tissue from patient 1 at 7 years of age demonstrating features of *LIP*. The fragment of lung parenchyma measured 2.5×1.5×0.3 cm. Although there is relative preservation of histological architecture, there is a marked and diffuse interstitial mononuclear cellular infiltrate with a follicular pattern that broadens the alveolar septa but largely spares the alveolar spaces. The peribronchial areas are also involved with this cellular infiltrate. **b** Axial chest computerized tomography scan of P1 showing centrilobular nodules (*white arrows*) and subpleural nodules (*black arrows*)

Table 3 Laboratory evaluation of 3 patients with STAT5B deficiency: Laboratory data of patients were compared with normal values from an age matched control sample

Patient	1		2		3		Normal range
Years of Follow up	23		10		10		
IgG (mg/dl)	1130–2948[a]		1530–2060[a]		2370–3250[a]		984–1144
IgA (mg/dl)	223–171[a]		72–119[a]		323–592[a]		112–252
IgM (mg/dl)	72–138[a]		87–125[a]		96–202[a]		82–220
IgE (UI/dl)	17		1095		28		
Specific IgG antibodies	EBV:+ CMV:+		Measles:+ Varicella:+		nd		
Tetanus toxoid (UI/ml)	0.2		0.1		0.55		>0.1
Pneumococcal (mg/L)	137		nd		234		>113
Autoantibodies	AMA+		ANA+ NUMA+ Cenp B + Histone+		ANA+ IgA TG + IgG /IgA DPG +		
Isohemagglutinins	1/512	1/256[a]	1/1024	1/128[a]	nd		
C3 /C4 (mg/dl)	120/20		135/ 23		185 / 39		90–150/15–35
Lymphocyte count / mm^3	1600–605[a]		2300		570–1364[a]		
CD3 cells/ mm^3 (%)	960 (60)	242 (40)[a]	1311 (57)		627 (46)	234 (41)[a]	1543–2484 (65–85)
CD4 cells/mm^3 (%)	768 (48)	181 (30)[a]	713 (31)		463 (34)	171 (30)[a]	771–1180 (36–46)
CD8 cells/mm^3 (%)	128 (8)	43 (7)[a]	483 (21)		122 (9)	34 (6)[a]	629–1128 (19–40)
Naïve CD4 (%)	29		nd		7		34.8–70.3
Memory CD4 (%)	70		nd		91		27.3–49.8
TCRγδCD3 (%)	4		nd		2		2–4
Activated CD4DR (%)	26		51		45		1.0–5.0
NK cells/mm^3 (%)	256 (16)	121 (20)[a]	184 (8)		272 (20)	28 (5)[a]	241–555 (7–23)
Tregs (%)	0		0.1		1.0		1.1–3.1
CD19 cells/mm^3 (%)	368 (23)	163 (27)[a]	644 (28)		436 (32)	228 (40)[a]	278–281 (7–23)
Naïve B cell (%)	67		nd		33		59–81
IgM memory (%)	5		nd		3		10.5–20,9
IgD^-CD27^+Memory (%)	14		nd		49		14.3–21.9
IgD^-CD27^-Memory (%)	14		nd		15		1.9–5.0
Plasma cells (%)	1.8		nd		2.2		
Proliferative response (cpm)							
PHA	98.000		nd		24.000	42.000[a]	74.000–135.000
PHA+IL-2r	113.000		nd		50.000 / 65.000[a]		
aCD3	72.000		nd		81.000–22.000[a]		55.000–91.000
aCD3+IL2r	nd		nd		55.000		
I+PMA	42.000		nd		19.000–77.000[a]		61.000–149.000
PWM	80.000		nd		21.000		
Perforin	Normal		nd		Normal		
NK Citotoxicity assay	Normal		nd		nd		

Abbreviations: *nd* no data, *ANA* anti-nuclear antibodies, *AMA* anti-mitochondrial, *NUMA* anti-mitotic apparatus, *Cenp B* anti-centromere B protein, *IgA TG* anti-transglutaminase), *IgG/IgA DPG* anti-deaminated gliadin peptide

[a] Assays were performed multiple, seperate occasions; these are expressed as a range

memory B cells and increased levels of $CD27^-IgD^-$ class-switched memory B cells. NK cell counts were normal

P2 Hypergammaglobulinemia (IgG, IgA, IgE isotypes) and positive autoantibodies (See Table 2) at 6 years old. T cell counts were normal but had an impaired proliferative response to mitogens, specific antigens and anti-CD3. NK cell counts were normal.

