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Human Growth Disorders Associated with Impaired GH action: Defects in STAT5B and JAK2

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Abstract

Growth hormone (GH) promotes postnatal human growth primarily by regulating insulin-like growth factor (IGF)-I production through activation of the GH receptor (GHR)-JAK2-signal transducer and activator of transcription (STAT)-5B signaling pathway. Inactivating *STAT5B* mutations, both autosomal recessive (AR) and dominant-negative (DN), are causal of a spectrum of GH insensitivity (GHI) syndrome, IGF-I deficiency and postnatal growth failure. Only AR *STAT5B* defects, however, confer additional characteristics of immune dysfunction which can manifest as chronic, potentially fatal, pulmonary disease. Somatic activating *STAT5B* and *JAK2* mutations are associated with a plethora of immune abnormalities but appear not to impact human linear growth. In this review, molecular defects associated with STAT5B deficiency is highlighted and insights towards understanding human growth and immunity is emphasized.

Keywords

STAT5B deficiency; IGF-I deficiency; growth hormone insensitivity; JAK2

1. Introduction

The human growth hormone receptor (GHR) relies on the cytosolic JAK2 (Janus kinase 2)-STAT5B (signal transducer and activator of transcription 5B) signaling pathway to transduce signals from the circulating growth hormone (GH), to the *IGF1* (insulin-like growth factor-I) gene important for normal human growth. Multiple other pathways are activated by the GHR-JAK2 system, including STAT1, STAT3, STAT5A, the MAPK (mitogen-activated protein kinase) and the PI3K (phosphoinositide-3 kinase) pathways. GHR belongs to the Type 1 cytokine receptor superfamily which includes the prolactin receptor, the erythropoietin receptor, leptin receptor and a number of interleukin receptors. Type 1 cytokine receptors lack intrinsic kinase activities and require the recruitment of one or a combination of the 4-membered JAK family of kinases for initiating signal transduction. For the homo-dimeric GHR, conformational changes are induced upon binding of GH, leading

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to the trans-activation of the two associated JAK2, one per GHR monomer (1,2). The activated JAK2 phosphorylates the 7 tyrosines located within the intracellular human GHR (3), permitting cytosolic components such as STAT5B, to dock to GHR.

Congenital mutations in many signaling components downstream of the GHR-JAK2 system have been identified, expedited, in part, by increased accessibility of next-generation sequencing in clinical settings. Clinical phenotype associated with inactivating *STAT5B* mutations (MIM245590) support STAT5B signaling as a key GHR signaling pathway for normal human growth. Patients with molecular STAT5B defects, similar to GHR deficient patients, present with growth hormone insensitivity (GHI) syndrome (MIM262500), severe insulin-like growth factor (IGF)-1 deficiency (IGFD), and profound postnatal growth failure (4). An additional co-morbidity of STAT5B deficiency absent in patients with *GHR* defects, is immune dysfunction which can potentially be fatal (4). In this review, the spectrum of STAT5B deficiency and insights gained will be discussed. *JAK2* defects, which, to date, have been predominantly somatic and associated with immune disorders, will be briefly summarized.

2. *JAK2* gain-of-function (GOF) defects are not associated with growth failure

The human *JAK2* gene, located on chromosome 9p24.1, encodes for a large multi-domain protein of 1132 amino acid residues. JAK2 carries a N-terminal FERM (4.1, ezrin, radixin, moesin) domain which binds to GHR and other receptors, a SH2 domain that binds STATs, a pseudokinase inhibitory domain and the C-terminal tyrosine kinase domain (Figure 1). Through its FERM domain, JAK2 interacts with the intracellular GHR Box 1 region (Leu298 - Pro304). Insights into how JAK2 is activated by GHR was elegantly presented in studies from Brooks et al (2), who provided evidence supporting pre-formed human homo-dimers of GHR. Brooks and colleagues proposed that, in the absence of the GH ligand, the parallel configuration of the dimeric intracellular GHR domains are “locked” together at the Box 1 regions via the two associated inactivated JAK2. The two JAK2 trans-inhibit each other through apposition of pseudokinase inhibitory domain of one JAK2 with the kinase domain of the other (2). The binding of GH induces conformational changes that force the two JAK2 apart and, with the removal of the trans-inhibition, the juxtaposition of the kinase domains triggers JAK2 auto trans-phosphorylation and subsequent downstream signaling (2). Of note, activated JAK2, depending on the receptor system and ligand, is known to phosphorylate all members of STAT family except for STAT6 (5). In contrast to the human GHR-JAK2 interaction, a recent study suggest, interestingly, that rabbit GHR undergo ligand induced dimerization and associated JAK2 dimerizes through its pseudokinase domain (6).

To date, somatic and germline dominantly inherited gain-of-function (GOF) JAK2 mutations are well established causes of a number of hematologic disorders (myeloproliferative neoplasma, thrombocythemia-3, thrombocytopenia, polycythemia vera). Recurrent somatic heterozygous mutations include Val617Phe (V617F) located in the pseudokinase domain (exon 14) which was first described in 2005 (7–10), mutations in exon 12 (11), and, more recently, a 4 amino-acid-deletion and variable 1-amino-acid insertion in exon 13 (12) (Figure

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1). The V617F is the most prevalent of the JAK2 defects and has also been reported in pediatric cases of essential thrombocythemia (13–15). Described germline inherited GOF variants, similarly located in the pseudokinase domain, include V617I mutation, associated with hereditary thrombocytosis (16), H608N with essential thrombocythemia (17), G571S with clonal hematopoiesis (18) and compound heterozygous E846D and R1063H with hereditary erythrocytosis (19). Stature was not reported in affected patients.

