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Molecular and Cellular Endocrinology 559 (2023) 111799

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Atypical STAT5B deficiency, severe short stature and mild immunodeficiency associated with a novel homozygous STAT5B Variant

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ARTICLE INFO

Keywords:
STAT5B
Immune deficiency
Growth hormone insensitivity

ABSTRACT

STAT5B deficiency, a rare autosomal recessive disorder characterized by severe growth hormone insensitivity (GHI) and immunodeficiency, can manifest as fatal pulmonary complications. We describe atypical STAT5B deficiency associated with a novel homozygous frame-shift *STAT5B* variant [*c.1453delG,* p.(Asp485Thrfs*29)] identified in a young 17.6 yr old female subject who had severe postnatal growth impairment, biochemistries typical of GHI, an immune profile notable for hypergammaglobulinaemia and elevated B lymphocytes, and lack of pulmonary disease. Marked elevation of serum prolactin and pathologically diagnosed eczema were evident. In reconstitution studies, the STAT5B p.(Asp485Thrfs*29) was expressed although expression was reduced compared to wild-type STAT5B and a previously identified STAT5B p.(Gln368Profs*9) variant. Both truncated STAT5B peptides could not be activated by GH, nor mobilize to the nucleus. We conclude that an intact, functional, STAT5B is essential for normal GH-mediated growth, while expressed loss-of-function STAT5B variants may alleviate severe immune and pulmonary issues normally associated with STAT5B deficiency.

1. Introduction

STAT5B (signal transducer and activator of transcription 5B) deficiency (MIM 245590) is a rare autosomal recessive condition of Growth Hormone Insensitivity (GHI) with immunodeficiency. First described in 2003 (Kofoed et al., 2003), patients carrying homozygous inactivating *STAT5B* variants are characterized by proportionate severe postnatal growth failure, low serum insulin-like growth factor-I (IGF-I) and IGF binding protein 3 (IGFBP-3) despite normal or elevated concentrations of serum growth hormone (GH), consistent with the diagnosis of GHI (Hwa et al., 2011; Hwa, 2016, 2021). In contrast to well-established GHI due to pathogenic variants in the gene encoding the GH receptor (*GHR*) (Rosenfeld et al., 1994; David et al., 2011; Andrews et al., 2021), unique features of STAT5B deficiency include eczema, elevated serum prolactin concentrations, elevated IgE, and a progressive immunodeficiency involving dysregulated T lymphocyte populations (Cohen et al., 2006; Jenks et al., 2013; Bernasconi et al., 2006; Bezrodnik et al., 2015; Foley et al., 2021), B lymphocytes (Pelham et al., 2022), and NK cells (Vargas-Hernandez et al., 2020), with potentially fatal pulmonary complications by 30 years of age (Bezrodnik et al., 2015). A recent report

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https://doi.org/10.1016/j.mce.2022.111799
Received 17 August 2022; Received in revised form 11 October 2022; Accepted 12 October 2022
Available online 17 October 2022

indicated that pulmonary issues involved, at least in part, dysregulated alveolar macrophages associated with symptoms consistent with pulmonary alveolar proteinosis (PAP) (Krone et al., 2022). Interestingly, dominant-negative *STAT5B* missense variants confer GHI and short stature less pronounced than homozygous inactivating *STAT5B* variants, and result in only a mild degree of immune dysregulation (Klammt et al., 2018).

STAT5B, a member of the 7-membered STAT family of transcription factors, transduces signals of many growth factors and cytokines (Levy and Darnell, 2002; Schepers et al., 2012), including GH. Pituitary GH promotes normal human postnatal growth by direct biological effects and indirectly through increasing the production of circulating and peripheral IGF-I. The binding of GH to cell surface GHR activates associated JAK2 (Janus kinase 2) and initiates a cascade of signalling events (Brown et al., 2005; Brooks et al., 2014).

Of the multiple signalling pathways activated, recruited cytosolic STAT5B docks to 3 redundant phosphorylated tyrosines on the intracellular domain of GHR (Derr et al., 2011). Subsequently, STAT5B itself becomes phosphorylated on tyrosine 699 (Y699) by JAK2 and then homodimerizes, translocates into the nucleus and binds to DNA sequences for gene regulation including *IGF1*, *IGFBP3* and *IGFALS*. Secreted IGF-I, predominantly contributed by the liver, circulates mainly in a ternary complex, with IGFBPs, i.e. especially IGFBP-3, and the acid labile subunit (ALS), to be delivered to target tissues.

To date, nine of the ten reported homozygous *STAT5B* variants are associated with IGF-I, IGFBP-3 and ALS deficiencies and severe postnatal growth failure, strongly implicating STAT5B as the key GH-induced transcription factor for normal human linear growth (Hwa, 2021; Foley et al., 2021). No endocrine data was provided for the most recent homozygous STAT5B c.121C > T, p.(Gln41*), variant, identified in a young infant who exhibited postnatal growth delay (Pelham et al., 2022). Seven of the 10 variants result in predicted protein truncation due to early protein termination and the remaining three are missense variants. Two of the missense STAT5B variants [p.(Leu151Pro), p.(Phe646Ser)] were reported in teens with less severe immune deficiencies (Bezrodnik et al., 2015; Scaglia et al., 2012; Acres et al., 2019), although it is of note that the patient carrying p.(Leu151Pro) now has progressive pulmonary issues (Pelham et al., 2022). Only one STAT5B deficient patient, carrying a homozygous frameshift mutation [p.(Gln368Profs*9), in exon 9], is known to have survived beyond 30 years of age and, interestingly, had a mild subclinical immune deficiency (Vidarsdottir et al., 2006; Walenkamp et al., 2007). A recent nonsense mutation [p.(Trp631*)] was reported in very young siblings who lacked overt immune deficient symptoms although skewing of immune profiles were emerging (Foley et al., 2021).

