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REVIEW ARTICLE

Unraveling the immunogenetics of STAT proteins: Clinical perspectives on gain-of-function and loss-of-function variants

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Abstract

Signal Transducer and Activator of Transcription (STAT) proteins play pivotal roles in immune regulation. The dysregulation of these proteins, attributed to both gain-of-function (GOF) and loss-of-function (LOF) variants, has emerged as a substantial and intricate area of research. This comprehensive review delves into the intricate details of the diverse clinical spectrum associated with *STAT* variants and the immunological findings linked to these genetic alterations. Although this review does not encompass the treatment of each individual disease, we discuss investigative approaches ranging from immunophenotyping assessment to evaluation of STAT protein activity. These investigations play a crucial role in identifying affected patients and understanding the complexities of STAT.

Keywords: STAT Proteins, gain-of-function variants, loss-of-function variants, immune dysregulation, primary immunodeficiency

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Introduction

Signal transducer and activator of transcription (STAT) molecules are a family of transcription factors that transduce signals from cell membrane receptors to the nucleus.[1] Together with the Janus kinases (JAK) family proteins, STAT molecules contribute to several crucial biological functions including immune responses, hematopoiesis, adipogenesis, angiogenesis, stem cell maintenance, and cell growth.[2] In humans, seven STAT proteins are expressed: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. These STAT proteins differ in size and function but they all share an N-terminal domain (ND), a coiled-coil domain (CCD), a DNA-binding domain (DBD), a linker domain (LD), a Src Homology 2 (SH2) domain, and a C-terminal transactivation domain (TAD) (**Figure 1**).[3-5]

Canonical STAT signaling is initiated when a ligand binds to its cognate receptor, causing a conformational change in the receptor and activating associated JAK molecules (JAK1, JAK2, JAK3, or TYK2).[3,6] This activation enables the kinases to trans-phosphorylate each other and also phosphorylates tyrosine residues in the cytoplasmic domain of the receptors. The phosphorylated tyrosine residues create a docking site for the SH2 domain of STAT proteins.[3,7] The STAT molecules are subsequently recruited and phosphorylated. The SH2 domain of a STAT partner molecule recognizes these phosphorylated tyrosine residues,