3. STAT5B gene and protein

The human GHR-JAK2 system activates STAT1, STAT3, STAT5A, and STAT5B of the 7 membered family of mammalian STAT proteins, a well-established family of cytosolic proteins that mediates biological actions of multiple growth factors and cytokines in many cell types (20). Availability of next-generation genomic sequencing in clinical settings has led to identification of pathological molecular defects, germline and somatic, in all but the *STAT5A* genes (21–25). All STAT defects are associated with distinct immuno-deficiencies but only STAT5B deficiency consistently confer GHI, IGF-I deficiency and postnatal growth failure (4).

The human *STAT5B* gene, on chromosome 17q21.2, is within a ~204 kb DNA region that includes the *STAT5A* and *STAT3* genes. The *STAT5B* and *STAT5A* genes (77.23 kb and 24.4 kb, respectively) are only ~11 kb apart (26), suggesting evolutionary duplication of an ancestral gene. The STAT5B protein, 787 amino acid residues, is encoded by 19 exons, while the STAT5A is slightly larger (794 amino acids). The two proteins share a remarkable ~96% amino acid identity, are often considered interchangeable and collectively referred as STAT5. The identification of pathological inactivating *STAT5B* mutations clearly indicate STAT5B and STAT5A have non-redundant as well as redundant roles. STAT5A, furthermore, is unable to compensate for loss of STAT5B despite their high degree of identity. Human mutations in *STAT5A*, as noted above, have yet to be identified.

Human STAT5B, typical of members of the STAT family (20), carries discrete protein modules (Figure 2A). The N-terminal domain (ND) and coiled-coiled domain (CCD) mediate protein-protein interactions, with the 4 alpha-helixes of CCD also serving as an unconventional nuclear localization signal (27). The common modular src-homology 2 (SH2) domain is necessary for binding phosphorylated tyrosines (Y), thus permitting STAT5B to dock on phospho-Y of activated receptors and to homo-dimerize when STAT5B itself is phosphorylated at Tyr699 (Y699) by JAK2 (Figure 2B). In the nucleus, the DNA binding domain (DBD) is essential for binding DNA elements and the transcriptional activation domain (TAD) drives transcriptional activities. In addition to Y699, the phosphorylation of S128 and S193 and acetylation of L701, have been reported as other mechanisms for regulating STAT5B activities (28–31). Pathophysiological mutations have been described in all domains except ND (Figure 3).

4. *STAT5B* molecular defects

The binding of GH to cell surface homo-dimeric human GHR activates the associated JAK2 (1,2), which initiate signaling cascades by phosphorylating the 7 tyrosines within the GHR

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intracellular domain. The docking of STAT5B to any one of three specific phosphorylated GHR tyrosines (Y534, Y566, Y627) suggest a redundancy in GHR tyrosine selection by STAT5B (32). This redundancy may explain why damaging *GHR* mutations in the intracellular domain, including dominant-negative *GHR* mutations (33–38), frequently involve frameshifts that abrogate the JAK2 binding site and/or the three critical GHR tyrosines (39–42). The sequence of events, upon STAT5B docking to GHR, involves STAT5B Y699 phosphorylation by JAK2, homo-dimerization of phosphorylated STAT5B which then mobilize to the nucleus to drive transcriptional activities (Figure 2B). Mutations in STAT5B disrupting this sequence of events are described below.

(a) Homozygous Loss-of-Function (LOF) STAT5B defects

All loss of function (LOF) mutations in STAT5B described to date are germline. The identification of these congenital inactivating *STAT5B* mutations associated with GHI, IGF-I deficiency and severe growth failure, provided definitive evidence that STAT5B signaling is key for normal GH-GHR-JAK2 mediated postnatal growth (Table 1; (43,44)). The first homozygous inactivating *STAT5B* mutation was a missense mutation, p.Ala630Pro (p.A630P), reported in 2003. The 16 year old female subject, from a consanguineous pedigree, had normal GHR but had clinical features that were reminiscent of patients with GHR defects (45). Additional features of chronic pulmonary disease were reported (45). The autosomal recessive (AR) p.A630P mutation, located in the SH2 domain of STAT5B, disrupted the core anti-parallel ß-sheets that forms the pocket for binding phosphate groups on activated receptors and other proteins (46–48). The amino acid substitution caused thermodynamic instability of both the SH2 domain and the entire protein (49,50). Mutant STAT5B p.A630P was thus very poorly detected and could not be activated in either primary cells from the patient (45,51) or in reconstituted systems (52). Conclusions drawn from these cell-based analyses were consistent with *in vivo* clinical IGF deficiency and GHI presentations, supporting the hypothesis that the presence of functional STAT5A (52) could not compensate for loss of functional STAT5B.