We now report a novel homozygous frameshift mutation leading to a truncated *STAT5B* variant [*c.1453delG*, p.(Asp485Thrfs*29), exon 12] in a teenage female patient, who presented with GHI but lacked the severe immune and fatal pulmonary complications typically associated with STAT5B deficiency. This new case expands the clinical spectrum of STAT5B deficiency and provides valuable opportunities to better understand biological properties of rare natural STAT5B variants towards improving phenotype-genotype correlations and patient management.

2. Case report

Consent Statement: Informed consent has been obtained from the patient for publication of the case report and accompanying images.

A 17-year-old female was referred for evaluation of severe short stature and primary amenorrhoea. She was the second child of consanguineous parents (Fig. 1) with heights close to the 10th percentile [father 165.5 cm (−1.3 SDS), mother 151.9 cm (−1.4 SDS), target height 152.2 cm (−1.3 SDS)]. In her family, an older brother (height −0.8 SDS) and two paternal aunts (height −1.4 SDS) subsequently proved to be carriers of the STAT5B variant (see below; Table 1). The father and two paternal aunts had chronic eczema.

The index patient was born at term with a birth weight of 2800 g (−1.6 SDS) (Niklasson et al., 1991), and has had eczematous pruritic lesions from infancy. Short stature became evident from two years of age although there was no history of severe or recurrent infection or pulmonary disease. She was first referred to an endocrinologist at the age of 15.2 years, when height was 121.8 cm (−6.2 SDS) and weight 32.3 kg (−4.1 SDS) [body mass index (BMI) 21.8 (0.3 SDS)] (Neyzi et al., 2015). Body proportions were normal. Pubertal development was delayed (Tanner stage B2P2), and bone age was delayed by 4 years.

At 17.6 years height was 129.3 cm (−5.2 SDS), weight 43.7 kg (−1.9 SDS), BMI 26.1 (1.5 SDS), sitting height/height ratio 0.54 (1.5 SDS) (Fredriks et al., 2005), arm span 123.8 cm (arm span minus height −1.4 SDS) (Gerver et al., 2020), and head circumference 52 cm (−1.9 SDS) (Neyzi et al., 2015). Pubertal development was Tanner stage 4 and menarche had not occurred yet. She had midface hypoplasia, frontal bossing (Fig. 2A and B), high-pitched voice, normal intelligence, no

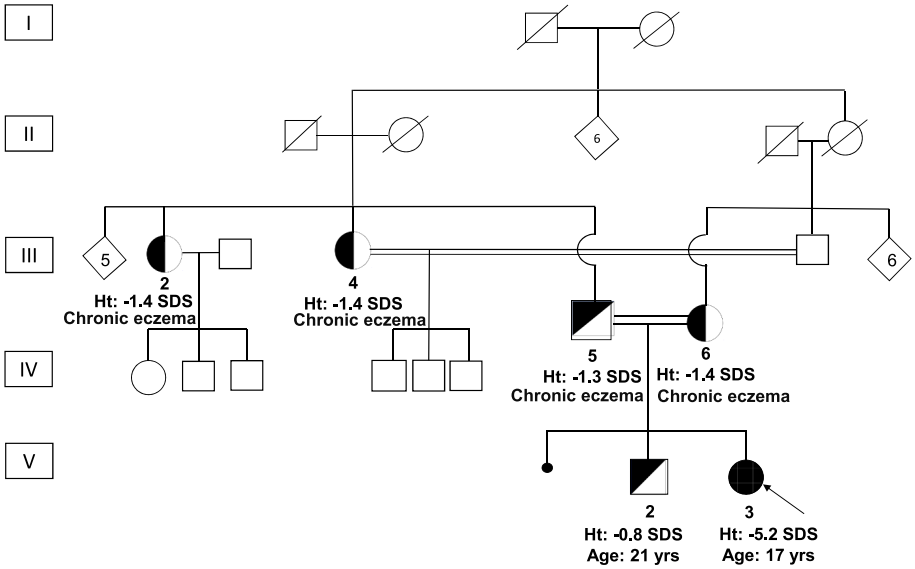

Fig. 1. Pedigree and segregation of the novel *STAT5B* frameshift variant. The index patient (arrow), part of generation V (left), is the only individual who is homozygous for the variant (filled circle). Other individuals in the family who were genetically identified to carry the heterozygous variant, are indicated as half-filled symbols. Consanguinity shown as double lines. Circle symbols, female; square, symbols male. Key phenotype of height (Ht) and chronic eczema are indicated.

Table 1
Clinical and biochemical characteristics of the index patient and family members.