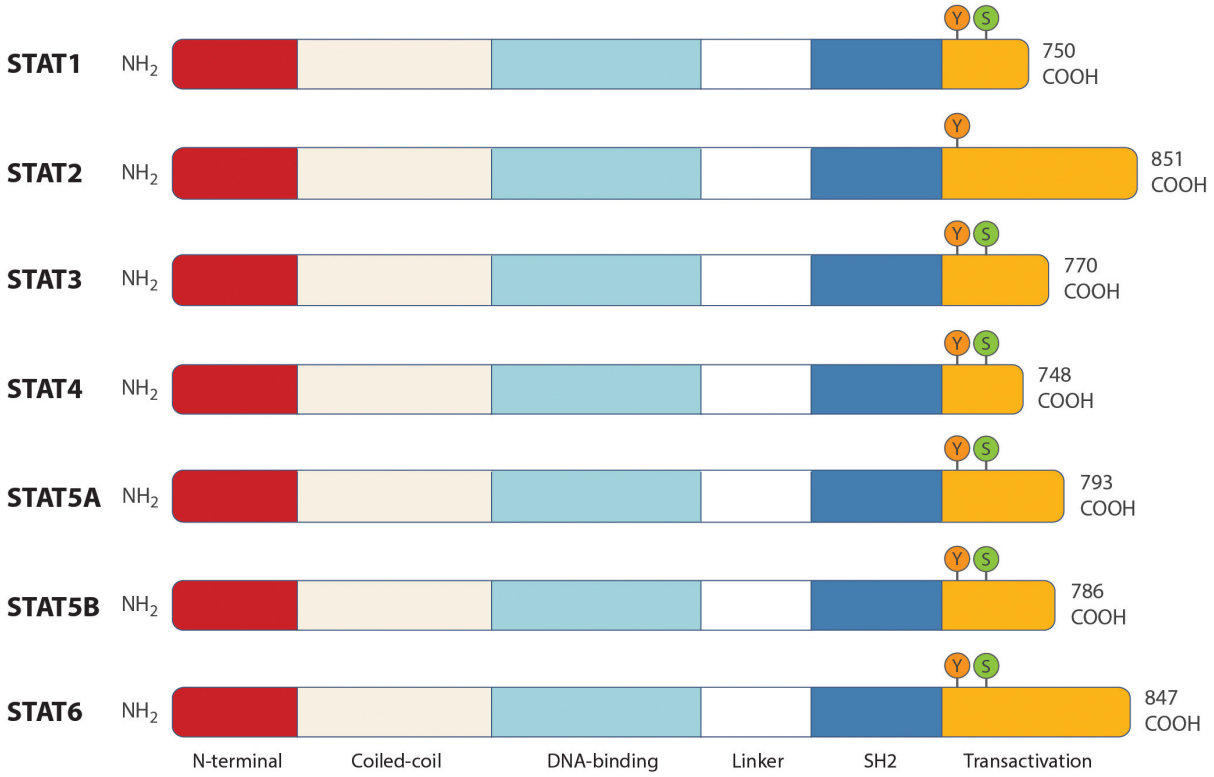

Figure 1. Linear depictions of human STAT proteins in their α isoforms are illustrated, showcasing the distinct domains of the STAT protein family: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6, each distinguished by different colors. The transactivation domain of each STAT includes phosphorylation sites, consisting of tyrosine (Y) and serine (S) residues. The number found at the end of each STAT protein indicates the total number of amino acids constituting the entire protein.

leading to a reorientation that allows the STAT proteins to dimerize, forming either heterodimers or homodimers. Some STATs can heterodimerize with more than one partner, primarily determined by the activating ligand.[3] This specific combinatorial usage of STATs, along with their cellular expression, allows over 50 different cytokines to transmit a variety of signals through just seven STAT proteins (**Table 1**).[8] Eventually, STAT complexes translocate to the nucleus, bind specific DNA sequences, and regulate the transcription of various genes (**Figure 2**). STAT complexes bind to a specific DNA regulatory sequence. All STAT proteins recognize the same palindromic DNA motif, 5′-TTCN$_{2\text{-}4}$GAA-3′, referred to as the gamma interferon activation site (GAS).[4,9] The preference of each STAT protein in GAS binding depends on the specificity of the GAS element. Different nucleotide sequence make-up of the GAS along with the nucleotide sequence surrounding the GAS determine the level of specificity.[4,9] STAT heterodimers preferentially bind to GAS variants, however different from homodimer complexes of STAT, and with varying affinities.[8] These differences allow each cytokine-regulated STAT to control and fine-tune the expression of specific gene sets, enabling the different STAT molecules to regulate unique cellular functions.[4] However, it is also possible for multiple STAT proteins to bind to the same GAS motif.[4,10,11] This might be due to subtle differences in the molecular structure of the STAT molecules and in the GAS variants themselves.[4]

Inborn errors of immunity in the STAT genes were first described in 2001.[12] The clinical phenotypes of STAT defects exhibit significant heterogeneity, depending on the specific STAT molecule affected and whether the genetic modification leads to a loss-of-function (LOF) or a gain-of-function (GOF) phenotype. Interestingly, genetic variants within the same gene can lead to a wide range of clinical presentations (**Table 2**). Defects have been identified in STAT1, STAT2, STAT3, STAT4, STAT5B and STAT6. This review provides a comprehensive overview of the function of all human STAT proteins as well as the clinical heterogeneity observed among patients with different STAT defects and among patients with distinct mutations in the same STAT gene. Additionally, we discuss immunological laboratory tests that can assist in the identification of patients with STAT defects. However, the treatment of each disease is not covered in this review.

Table 1. Activators of STAT and STAT heterodimerization partners are listed, with known activators for heterodimerization specified in parentheses. The table is adapted from reference.[8]

STATs	Activators	Heterodimerization partners
STAT1	Type I IFN Type II IFN IL-6 IL-10 IL-27 IL-35 GH	STAT2 (IFN) STAT3 (IL-6, IL-27, GH) STAT4 (IL-35)
STAT2	Type I IFN	STAT1 (IFN)
STAT3	IL-2 IL-5 IL-6 IL-10 IL-23 IL-27 M-CSF G-CSF	STAT1 STAT5A/B
STAT4	IL-12 IL-23 IL-35	STAT1 (IL-35) STAT3 (IL-23)
STAT5A/B	IL-2 IL-7 IL-15 IL-21 M-CSF GM-CSF GH	STAT3 (IL-2, IL-7, G-CSF, M-CSF)
STAT6	Type I IFN IL-3 IL-4 IL-13	STAT2 (IFN)

GH; growth hormone, G-CSF; granulocyte colony stimulating factor, M-CSF; macrophage colony-stimulating factor, GM-CSF; Granulocyte-macrophage colony-stimulating factor.