Seven other homozygous inactivating *STAT5B* mutations have since been reported, involving 4 male and 6 female GHI patients and includes 2 sets of siblings (53–60). These rare AR STAT5B mutations suggest that STAT5B haploinsufficiency minimally perturbed IGF-I expression, growth, and immune functions (43). The molecular defects, located in different domains of the STAT5B protein (Table 1; Figure 3). The one nonsense (57) and 4 frameshift (53–56) mutations are predicted to be pathogenic as a result of early protein termination. In addition to p.A630P, two other missense mutations, p.F646S in the SH2 domain (45,59) and p.L151P in CCD (60), were functionally compromised, consistent with clinical phenotype. All *STAT5B* LOF mutations were ultimately unable to drive transcription. It remains to be determined if predicted truncated peptides are expressed *in vivo*.

In humans, the inability of STAT5A to compensate for loss of STAT5B is of note and is in contrast to mouse germline *Stat5b*$^{-/-}$ knock-out models (61,62). Sexual dimorphic growth patterns were observed in *Stat5b*$^{-/-}$ knock-out mice, with both male *Stat5b*$^{-/-}$ and female *Stat5b*$^{-/-}$ mice comparable to size of wild-type *Stat5b*$^{+/+}$ females, all of whom were smaller

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than wild-type *Stat5b*$^{+/+}$ males. Growth of *Stat5a*$^{-/-}$ mice were consistently normal (62,63). Only *Stat5a*$^{-/-}$ and *Stat5b*$^{-/-}$ double knock-out mouse models (62) conferred the severity of growth failure, IGF-I deficiency, and immune dysregulation observed in the human STAT5B deficiency state. Interestingly, a recent study provided evidence that, in mice, *Stat5b* was the dominant paralog over *Stat5a*, and, further, that this was likely a consequence of higher expression of Stat5b compared to Stat5a rather than functional differences (64), a hypothesis first proposed over 20 years ago (62). Whether similar asymmetric expressions could account for the severe co-morbidities in clinical STAT5B deficiency remain unresolved. Clearly, the loss of functional STAT5B has significant pathophysiological consequences for humans not observed in mouse *Stat5b*$^{-/-}$ models.

(b) Dominant-negative LOF STAT5B defects

STAT5B deficiency is established as an autosomal recessive disorder. The first germline dominant-negative inactivating STAT5B mutations were recently reported in 3 unrelated patients who exhibited GHI, IGF-I deficiency and post-natal growth deficits less severe than those with “classical” STAT5B deficiency (65). Whole exome sequencing analysis confirmed that, in each case, the heterozygous *STAT5B* variant was the top candidate. Each of the LOF mutation was a missense variant, with the two familial mutations, p.Q474R (p.Gln474Arg, exon 12) and p.A478V (p.Ala478Val) located in the DBD, and the one *de novo* mutation, p.Q177P (p.Gln177Pro, exon 5) located in the CCD (Table 1, Figure 3).

In vitro functional studies demonstrated that all three expressed mutants were robustly tyrosine phosphorylated upon GH-stimulation. However, the two DBD mutations were unable to bind DNA, while the p.Q177P was impaired in nuclear localization, the first reported STAT variant with a nuclear localization defect (65). The Q177P substitution in the first alpha-helix of the CCD, surprisingly, did not disrupt protein structure although prolines are known to be incompatible with alpha-helix structures. Instead, the functional integrity of the 4-helix bundle of CCD was disrupted (27). The p.Q474R and p.A478V mutations, both in highly conserved amino acid residues, are in positions analogous to dominant-negative STAT3 DBD missense mutations (66–68) associated with hyper-IgE syndrome (MIM 147060) (68,69). Importantly, each of the STAT5B mutant retained the capability to interact with wild-type STAT5B and inhibit normal dimeric STAT5B functions (65).

One dominant-negative STAT5B mutation, p.Gln206Arg (p.Q206R, exon 5, alpha-helix 2, CCD) was recently reported in a 33 yr old male patient with severe autoimmune lymphoproliferative syndrome-like features from childhood (70) but whose height was not reported. The mutation was inherited from his severely immunocompromised mother. The p.Q206R was shown to translocate to the nucleus upon IL-2 stimulation and exhibited dominant negative effects on IL-2-stimulated T-cell functions. It remains unclear if the severity of immune dysregulation could be due solely to the heterozygous STAT5B p.Q206R.

It is of note that three AR STAT5B mutations (Table 1), similar to p.Q177P, disrupt the first alpha-helix of CCD (exon 5). The frameshift c.424_427del (p.L142Rfs*20) (54) and nonsense p.R152* (57) mutations are clearly pathogenic and likely result in total STAT5B deficiency. The recently described missense p.L151P mutation was found to be normally

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expressed in patient B cells but IL2-induced phosphorylation of peripheral blood mononuclear cells was blunted (60). Whether the STAT5B p.L151P can translocate to the nucleus is unknown nor is potential dominant-negative effects known, as the growth phenotype of the carrier parents and the one sibling who was wild-type for STAT5B, were not reported. Nevertheless, it is striking that the first alpha-helix of the CCD, encoded by exon 5, appears to be vulnerable to molecular disruptions.

The rarity of pathological defects in *STAT5B* has heighten awareness of heterozygous STAT5B variants identified in genomic screens of patients with milder GHI and diagnosed with idiopathic short stature (ISS). Two such variants, however, were proven to be functionally benign (p.V498M, exon 13, Linker, (71); and p.E315A, exon 8, CCD (72)). A third recently reported heterozygous variant, p.K632N (exon 15, SH2) was functionally inert when evaluated in homozygous state but did not display dominant-negative effects, raising questions of the contribution of this heterozygous variant to clinical phenotype (73). As part of a genetic analysis of a cohort of 89 patients with ISS, a heterozygous p.T479A (exon 12, DBD) was reported in one patient with a height SDS of −3.56 but pathogenicity remains unclear (74). In each of these cases, it is possible that digenic or oligogenic effects may explain the short stature phenotype of the patient.