Parameter	Index patient	Father	Mother	Aunt 1	Aunt 2	Brother
STAT5B p.(Asp485Thrfs*29)	Homozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Age, years	17.6	51	46	49	48	21
Height, cm (SDS)	129.3 (−5.2)	165.5 (−1.3)	151.9 (−1.4)	151(-1.4)	150.5 (−1.4)	171.4 (−0.8)
Weight, kg	43.7 (−1.9 SDS)	61	107	71	59	92
BMI, kg/m^2	26 (1.5 SDS)	22	49	31	26	32
Head circumference, cm	52 (−1.9 SDS)	55.3	56	54.6	52.5	56.5
Waist/Hip ratio	0.92	0.81	0.95	0.83	0.86	0.87
Percent body fat	21.3%	12.8%	48.2%	36.6%	31.3%	24.4%
Gestational age (weeks)	40	N/A	N/A	41	40	39
Birth weight (kg)	2.8	N/A	N/A	4.5	3.2	2.8
Puberty	Delayed	Normal	Normal	Normal	Normal	Normal
Eczema	Yes	Yes	No	Yes	Yes	No
Varicella (age/severity)	3 years/Normal	N/A	10 years/Normal	N/A	8 years/Normal	7 years/Normal
Chronic pulmonary disease	No	No	No	No	No	No
Other	anaemia	Pollen allergy anaemia	anaemia	Pollen allergy	Pollen allergy	No
Insulin (μIU/mL)	22.9	3.5	7.37	2.45	3.65	1.5
Glucose (mg/dL)	93	92	91	74	77	82
Baseline GH (ng/mL)	0.74	0.34	2.1	0.08	0.54	1.5
Stimulated GH (ng/mL)	3.8	N/A	N/A	N/A	N/A	N/A
IGF-I (nmol/L)	4.7	11.3	9.5	12.3	14.0	21.1
IGF-I SDS	−5.7	−0.9	−1.8	−0.6	−0.3	−1.3
IGF-II SDS	−4.0	−3.6	−0.7	−1.5	−1.7	−0.04
IGFBP-2 SDS	1.3	2.3	0.9	0.9	1.4	−0.6
IGFBP-3 SDS	−6.2	−2.0	−0.2	−0.6	−0.5	−1.0
ALS SDS	−6.0	−2.1	−1.7	−0.6	−0.7	0.2
Prolactin (ng/mL)	225	14.9	19.1	6.8	9.43	8.09

N/A, not available.

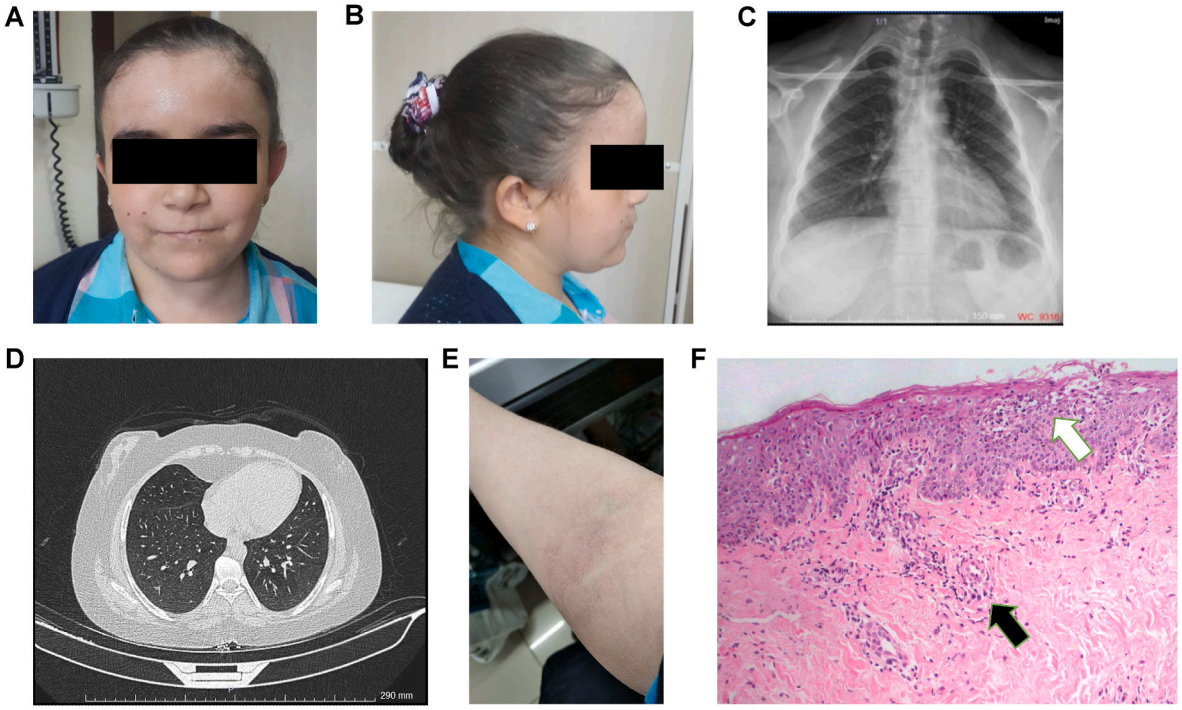

Fig. 2. Clinical features of the index patient. **A** and **B**. Facial characteristics (frontal bossing, depressed nasal bridge) resemble patients with classical GHI syndrome; **C**. Lung radiograph (age 23.8 yrs) and **D**. CT scan (age 23.8 yrs) lack apparent abnormalities such as ground glass or nodules; **E**. Skin abnormalities on antecubital fossa; **F**. Hematoxylin and eosin stain of sections from biopsied skin lesions. Note the presence of focal parakeratosis, irregular acanthosis, spongiosis (White arrow), and perivascular inflammatory infiltrate (black arrow) consistent with eczema (x 200).

hearing loss and without truncal obesity.

Biochemical evaluation (Table 1) revealed extremely decreased concentrations of IGF-I (<25 ng/mL), IGF-II (−4.0 SDS), IGFBP-3 (−6.2 SDS) and ALS (−6.0 SDS). In heterozygous carriers, almost all of these parameters were in the lower half of the reference range, except for an extremely low serum IGF-II level in the father. Serum prolactin levels in the index case were persistently elevated [97–225 ng/mL (reference range, 2.5–25 ng/mL)]. Thyroid function was normal. Results of two non-primed GH stimulation tests (L-dopa and clonidine) showed peak GH values of 1.8 and 3.8 ng/mL, respectively, suggestive for GH deficiency. An MRI of the pituitary and hypothalamus showed no abnormalities. Recombinant human GH (rhGH) was started at 15.2 years at a dose of 35 mcg/kg.d, but did not significantly improve serum IGF-1 level (34–64 ng/mL) and growth was only transiently accelerated at the end

of the first year of treatment (annualized growth velocity of 5.6 cm), decreasing to 1.9 cm/year in the second year of therapy, after which treatment was stopped.