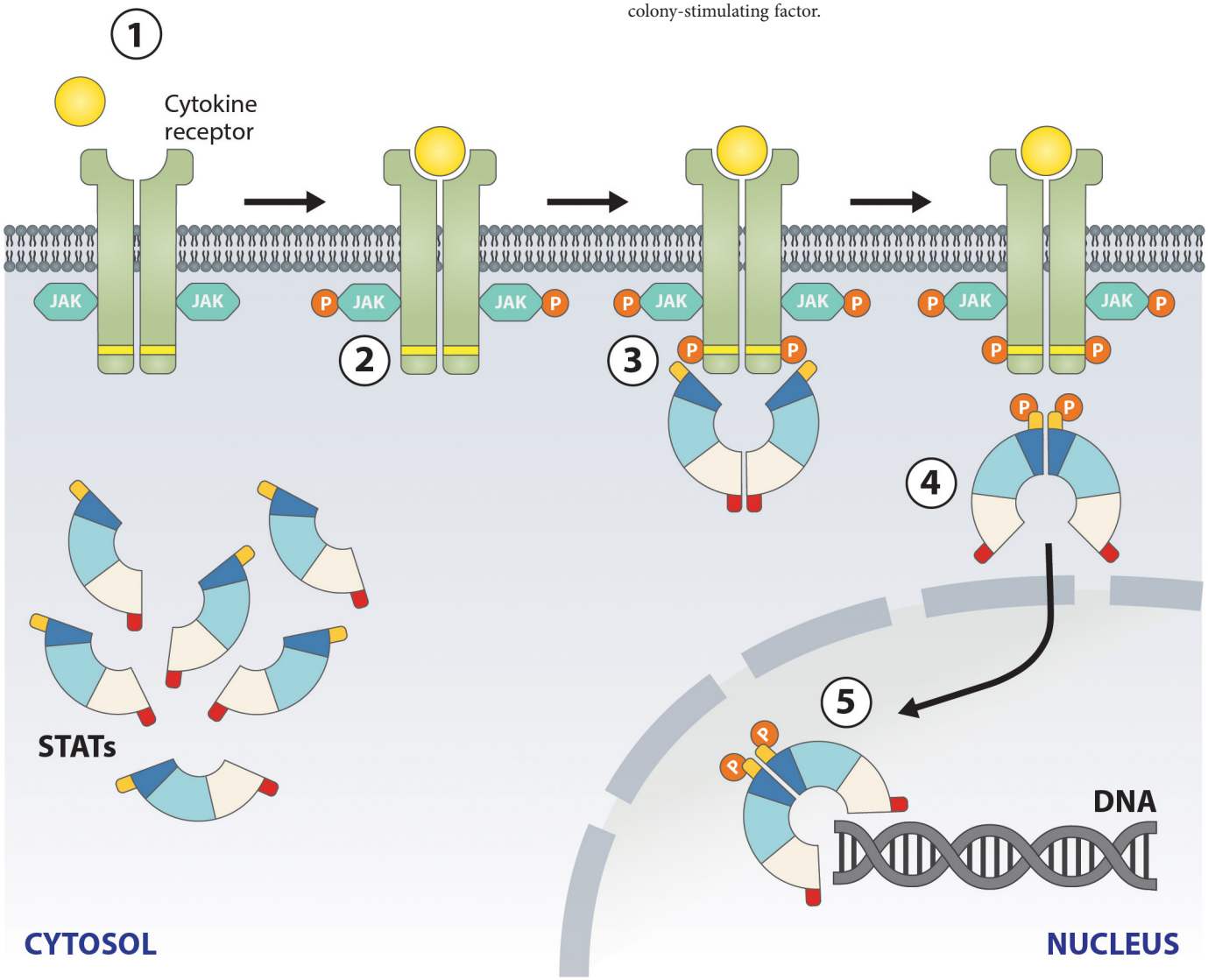

Figure 2. In the classical JAK/STAT signaling pathway, the process occurs through a series of distinct steps. Initially, ligand binding to receptors triggers a conformational change, activating JAK molecules (Step 1). These activated JAKs then phosphorylate each other and the receptor cytoplasmic domains (Step 2), creating a docking site for STAT proteins. Subsequently, recruited STAT proteins undergo tyrosine phosphorylation (Step 3), leading to STAT dimerization (Step 4). These dimerized STAT complexes then translocate to the nucleus (Step 5), where they bind to specific DNA sequences to regulate gene transcription.