P3 Hypergammaglobulinemia, mainly IgG and IgA isotypes with normal tetanus toxoid and pneumococcus specific

antibody response, normal IgE and IgM serum levels. Positive autoantibodies (See Table 2). Low levels of IgM memory B cells and increased levels of switched memory B cells ($CD27^{-}IgD^{-}$ and $CD27^{+}IgD^{-}$). Reduced counts of CD4 and CD8 T cells, with skewing of the CD4 cells to a memory phenotype. Decreased number of regulatory T cells. In vitro T cell proliferative response to PHA and anti-CD3 was decreased but could be improved by addition of IL-2 stimulation.

Discussion

In 2003, we described P1 as short stature and suffering from growth hormone insensitivity syndrome (GHIS), facial dysmorphism, severe varicella infections, lymphoid interstitial pneumonitis and a homozygous missense mutation in the *STAT5B* gene, which encodes a key component of the IL-2R signaling pathway [10]. Since that time we evaluated two additional patients with biallelic *STAT5B* mutations for whom GHIS was associated with susceptibility to infections, autoimmune manifestations and eczema [12].

Human STAT5A and STAT5B share over 90 % sequence identity in their cDNA and proteins, suggesting that they may have been generated by gene duplication. However, the STAT5A and STAT5B proteins differ in the last six amino acids of the DNA binding domain and in the 20 amino acids of the transactivation domain. These differences have important biological and clinical implications, as demonstrated by mice lacking Stat5a or Stat5b and by identification of STAT5B deficient patients [16, 17]. The interaction of GH with its receptor (GHR) triggers JAK2 activation and STAT5B phosphorylation, leading to the production of IGF-1, a key factor for body growth. Patients with GHR deficiency display the same extrahematological manifestations as STAT5B deficient patients with postnatal growth retardation and GH insensitivity but do not have immune deficiency [18, 19]. Interestingly, patient 1 had history of prenatal growth retardation. Stat5b deficient mice fail to develop sexually dimorphic body growth, in contrast, our patients all exhibited normal sexual development [17].

STAT5B deficient patients also display various autoimmune and allergic signs. All of our patients had severe eczema and chronic diarrhea. Patient 3 suffered from autoimmune disease (thyroiditis, celiac disease and psoriasis). Patient 1 had biopsy-proven lymphocytic interstitial pneumonitis and died due to respiratory failure when she was 30 years old. Patient 2 also had clinical evidence of LIP but did not undergo lung biopsy.

Previous work in murine models has demonstrated that Stat5b is a critical transducer of IL-2 mediated signals that are required to sustain FOXP3 expression in T_{REG} cells and to maintain T_{REG} cells themselves [20]. Double knock-out *Stat5a/b* mice have very few T_{REG} cells in the thymus and periphery leading to signs of autoimmunity and lymphocytic infiltration in multiple target organs [4, 21, 22]. These data are consistent with the role of STAT5B in the IL-2 induced up regulation of FOXP3 [23]. The deficiency and impaired function of regulatory T cells described in patient 1 is therefore likely a direct result of impaired IL-2 signaling, accounting for the signs of immune dysregulation associated with this defect.

The clinical manifestations common to all three patients including failure to thrive, growth failure, eczema, chronic diarrhea, severe varicella and recurrent Herpes zoster infections indicate that STAT5B modulates cytokine biological pathways key to immune response, and that its impairment can result in a combined immunodeficiency.

The infectious spectrum was different between our patients. Patients 1 and 2 had recurrent bacterial and viral pneumonias and chronic lung disease. Patient 3 had less severe infections, reporting only otitis at an early age but in contrast, she developed more significant autoimmune disease including autoimmune thyroiditis, psoriasis, alopecia, and celiac disease. The susceptibility to Herpes family viruses (Varicella and zoster) may reflect a block in IL-15 signaling caused by STAT5B deficiency that leads to a numerical and likely functional deficiency of NK cells in many patients with this disorder.