Serum gonadotropins and pelvic ultrasound revealed no aetiology for the primary amenorrhoea. A chest radiograph (Fig. 2C) and thorax CT (Fig. 2D), showed normal findings and ruled out any subclinical changes in the lungs. Her cardiovascular, respiratory and abdominal examinations were normal.

Examination of the skin revealed generalized ichthyosis and lichenification on her forehead, trunk, forearms (Fig. 2E), and erythema and papules on her hands. An incisional biopsy of skin lesions revealed a diagnosis of chronic dermatitis with irregular epidermal acanthosis and parakeratosis as well as a discrete spongiosis (Fig. 2F). In the papillary dermis, lymphocytic and plasmacytic infiltrates were present with no deposits of IgG, IgA, IgM and complement or fibrinogen on direct immunofluorescence analysis. The eczema in the father and aunts was milder than that of the index patient.

3. Material and methods

3.1. Consent and regulatory compliance

For sample procurement, informed consent from patient and family members were obtained in compliance with the Declaration of Helsinki and approved by the Ethical Review Committee of the Leiden University Medical Centre and Institutional Regulatory Board of the Cincinnati Children's Hospital Medical Center. Samples include whole blood and a skin biopsy from a non-lesion area to establish primary fibroblast cultures. Consent from patient was obtained to publish images.

3.2. GH related biochemical parameters in serum

Determination of serum levels of IGF-I, IGF-II, IGFBP-2, IGFBP-3, ALS, and ternary complex formation studies were performed as described previously (Isik et al., 2017).

Details on Sanger Sequencing and Whole Exome Sequencing (WES) Analysis, Antibodies, Generation of Recombinant FLAG-tagged STAT5B Variants, Cell Culture, Western Immunoblotting and Immunocytochemistry are presented in the Supplemental Data.

4. Results

4.1. Identification of the homozygous STAT5B c.1453delG, p.(Asp485Thrfs*29) variant

The severe postnatal growth failure of the index patient with low SDS values of serum IGF-I, IGFBP-3 and ALS, and significantly diminished 150 kD ternary complex formation (Supplementary Fig. 1), suggested a molecular defect along the GH-IGF-I growth axis. The detection of persistently elevated prolactin concentrations and chronic eczema, further suggested *STAT5B* as a potential candidate gene despite lack of the severe symptomatic immune or pulmonary issues normally associated with classical STAT5B deficiency (Hwa, 2021). Sanger sequencing analysis of patient's *STAT5B* gene revealed a homozygous single base deletion in exon 12 of the *STAT5B* gene (*c.1453delG*). The parents, two paternal aunts and the patient's brother were heterozygous for the same variant (Fig. 1 and Supplementary Fig. 2). The single nucleotide deletion leads to a frameshift and premature protein termination [p.(Asp485Thrfs*29)]. The variant, as expected, was predicted as deleterious according to *in silico* analysis (PROVEAN) (http://provean.jcvi.org/index.php). WES analysis performed for homozygous rare variants that could explain the apparent GH deficiency (*HESX1, OTX2, LHX3, LHX4, SOX3, FGF8, FGFR1, GLI2, PROP1, POU1F1, IGSF1, GHRH, GHRHR, BTK, GH1, RIEG, GLI3, RNPC3*) was unrevealing.

4.2. Mildly dysregulated immune profile associated with homozygous STAT5B c.1453delG, p.(Asp485Thrfs*29)

The immunoglobulin profile of the index patient was consistent with hypergammaglobulinaemia concordant with increased $CD19^+$ B-lymphocytes numbers (Table 2 (Ikinciogullari et al., 2004),), as previously reported for other STAT5B deficient patients (Hwa, 2021). Interestingly, the IgE concentration which can be elevated in STAT5B deficiency (autosomal recessive as well as dominant-negative inheritance), was well within the normal range. T-lymphocytes were relatively normal, based on absolute lymphocyte counts although the percentage of $CD3^+$ and $CD4^+$ T-lymphocytes were modestly below normal (Table 2) and contrary to described T-lymphopenia associated with autosomal recessive STAT5B deficiency (Hwa, 2021; Cohen et al., 2006; Jenks et al., 2013). The subpopulation of T lymphocytes, $CD4^+CD25^+$ $FOXP3^+$, which are typically below normal in STAT5B deficiency (Hwa, 2021; Cohen et al., 2006; Jenks et al., 2013), was within normal ranges in our patient (Table 2). Similarly to reported STAT5B deficient cases, *in vitro* T lymphocyte proliferative blastogenesis was normal in response to stimulation with CD3 (carbofluorescein diacetate succinimydil ester, CFSE, flow cytometry; data not shown). The NK cell population was also within normal ranges (Ikinciogullari et al., 2004). Collectively, the immune profile of our patient is considerably less perturbed than might be predicted for a homozygous truncated loss-of-function *STAT5B* variant.

4.3. Stable, reduced expression of FLAG-STAT5B p.(Asp485Thrfs*29) in reconstitution system

The identified p.(Asp485Thrfs*29) variant is located within the DBD (DNA binding domain) region of STAT5B, schematically depicted in Fig. 3A. To assess whether the truncated STAT5B-p.(Asp485Thrfs*29) was stably expressed, N-terminally FLAG-tagged STAT5B-p.(Asp485Thrfs*29) (hereafter, F-D485Tfs*29) was compared to wild-type F-STAT5B, the F-Q368Pfs*9 variant, and recently described F–W631* variant (Foley et al., 2021), in our established HEK293 (hGHR) reconstitution system. To perform comparative expression and GH-induced STAT5B phosphorylation analyses, immunologically-

Table 2
Immunological results of the index patient and the parents compared to reference data[a].