(c) Somatic Gain-of-Function (GOF) STAT5B defects

To date, GOF STAT5B mutations have been somatic and are associated with hematological malignancies, predominantly of T-cell origin, with recurring mutational “hot spots” in the SH2 and TAD modules (47). The p.N642H mutation, first reported in 2015 (75–77), is the most common GOF STAT5B mutation and has now been detected in over 150 patients. Recent crystallography studies of the human STAT5B core regions (CCD-DBD-Linker-SH2, amino acid residues A136 – Q703), comparing wild-type and p.N642H structures, suggested the $\mathrm{STAT5B}^{\mathrm{N642H}}$ can adopt a hyper-activated and hyper-inactivated state which enhanced affinity for self-dimerization and is resistance to dephosphorylation (47,48). Other reported GOF are schematically shown in Figure 3.

5. STAT5B deficiency: spectrum of GHI and severe postnatal short stature

The post-natal growth impairment associated with STAT5B deficiency is remarkably similar to those of patients carrying *GHR* defects (65). Severe height deficits as consequences of AR STAT5B deficiency are comparable to AR GHR deficiency (78) (Figure 4). For the 11 subjects carrying DN STAT5B mutations (65), the mean height SDS strikingly resembled subjects carrying dominant-negative *GHR* mutations (33,35–38,79) and subjects carrying *GHR* pseudo-exon 6 (80–82).

Birth size, where documented (both AR and DN STAT5B), was normal for gestation (Table 1), indicating that STAT5B, similar to GHR, is not essential for *in utero* growth. It was notable, however, that 6 out of 9 subjects carrying homozygous STAT5B, and 1 out 2 carrying DN-STAT5B, were documented to be born before 37 weeks of gestation. Postnatal growth failure, as noted (Table 1), was significant and consistent with IGF deficiency. At first report, height SDS of reported probands ranged from −2.9 SDS to −9.9 (Table 1). Bone age, when measured, was significantly delayed (54,57,65). Puberty was also consistently

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delayed (Table 1), reflective of the low levels of circulating IGF-I. Mild facial dysmorphic features, such as a prominent forehead, depressed nasal bridge and high-pitched voice, were noted for some of the subjects carrying AR STAT5B mutations (45,54,57).

For subjects carrying AR STAT5B mutations, basal GH levels were normal (ranging from 0.1 to 17.6ng/ml), and when stimulated, GH concentrations were often elevated (4.0 – 53.8 ng/ml). Serum IGF-I, IGFBP-3 and ALS concentrations in all cases were abnormally low, and remained low after GH treatment, during IGF-I generation tests (45,55,83) or during GH therapy (54,56). Some of the subjects underwent growth hormone therapy (1yr to 4yr), but growth response was uniformly poor (45,54). Recombinant human IGF-I, a therapeutic option to by-pass blockage in GH signaling, had limited efficacy in one treated AR STAT5B proband (54) and two of the DN STAT5B probands (65). Interestingly, serum prolactin levels, when recorded, were abnormally high in majority of AR STAT5B subjects (Table 1). It is likely that the *STAT5B* mutations disrupted the negative feedback loop for PRL production, although the mechanisms remain to be clarified.

Collectively, clinical observations of AR and DN STAT5B deficient patients fully supported the critical importance of the GHR-STAT5B signaling pathway for post-natal linear growth and IGF-I production in response to GH. The identification of DN STAT5B mutations, moreover, has expanded the spectrum of growth impairments from severe (AR mutations) to mild (DN STAT5B).

6. Impaired Immune Functions associated with STAT5B deficiency

A distinguishing feature in the patients carrying *STAT5B* mutations is symptoms of immune dysfunction with potential fatal pulmonary involvement (Table 1). Shared symptoms amongst the 11 AR STAT5B deficient probands include severe eczema (9 out of 9), auto-immune thyroiditis (7 out of 11), chronic pulmonary disease (8 out of 11) manifesting as early as the first year of life (54,57,58), and confirmed lung fibrosis and/or lymphoid interstitial pneumonia (LIP) (7 out of 9), a condition of unknown etiology that is rare in children and associated with autoimmune disease (84). In the severest cases, corticosteroid and oxygen treatments temporarily stabilize worsening pulmonary functions. Only one patient, a 17.5 year old male, has been reported to undergo a lung transplantation which appeared to have alleviated impaired pulmonary function and the requirement for oxygen (54). Overall, however, long-term prognosis for AR STAT5B deficiency is poor. The first described patient, carrying p.Ala630Pro (45), succumbed and died before the age of 30 years as consequences of progressive pulmonary fibrosis and respiratory failure (85); not officially reported, four other AR STAT5B deficient patients have since succumbed.

In subjects carrying DN STAT5B mutations, eczema was milder and other extrapulmonary disease was absent or transient, except in one proband diagnosed with thyroiditis and celiac disease (65). In general, immune dysregulation was milder, with elevated IgE concentrations detected in 8 of 9 DN STAT5B deficient patients. The immune profiles of the 3 probands were otherwise normal (65). Elevated IgE concentrations was also detected in 5 of 8 evaluated AR STAT5B deficient patients (4,60,85,86).