Table 2. STAT variants and associated major clinical phenotypes.

Gene	OMIM	Inheritance	Effect	Clinical phenotype
STAT1	613796	AR	LOF	Mycobacterial and viral infections
STAT1	614892	AD	LOF	MSMD
STAT1	614162	AD	GOF	CMC and immune dysregulation
STAT2	616636	AR	LOF	Viral infections and systemic inflammation
STAT2	618886	AR	GOF	Type I interferonopathy
STAT3	615952	AD	GOF	Immunodeficiency with autoimmunity and lymphoproliferation
STAT3	147060	AD	LOF	Hyper-IgE syndrome
STAT4		AD	LOF	Vulnerable to *Coccidioides* and *Paracoccidioides* spp.
STAT4	620443	AD	GOF	Disabling pansclerotic morphea
STAT5B	245590	AR	LOF	Growth hormone insensitivity and immune dysregulation
STAT5B	618985	AD	LOF	Growth hormone insensitivity
STAT6	620532	AD	GOF	Early onset allergy

AD; autosomal dominant, AR; autosomal recessive, MSMD; Mendelian susceptibility to mycobacterial disease, CMC; chronic mucocutaneous candidiasis.

STAT1

In the early 1990s, STAT1 was discovered as cytoplasmic IFN-induced transcription factor.[3,13] The human *STAT1* gene encodes two STAT1 isoforms: STAT1α and STAT1β. STAT1α is the full-length isoform and consists of 750 amino acids whereas STAT1β is the truncated isoform that lacks 38 amino acids of the TAD.[14,15] The function of human STAT1α has been extensively investigated and this isoform is considered as transcriptionally active. The functional attributes of STAT1β are far less explored, with prevalent conceptualization of its transcriptional inactivity and role as a negative regulator of STAT1α activity.[15,16] Nevertheless, multiple investigations have revealed that STAT1β is also exhibits transcriptional activity, but modulates cellular processes different from those governed by STAT1α.[14,17,18]

STAT1 is a downstream mediator of several cytokines and hormones and is necessary for the regulation of a variety of biological activities, including cell growth and differentiation, antiviral response, and immune system homeostasis.[19] STAT1$^{-/-}$ mice are severely susceptible for viruses and other pathogens due to a complete unresponsiveness to type I and type II IFNs.[20-22] Type I IFNs (including IFN-α and IFN-β) are well-known for their antiviral and immunomodulatory roles, both at the level of innate and adaptive immunity.[23,24] Type II IFN, known as IFN-γ, is the crucial effector cytokine for antiviral and antimycobacterial immunity, but also a key regulator of several other immune functions, including maintenance of Th1 cells.[25,26] Besides IFNs, STAT1 activation is downstream of several other inflammatory and anti-inflammatory cytokines such as IL-6, IL-10, IL-27, and IL-35, and can heterodimerize with STAT2, STAT3, and STAT4 (**Figure 3**).[8,27-32] While both GOF and LOF variants have been identified in *STAT1*, there is a considerably higher number of reported GOF variants compared to LOF variants. The GOF or LOF phenotype of a missense variant in *STAT1* is not related to the domain where the variants are harbored. However, it is noteworthy that nonsense, frameshift, and indel variants are commonly related to the LOF phenotype.[33]

STAT1 LOF variant is a genetic cause of Mendelian susceptibility to mycobacterial disease (MSMD), an inborn immunity disorder marked by a unique susceptibility to infections induced by less virulent mycobacteria. MSMD was first described in 1996 and is caused by interruption of the IL-12/IFN-γ signaling pathway.[34] Autosomal dominant (AD) STAT1 deficiency has been identified as a cause of "isolated" MSMD, distinguished by an exclusive predisposition to one or more mycobacterial and associated infections.[35] Conversely, autosomal recessive (AR) STAT1 deficiency results in "syndromic" MSMD, marked by the combination of the mycobacterial disease infectious phenotype with other clinical features, such as type I interferonopathy.[35,36]

AD heterozygous *STAT1* LOF deficiency is currently described in 47 patients from 20 variants and 3 splice site mutations.[37-40] These patients are susceptible for infections with mycobacteria.[36,41] Interestingly, the AD *STAT1* variants have a dominant negative effect on IFN-γ signaling, while IFN-α/β signaling seems unaffected.[12,42] As a result, mycobacterial infections were identified in all patients, with *M. bovis*-BCG infection being the most prevalent in more than half of the cases. Mycobacterial infections are primarily manifested in disseminated, skin, and bone sites. Meanwhile, viral infections were documented in three patients, while no bacterial or fungal infections were reported.[37] The onset of the disease is variable, usually occurring in childhood but possibly also in adulthood. Importantly, the penetrance of AD STAT1 LOF deficiency is not complete, Bustamante et al. described a cohort in which 5 out of 17 tested individuals were asymptomatic (**Figure 4**).[42]