Parameter	Index patient	Father	Mother
Immunoglobulins		N/A	N/A
IgG (mg/dL) (N: 751–1560)	1550		
IgG1 (mg/L) (N: 1940–8420)	**12,500**		
IgG2 (mg/L) (N: 640–4950)	1800		
IgG3 (mg/L) (N: 230–1960)	1940		
IgG4 (mg/L) (N: 230–1960)	< **78**		
IgA (mg/dL) (N: 85–453)	240		
IgE (mg/dL) (N: 0–165)	18		
IgM(mg/dL) (N: 46–304)	97.2		
[a]**T-lymphocytes**			
$CD3^+$ (N: 700–1900/mm^3) (N: 58–82%)	1120/mm^3 **(46.1%)**	1355/mm^3 (65.9%)	1983/mm^3 (69.2%)
$CD4^+$ (N: 400–1300/mm^3) (N: 26–48%)	600/mm^3 **(24.5%)**	986/mm^3 (48.1%)	1014/mm^3 (35.4%)
$CD4^+CD25^{high}FOXP3^+$ (N: 2–5%)	27/mm^3 (4.5%)	10/mm^3 (2.2%)	11/mm^3 (1.9%)
$CD8^+$ (N: 200–700/mm^3) (N: 16–32%)	520/mm^3 (21.2%)	369/mm^3 (17.7%)	969/mm^3 (26.5%)
[a]**B lymphocytes**			
CD 19 (N: 100–400/mm^3) (N: 10–30%)	**1040/mm^3 (42.6%)**	442/mm^3 (21.5%)	378/mm^3 (13.2%)
[a]**NK-lymphocytes**			
CD16 + 56+ (N: 100–400/mm^3) (8–30%)	210/mm^3 (8.6%)	222/mm^3 (10.8%)	432/mm^3 (15.1%)
***In vitro* T cell proliferative response**	Normal	Normal	Normal

[a] Reference ranges are derived from Ikinciogullari et al., 2004

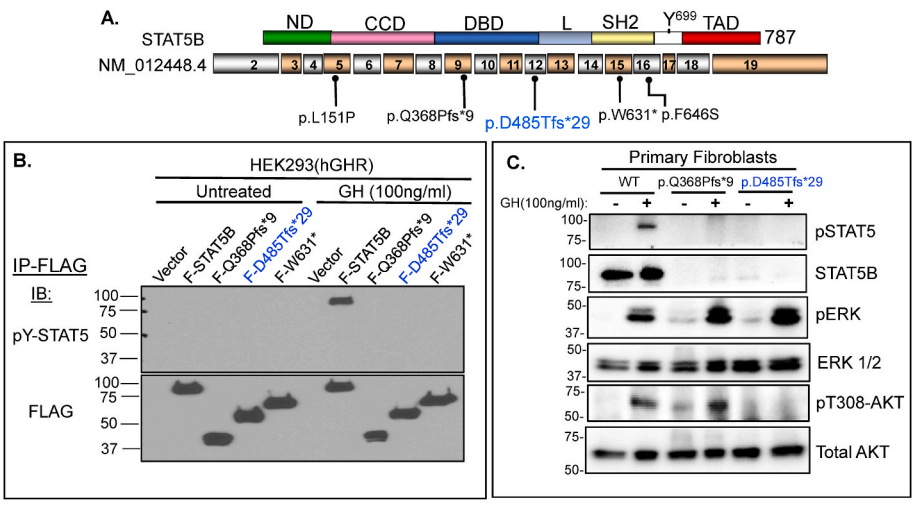

Fig. 3. *In vitro* expression analysis of STAT5B p.(Asp485Thrfs*29) variant. **A.** Schematic of STAT5B mRNA (transcript NM_012448.4; exons 2–19, indicated) and protein (787 total amino acids), with location of p.(Asp485Thrfs*29) (this report, blue) relative to other homozygous STAT5B variants not yet known to be associated with pulmonary diseases. ND, N-terminal domain; CCD, coiled-coiled domain; DBD, DNA binding domain; L, Linker; SH2, src-homology 2; TAD, transcriptional activation domain; Y699, tyrosine at position 699 that is phosphorylated when STAT5B is activated. **B.** Reconstitution immunoblot assays with N-terminally FLAG-tagged STAT5B variants overexpressed in HEK (hGHR), GH-treated or untreated. From collected total cell lysates, immunologically equivalent F-STAT5B variants (F-STAT5B, 0.1 mg; F-Q368Pfs*9, 0.1 mg; F-D485Tfs*29, 1.0 mg; F–W631*, 0.5 mg) were immunoprecipitated prior to immunoblot (IB) analyses. **C.** Established primary dermal fibroblasts carrying homozygous wild-type (WT), p.(Gln368Profs*9), or p.(Asp485Thrfs*29), were treated with GH, 100 ng/mL, 20 min, and IB analysed (500 μg per lane).

equivalent FLAG-tagged variants were immunoprecipitated (IP) from transfected cell lysates (Fig. 3B). The results revealed that although all 3 truncated variants were stably expressed, expression was variable, as detection of immunologically-equivalent F-D485Tfs*29 protein required IP from 10-fold higher quantity of cell lysates (i.e. 1 mg) than that for detection of wild-type F-STAT5B (0.1 mg cell lysates). Thus we conclude that the expression of F-D485Tfs*29 is at least 10-fold lower than wild-type F-STAT5B while expression of F-Q368Pfs*9 was surprisingly comparable to that of F-STAT5B (Fig. 3B). The intermediate expression of F–W631* was as previously reported (Foley et al., 2021). None of the truncated variants were phosphorylated upon rhGH treatment.