Many of the features of STAT5B deficiency are similar to those found in CD25 deficient patients including eczema, chronic diarrhea, autoimmunity and increased susceptibility to infections, likely because interleukin 2 receptor signaling requires STAT5B [1, 24].

Previous reports show that the clinical presentation of STAT5B deficiency is more variable and less severe than that of CD25 deficiency [1, 25–27]. In this follow-up we observed a disease progression in some of our STAT5B deficiency patients that was as severe as that has been described in patients with CD25 deficiency (severe lung compromise, infections and autoimmune manifestations).

Murine models, using *Stat5a/b* double-knock-out mice showed severe impairment of lymphoid development while Stat5b deficient mice showed less dramatic immunologic alterations, characterized by decreased numbers of T cells, low proliferative response and very low natural killer cell count and function [2, 20, 28]. Our patients had T cell lymphopenia with a skewing toward an activated, mature phenotype and variable functional compromise as measured by *in vitro* proliferation to mitogens and antigens.

Increased T lymphocyte apoptosis has been described in *Stat5a/b* double knockout mice and in human STAT5B deficiency. Increased apoptosis is likely the result of an inability to respond to important T cell growth factors including IL-2 and IL-7 and may contribute to the T cell lymphopenia observed in this disorder [15, 29]. Defective effector T cells may therefore

account for much of the patient's broad and profound susceptibility to infections. These observations indicate that Stat5a and Stat5b play largely redundant roles in the development and function of the immune and endocrine systems in mice, whereas STAT5B has unique, non-redundant functions in growth and immunity in humans [16].

All patients had low levels of $FOXP3^+$ T_{REG} cells. Regulatory T cells in patient 1 and her parents exhibited impaired suppressive function [15]. The autoimmunity observed in these three patients is consistent with the hypothesis of STAT5B's essential role in the development of T_{REG} cells and their involvement in homeostasis and immune tolerance [30].

Several previous studies have indicated that Stat5b deficiency may produce alterations not only in T cells but also in the B cell compartment in murine models [31]. There are no reports of B cell compartment impairment in humans. Despite having normal B cell counts and adequate specific antibody responses, these patients had hypergammaglobulinemia, low IgM memory B-cells, and increased switched memory B-cell counts. It is unclear whether the predominance of switched memory B cells reflects a loss of peripheral tolerance, an abnormal selection processes, or whether their appearance is simply the result of enhanced activation and differentiation of B cells. A central finding in peripheral blood B cell phenotyping from these patients was a substantial increase of $CD27^-IgD^-$switched memory B cells. Enhancement of this subset has been described in patients with systemic lupus erythematosus, correlation has also been reported with the presence of autoantibodies and disease activity [32].

This is the first report of long-term follow up greater than 1 decade in patients with STAT5B deficiency. It demonstrates clearly that *STAT5B* mutations are associated with various clinical pathologies associated with high morbidity. Interestingly however, these cases also demonstrate that STAT5B is not absolutely required for embryonic viability in humans, and that despite having a significant combined immune defect, patients can live into adulthood with supportive care. This is likely the result of at least some functional redundancy of STAT5A. The progression and symptoms of these patients provide evidence that STAT5B proteins are essential mediators of the IL-2 cytokine family in the development, homeostasis and proliferation of different lymphocyte populations. Murine studies suggest that the immune defects associated with Stat5b deficiency could be corrected by bone marrow transplant (BMT) although to our knowledge, this has not yet been attempted in any patients [33].

Early diagnosis is critical for the prevention of permanent sequelae and improvement of clinical outcomes for STAT5B deficient patients. Any signs and symptoms of immunodeficiency should be treated with appropriate antimicrobial therapy and autoimmunity/atopic disease treated with immunosuppressants.

There remain many questions regarding the exact genotype/phenotype correlations of particular *STAT5B* mutations and regarding the optimal approach to clinical therapy for this disease. While there have been a relatively limited number of patients identified worldwide, we suspect that there may be additional patients who have hypomorphic variants of *STAT5B* that may have a milder clinical phenotype and may not yet have been identified. Give that disease causing, autosomal-dominant loss-of-function mutations of *STAT1* and *STAT3* have been described; we would predict that a similar process may occur for *STAT5* but that these patients may also have a milder clinical phenotype and may not yet have come to light. Hopefully, studies like this one will help to raise the clinical awareness of this disorder and lead to the identification of other patients.

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