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STAT5B mutations has been accepted by the International Union of Immmunological Societies as causal of human inborn error of immunity, with the most recent update in 2019 (87). Unlike the endocrine profiles which showed an absolute association between *STAT5B* mutations and IGF deficiency, abnormalities in the immunological profiles, where evaluated, were variable, even between siblings who carried the same homozygous *STAT5B* mutation (54,88).

The immune complications of AR STAT5B deficiency have been attributed, in part, to T-lymphopenia, with both CD4+ and CD8+ T-cells often below normal (51,54,57,59,85). Although multiple cytokines are known to activate STAT5 (89), specific roles of STAT5B and STAT5A are not always delineated. STAT5B defects have highlighted some key pathways in which STAT5B play a more significant role (51). Notably, in STAT5B deficiency, dysregulated IL-2 signaling significantly reduced T-regulatory cells (Treg) (45,51,90), which are a subset of CD4+ cells essential for the propagation and homeostasis of T-cell populations (91). In human Tregs, STAT5B was shown to be important for regulating expression of CD25, the α-subunit of the heterotrimeric IL-2 receptor (IL2R) complex, and for regulating the transcription factor, FOXP3 (51). In AR STAT5B deficient patients, $Treg^+FOXP3^+$ are abnormally low (45,51,90). Specifically, peripheral, naïve Treg subsets ($CD45RA^+$) were noted to be low, while dividing memory Treg subsets ($CD45RO^+$) were abnormally elevated and had reduced suppressive functionality. This skewing of Treg sub-populations likely contributed to the autoimmune phenotypes of STAT5B deficient patients (90). Further, the increased susceptibility to opportunistic infections experienced by STAT5B deficient patients could also be related to decreased CD25 on all T cells, as has been reported in patients with severe CD25 deficiency (92).

Recent evidence indicated the association of two of the AR STAT5B mutations, *c.1680delG* and p.F646S, with impaired maturation and cytolytic actions of NK (natural killer) cells (88,93). NK cells are early effectors against viral infections, pathogens and malignant cells, and dysregulation could, therefore, contribute to the susceptibility of STAT5B patients to infections, including specific viral infections such as *Herpes Zoster* (reported in a number of the patients). The majority of AR STAT5B deficient patients display low-normal NK cell numbers, although functional integrity has yet to be analyzed.

A recent follow-up report of siblings carrying AR STAT5B *c.1680delC* (Table 1), first reported in 2007 (53), demonstrated that initial indications of pulmonary disease in early childhood, has progressed in severity (88). It remains unclear how immune dysfunction led to pulmonary insufficiencies observed in AR STAT5B deficiency. Intriguingly, three of the reported AR STAT5B patients lacked severe immune and/or pulmonary problems (56,59,60). Immune dysfunction were variably detected, with the male patient carrying *STAT5B c.1102insC* (56) presenting $Treg^+FOXP3^+$ that were ~75% of normal healthy control (90), but, otherwise, appeared relatively healthy at 31 yrs when first reported (56). The female patient carrying *STAT5B* p.F646S (59) who had autoimmune thyroiditis, psoriasis, alopecia, and was diagnosed with Celiac disease at age 20 yr, presented with progressive immune dysregulation but no pulmonary disease at the time of report (85). The AR STAT5B p.L151P patient, a 15 year old male, who similarly has severe eczema and

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autoimmune disease, lacked pulmonary issues (60). An explanation for the lack of chronic pulmonary disease in these three patients remains to be elucidated.

Perspective and Future Directions

The continued identification of patients with unequivocal defects in the GH-induced STAT5B signaling has advanced our understanding of the molecular basis of growth failure associated with primary IGF deficiency. In addition to AR inactivating STAT5B mutations, recent reports of DN STAT5B has broadened the spectrum of STAT5B deficiency. Collectively, in humans, the presence of STAT5A cannot compensate for the loss of STAT5B in terms of GH-dependent growth and IGF-I production. The GHI and IGF-I deficiency but mild immune dysregulation in DN STAT5B deficient subjects, furthermore, support the hypothesis that STAT5B-mediated growth functions can be delineated from its immune functions. Mechanism of how STAT5B regulate IGF-I production in humans still need to be fully elucidated. Currently, there is a lack of a unifying mechanism to explain the complex immune-related pathologies observed in STAT5B deficiency, although dysregulated Treg $^{+}$FOXP3^{+} likely contributes to autoimmunity and increased susceptibility to infections. New evidence of dysregulated NK cells (88,93) highlight the importance of investigating the contribution of different immune cell populations to clinical immune pathology associated with STAT5B deficiency. Understanding the mechanisms leading to progressive immune dysfunction and whether this process precede fatal pulmonary insufficiency, is essential to preventing mortality associated with STAT5B deficiency.

Therapeutic options to improve poor linear growth and immunodeficiency for STAT5B deficiency remain extremely limited. For immune dysregulation, a combination of steroids with either cyclosporine A (cell cycle inhibitor), tacrolimus (calcineurin inhibitor) or sirolimus (mTOR inhibitor) may provide temporary sparing from T-cell dysregulation as have been shown for patients with IPEX (Immune Dysregulation, Enteropathy, X-linked) and IPEX-like syndrome (94). A lung transplantation appeared to have, at least temporarily, alleviated problems caused by chronic pulmonary disease (54). Bone marrow transplants is currently being considered for STAT5B deficiency. For linear growth, efficacy of recombinant human IGF-I treatment remains uncertain, as linear growth response was poor in one treated AR STAT5B deficient patient (54) and two DN STAT5B deficient subjects (65).