To distinguish STAT5B from the highly homologous STAT5A, commercially available anti-STAT5B antibodies have been generated against a unique region in the C-terminus of the STAT5B peptide. These specific antibodies are, therefore, unable to detect native, truncated, STAT5B p.(Asp485Thrfs*29) and STAT5B p.(Gln368Profs*9) proteins in primary dermal fibroblasts established from index-cases (Fig. 3C). Furthermore, immunoblot analysis did not reveal GH-induced phosphorylated STAT5 in the STAT5B deficient fibroblasts, although the ERK1/2 pathway was robustly activated consistent with our previously reported STAT5B deficient fibroblasts (Kofoed et al., 2003), while the AKT pathway was detectably activated only in fibroblasts carrying STAT5B p.(Gln368Profs*9). Thus, despite variable expression in reconstitution systems, GH was not capable of activating the truncated STAT5B variants.

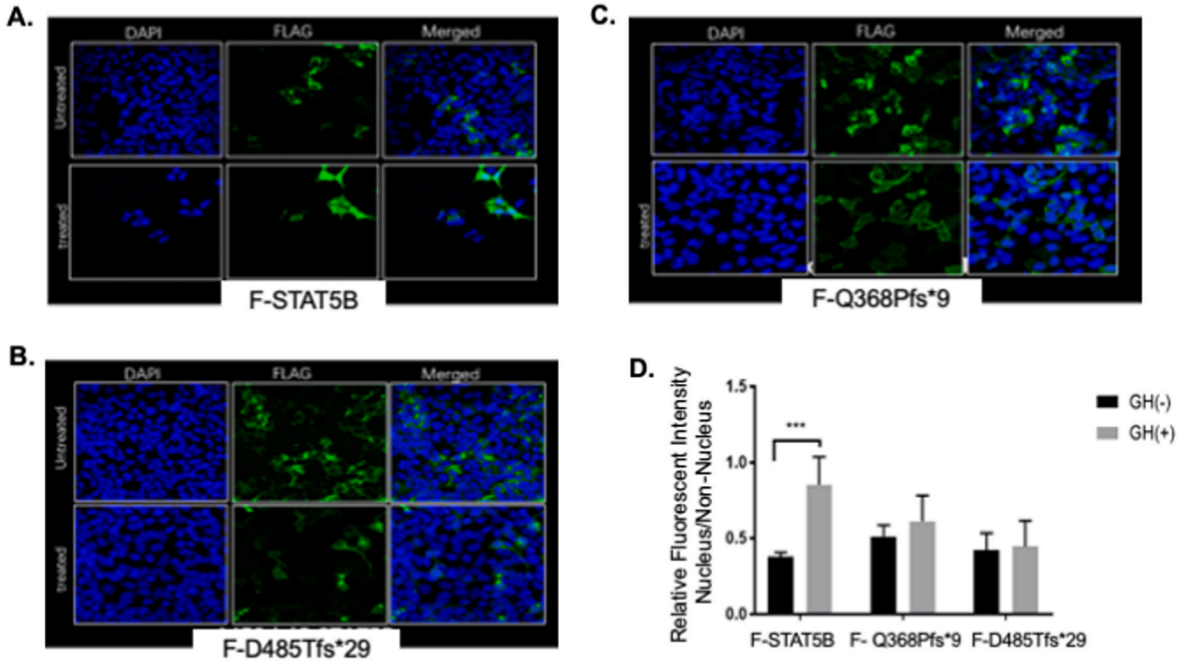

Fig. 4. Cellular localization of GH-stimulated FLAG-tagged STAT5B variants. Tracking of F-STAT5B variants by immunofluorescent. **A.** F-STAT5B; **B.** F-D485Tfs*29; **C.** F-Q368Pfs*9; **D.** Nuclear localization quantitated using NIS-element program (see Materials and Methods).

4.4. FLAG-STAT5B p.(Asp485Thrfs*29) and p.(Gln368Profs*9) cannot translocate to the nucleus

An intact coiled-coil domain (CCD) is important for nuclear localization of STAT5B (Klammt et al., 2018; Shin and Reich, 2013). An isolated N-terminal domain (ND)-CCD segment seems to be sufficient for constitutive nuclear localization of the closely related STAT5A (Iyer and Reich, 2008). To assess whether the truncated F-D485Tfs*29 and F-Q368Pfs*9, both of which carry intact ND and CCD domains, retained ability to translocate to the nucleus, we performed immunofluorescence assays of transfected HEK293(hGHR) cells, under rhGH treated or untreated conditions. Wild-type F-STAT5B, normally residing in the cytoplasm when unstimulated (untreated), translocated to the nucleus within 20 min of GH exposure (Fig. 4A). In contrast, both F-D485Tfs*29 (Fig. 4B) and F-Q368Pfs*9 (Fig. 4C) remained cytoplasmic after GH treatment. Quantitated (Imaris and NIS elements software), the average percentages of GH-induced nuclear translocation revealed a significant 2-fold increase of nuclear F-STAT5B after 20 min GH treatment, but no significant changes for either F-D485Tfs*29 or F-Q368Pfs*9 (Fig. 4D). Thus, for the F-D485Tfs*29 and F-Q368Pfs*9 variants, the presence of an intact ND and CCD was insufficient for passive or GH stimulated nuclear translocation.

5. Discussion

We report an atypical STAT5B deficiency phenotype associated with a novel autosomal recessive *STAT5B* truncating variant. The homozygous frame-shift *c.1453delG* variant resulted in reduced expression of a truncated STAT5B variant of ~50 KDa [p.(Asp485Thrfs*29)] that had lost the critical Y699 phosphorylation site and remained predominantly cytoplasmic, when assessed by reconstitution studies. Surprisingly, this loss of a full-length, functional, STAT5B did not confer the expected "classical" STAT5B deficiency phenotype of GHI with immune deficiency and pulmonary complications. Our young female subject was clearly GHI (severe post-natal growth impairment, delayed puberty and bone age, IGF-I, IGFBP-3 and IGFALS deficiencies, limited response to rhGH therapy) and in combination with the elevated serum prolactin this was consistent with autosomal recessive STAT5B deficiency reported to date. However, the patient's immune profile indicated that she lacked the T-lymphopenia and reduced Treg cells, which typically accompany STAT5B deficiency and, importantly, she remained free from overt signs of pulmonary issues. Altogether, the phenotype of our patient emphasized the critical importance of STAT5B for GH-stimulated growth and shows that key functions of STAT5B associated with growth can be delineated from immune and pulmonary functions.

Interestingly, serum IGF-II (not evaluated for the majority of STAT5B deficient patients), was extremely low, but in the same range as observed in patients with ALS-deficiency (Isik et al., 2017). Circulating IGF-II, like IGF-I, is produced predominantly by the liver and circulates in ternary complex with IGFBP-3 and ALS, but unlike IGF-I, is not regulated by GH. The results, therefore, suggests that the serum IGF-II concentration is largely determined by the actual degree of stabilising ternary complex formation (i.e. IGFBP-3 and ALS availability).

It was of note that serum GH concentrations in our patient were within normal range, but she failed two GH-stimulation tests. This is in contrast with most cases of STAT5B deficiency (Hwa et al., 2011). For our patient, genetic (WES) and physiological (MRI) assessments for potential GH deficiency, however, were unrevealing. Of note, a recent zebrafish model suggested existence of a positive feedback loop between Stat5.1, the STAT5B ortholog, and Gh mRNA expression, which was necessary for somatic growth of the zebrafish (Xiong et al., 2017). Whether a similar mechanism could explain, in part, the failed GH-stimulation tests and poor growth in our patient is unclear. Interestingly, response to rhGH therapy initially improved growth velocity to 5.6 cm/yr (with a modest rise of serum IGF-I, without normalization) but decreased to 1.9 cm/yr in the second year. A similar transient response was also reported in the STAT5B deficient patient carrying [p.(Gln368Profs*9)], whose modest growth response was attributed to coincident pubertal growth (Vidarsdottir et al., 2006; Walenkamp et al., 2007). Effectiveness of rhIGF-I therapy remains to be determined.

In addition to severe short stature, another striking common feature in all STAT5B deficient patients, including the present case, is eczema. For our patient, chronic dermatitis was definitely diagnosed by an incisional biopsy of skin lesions, showing lymphocytic and plasmacytic infiltrates, with irregular epidermal acanthosis and parakeratosis, and discrete spongiosis (Fig. 2E). This detailed pathological observation, a first report for STAT5B deficiency, is compatible with typical eczema. The role of STAT5B in skin immune-related issues is unknown, but there is evidence that aberrant thymic stromal lymphopoietin (TSLP) signalling, which includes the JAK/STAT5 pathway (Zhong et al., 2014), is associated with asthma and atopic dermatitis, amongst other immune diseases. TSLP signalling pathways are found on multiple cell types, including epithelial cells and immune cells, and most recently described in skin mast cells relevant to pruritis and atopic skin pathologies (Babina et al., 2021), although mechanistic roles of STAT5B have yet to be fully determined.

To date, of the 15 reported cases, summarized in (Hwa, 2021; Foley et al., 2021), the patient carrying STAT5B p.(Gln368Profs*9) is the only STAT5B deficient patient known to survive beyond 30 yrs of age. The first reported STAT5B deficient patient [p.(Ala630Pro) (Kofoed et al., 2003)] succumbed to progressive pulmonary fibrosis and respiratory failure before the age of 30 (Bezrodnik et al., 2015); four other patients who had progressive pulmonary dysfunctions evident as early as the first year of life, have also succumbed before age 30 yrs (unpublished; personal communication to VH). Intriguingly, a recent report of previously identified STAT5B deficient siblings [carrying a homozygous frameshift *c.1680delG,* p.(Glu561Argfs*17) variant (Hwa et al., 2007)], indicated that by age 6, the siblings had evident lung disease which developed in severity by the mid-teens, and comprised of PAP (pulmonary alveolar proteinosis), a syndrome of surfactant accumulation due to dysregulated GM-CSF signalling in alveolar macrophages, and includes features of lymphocytosis, bronchiectasis and fibrosis (Krone et al., 2022). Continued PAP therapeutic intervention has alleviated respiratory issues for the older sibling, while an initial successful allogeneic stem cell transplantation for the younger sibling did not prevent the patient from ultimately succumbing to sepsis 11 months post-transplant (Krone et al., 2022). More recently, a homozygous STAT5B p.(Gln41*) was clinically identified in a 20 month old patient who subsequently succumbed to fatal inflammatory lung disease despite undergoing HSCT (Pelham et al., 2022). These unfortunate outcomes suggested that severe, advanced, immune dysregulation in STAT5B deficiency, which include recent evidence of impaired NK cell maturation and functions (Vargas-Hernandez et al., 2020; Caldirola et al., 2018) and impaired humoral immune homeostasis (Pelham et al., 2022), may be difficult to entirely mitigate. It is, therefore, critically important to better understand how some STAT5B deficient patients, like our patient, organically circumvented these devastating co-morbidities.