The current application of next-generation genomic sequencing will likely expedite identification of new STAT5B defects, both germline and somatic. Early genetic detection of germline LOF *STAT5B* mutations and functional evaluation of the defects are essential towards improving understanding the pathophysiology of STAT5B deficiency and crucial for improving patient management.

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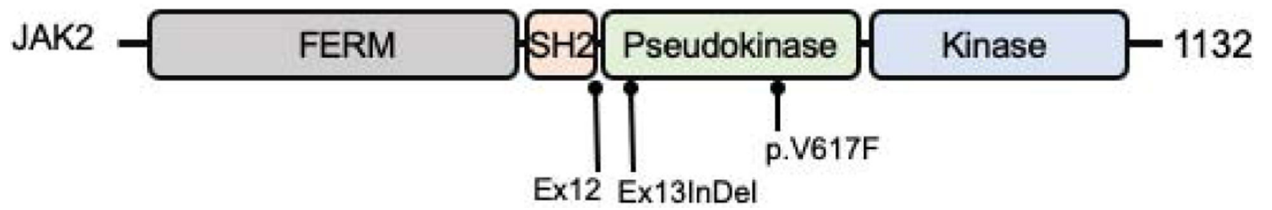

Figure 1.
Schematic of the human JAK2 protein. The three most common gain-of-function mutations are indicated. Ex12, exon 12; Ex13InDel, exon13 insertion/deletion; p.V617F, p.Val617Phe. FERM, amino acids 37–380; SH2, amino acids 401–482; Pseudokinase, amino acids 545–808; Kinase, amino acids 849–1124.

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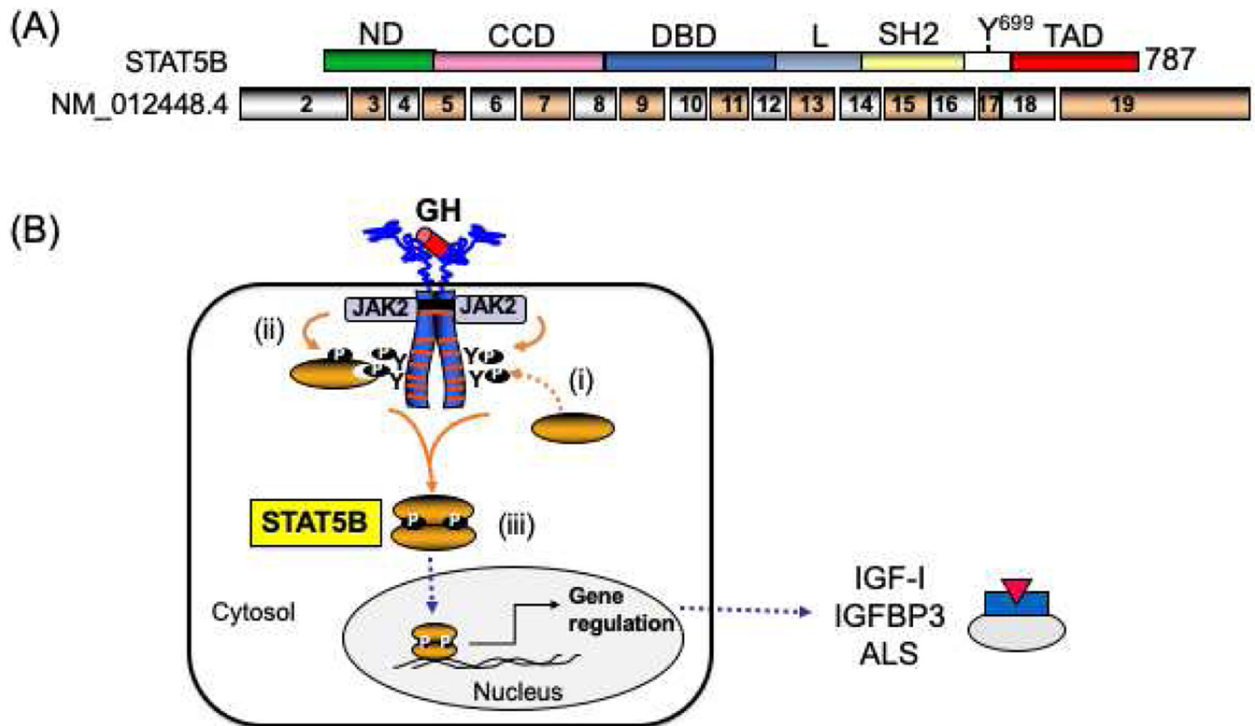

Figure 2.

Schematic of the human GH-induced activation of STAT5B. (A) Schematic of the human STAT5B peptide (upper schematic) and encoding exons (lower schematic). The domains indicated: ND, N-terminal domain; CCD, coil-coiled domain; DBD, DNA binding domain; L, linker domain; SH2, Src-homology 2 domain; TAD, transactivation domain. Tyrosine 699 (Y699) that can be phosphorylated by JAK2 and other kinases. (B) Recruitment and activation of STAT5B to the human growth hormone receptor (GHR): (i) Upon GH-GHR interactions, STAT5B docks to 3 of the 7 JAK2-phosphorylated tyrosines (Y) on activated intracellular domain of GHR; (ii) the recruited STAT5B is Tyr699 (Y699) phosphorylated by JAK2; (iii) Phosphorylated STAT5B homo-dimerize, translocate to the nucleus where it binds DNA and transcriptionally regulated genes such as *IGF1*, *IGFBP3* and *IGFALS*. Broken arrows, translocation process.