Our patient (currently over 20 yrs of age) has yet to manifest severe immune or pulmonary issues. This atypical STAT5B deficiency phenotype is most similar to the reported surviving 31 yr old STAT5B deficient patient (who is now in his 40's). The other patients who did not exhibit pulmonary issues at the time of first report, were younger, but suffer from significant immune issues such as autoimmunity (Table 3). For the patient with the p.(Phe646Ser) variant, at 27 years of age, on-going coeliac disease and immune dysregulation was noted (Caldirola et al., 2018). Whether the expressed, cytosolic, recombinant STAT5B p.(Asp485Thrfs*29) and p.(Gln368Profs*9) proteins detected in reconstitution studies exhibit bio-physiological properties that contribute to avoidance of the potential devastating immune and pulmonary effects of STAT5B deficiency, remains to be clarified. It is notable that, in contrast, the frame-shift p.(Glu561Argfs*17) variant associated with devastating pulmonary issues (Krone et al., 2022), was not detectably expressed in

Table 3
Comparative characteristics of STAT5B deficient patients with mild immune dysregulation and lacking severe pulmonary diseases. Phenotypic features indicated are at time of report.

Homozygous *STAT5B* variant	*c.1453delG*	*c.1102insC*	*c.452T > C*	*c.1892G > A*	*c.1937T > C*
Protein nomenclature:	p.Asp485Thrfs*29	p.Gln368Profs*9	p.Leu151Pro	p.Trp631*	p.Phe646Ser
Male/Female	F	M	M	F[a]	F[b]
Age, yr	17	31	8	4.9	14.8
Height SDS	−5.1	−5.9	−4.7	−6.6	−6.0
Birth size	AGA	AGA	AGA	AGA	NA
Growth Hormone Insensitivity	+++	+++	+++	+++	+++
IGF-I Deficiency	+++	+++	+++	ND	+++
Prolactin, elevated	+++	+++	Normal	ND	+++
Hypergammaglobulinemia	+++	No	No	No	+++
IgE, elevated	No	ND	+++	+++	No
T-cell lymphopenia	No	No	No	No	+++
Treg, reduced	No	No	Yes	No	No
Eczema/ichthyosis	Yes	Yes	Yes	Yes	Yes
Other Immune Issues	Anaemia	No	Autoimmunity	Autoimmunity	Autoimmunity
Severe Pulmonary Disease	No	No	No	No	No
Reference	Index Patient	Vidarsdottir S et al., 2006; Walenkamp MJE et al., 2007.	Acres MJ et al., 2019.	Foley CL et al., 2021	Scaglia PA et al., 2012; Bezrodnik L et al., 2015

+++, strong effect.
AGA, appropriate for gestational age; ND, not determined.
[a] The oldest of 3 siblings carrying homozygous *STAT5B* mutation. The two younger siblings presented similar phenotypic profiles.
[b] The patient, at age 27 yrs, is reported to have reduced Treg (Caldirola et al., 2018) and was diagnosed with coeliac disease at age 20 yrs. Pulmonary conditions were not discussed.

similar reconstitution assays (Hwa et al., 2007).

Three other homozygous variants, two missense (Scaglia et al., 2012; Acres et al., 2019) and one nonsense (Foley et al., 2021), are associated with patients under 25 years of age who lack severe pulmonary issues at the time of report. Since these pathological variants are located in the CCD and SH2 domains, while the p.(Asp485Thrfs*29) and p.(Gln368Profs*9) variants are both located in the DBD, there is no obvious correlation between genotype and a STAT5B deficient phenotype without severe immune and pulmonary dysfunction. The potential development of more fatal co-morbidity is still of concern in these young patients, however, as a recent update of the patient carrying one of these missense variants, p.(Leu151Pro) (Acres et al., 2019), indicated deteriorating lung functions for which a hematopoietic stem cell transplant (HSCT) is planned (Pelham et al., 2022).

In conclusion, identification of a young STAT5B deficient patient carrying a novel homozygous frameshift *STAT5B* variant who had the expected GHI phenotype but only mild immune complications and a surprising lack of pulmonary issues, expands the spectrum of STAT5B deficiency. This report, together with the STAT5B deficient patient carrying p.(Gln368Profs*9), highlights the separation of growth functions of STAT5B from immune and pulmonary functions and emphasizes the critical importance of STAT5B for GH-mediated growth. For our patient, and all young STAT5B deficient patients, continued monitoring is clearly essential. Finally, with clinical WES now more prevalent as part of diagnosis and clinical care, it is expected that new cases of STAT5B deficiency will be more rapidly identified and at earlier ages, as was reported for STAT5B p.(Trp631*) (Foley et al., 2021) and for STAT5B p.(Gln41*) (Pelham et al., 2022). Future studies will delineate how the STAT5B p.(Asp485Thrfs*29) and p.(Gln368Profs*9) variants from unrelated patients may prevent severe immune and lung complications.

Funding

This work was supported by the National Institute of Child health and Human Development, R21 HD098417 (to VH).

CRediT authorship contribution statement

Gonul Catli: Formal analysis, Data curation. **Wen Gao:** Formal analysis. **Corinne Foley:** Formal analysis. **Berk Özyilmaz:** Formal analysis. **Neslihan Edeer:** Formal analysis. **Gulden Diniz:** Formal analysis. **Monique Losekoot:** Formal analysis. **Andrew Dauber:** Formal analysis. **Bumin N.Dundar:** Formal analysis. **Jan M. Wit:** Project administration, Supervision, Formal analysis, Writing – original draft. **Vivian Hwa:** Project administration, Supervision, Formal analysis, Writing – original draft.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the participating patient and family members for their kind cooperation. We would also like to thank Shayne F Andrew for excellent research assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2022.111799.

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