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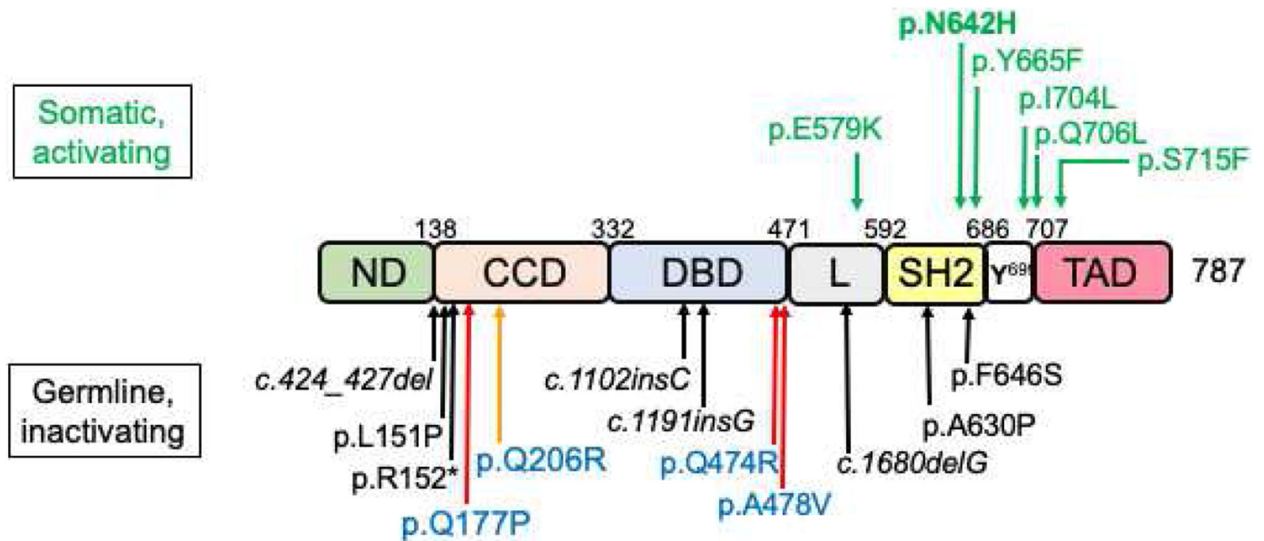

Figure 3.

Pathophysiological *STAT5B* mutations. Schematic of the human STAT5B protein (see Figure 2A for definition of the protein modules). Amino acid numbering is based on recent crystal structure of human STAT5B (47). Germline, inactivating, mutations are indicated below and somatic, activating, mutations above the schematic of STAT5B structure. Black arrows, homozygous; red arrow, dominant-negative; orange arrow, dominant-negative associated with immunity only; green arrows, gain-of-function mutations.

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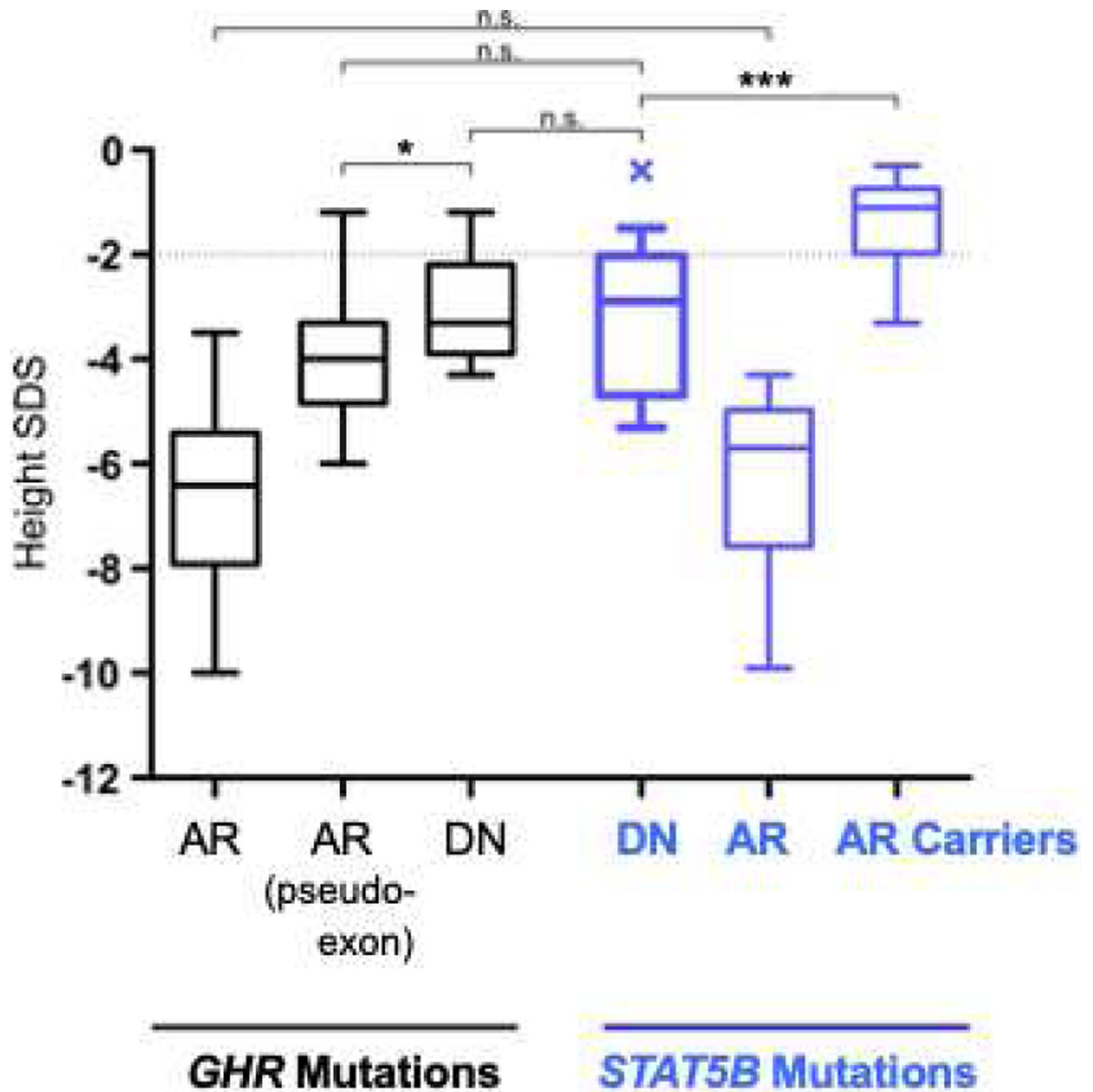

Figure 4.

Height of STAT5B deficient patients are comparable to GHR deficient patients. Height SDS values of GHI patients with *GHR* mutations (black) were compared with height SDS values of *STAT5B* mutation carriers (blue). Growth Hormone Receptor (*GHR*) Defects: AR, Autosomal Recessive *GHR* mutations (n=100); AR pseudoexon, *GHR* pseudoexon 6Ψ mutations (n=21); DN, Dominant Negative *GHR* mutations (n=16). *STAT5B* Defects: DN, Dominant Negative *STAT5B* mutations (n=11); AR, Autosomal Recessive *STAT5B* mutations (n=10); AR Carriers, *STAT5B* mutation carriers (n=14). Box (median, 25th and 75th percentiles) and whiskers (minimum and maximum values) plots; statistical analysis by

Student's t-test, * P<0.05; *** P<0.001; n.s., not significant. (Reproduced with permission from Klammt J, et al, Nat Commun 2018; 9:2105)

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Table 1.

Genetic and clinical phenotype of STAT5B deficient patients

STAT5B Exon	cDNA	Protein	Domain	Sex	Age (yr)	Height SDS	Birth	GHI	IGFD	Prolactin elevated	Hypergamma-globulinemia	T-cell lymphopenia	Pulmonary Disease	Reference
Homozygous														
5	*c.424_427del*	p.Leu142Argfs*20	CCD	M	6	−5.6	AGA	+++	+++	+++	+	+++	+++	(54)[#]
5	*c.424_427del*	p.Leu142Arqfs*20	CCD	M	2	−3.0	AGA	+++	+++	+++	No	+++	+++	(54)[#]
5	*c.452T>C*	p.Leu151 Pro	CCD	M	8	−3.2	AGA	+++	+++	Normal	No	No	No	(60)
5	*c.454C>T*	p.Arg152*	CCD	F	15.3	−9.9	NA	ND	ND	+++	+++	+++	+++	(57)
5	*c.454C>T*	p.Arq152*	CCD	F	12	−5.3	SGA	+++	+++	Normal	N0	No	+++	(58)
9	*c.1102insC*	p.Gln368Profs*9	DBD	M	31	−5.9	AGA	+++	+++	+++	No	No	No	(56)
10	*c.1191insG*	p.Asn398Glufs*16	DBD	F	16.4	−7.8	AGA	+++	+++	ND	ND	ND	+++	(55)
13	*c.1680delG*	p.Glu561Arqfs*17	Linker	F	4	−5.6	AGA	+++	+++	ND	ND	ND	+	(53)[$]
13	*c.1680delG*	p.Glu561Argfs*17	Linker	F	2	−5.8	AGA	+++	+++	ND	ND	ND	+	(53)[$]
15	*c.1888G>C*	p.Ala630Pro	SH2	F	16.5	−7.5	AGA	+++	+++	+++	+++	+++	+++	(45)
16	*c.1937T>C*	p.Phe646Ser	SH2	F	14.8	−5.95	NA	+++	+++	+++	+++	+++	No	(59)
Dominant-negative														
5	*c.530A>C*	p.Gln177Pro	CCD	M	14.5	−5.3	AGA	++	++	Normal	No	No	No	(65)
12	*c.1421A>G*	p.Gln474Arq	DBD	F	12.8	−4.5	AGA	++	No	+++	No	No	No	(65)
12	*c.1433C>T*	p.Ala478Val	DBD	M	1.8	−2.9	AGA	++	+++	Normal	No	No	No	(65)

+ to +++, increasing severity of indications.

AGA, appropriate for gestational age; ND, not determined

#, siblings

$, siblings