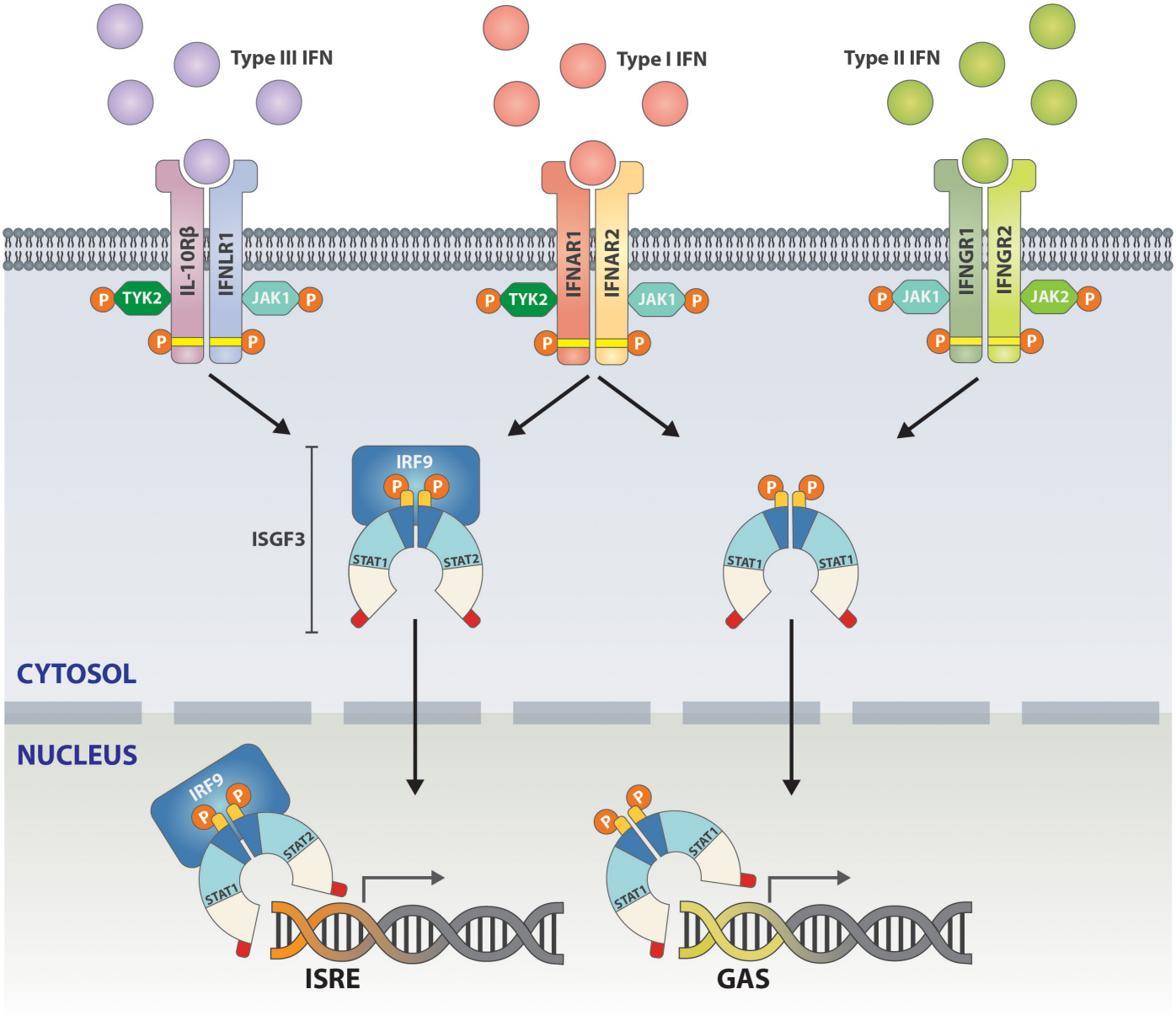

Figure 3. JAK/STAT pathway in interferon (IFN) signaling. All type I interferons (IFNs) engage a common receptor on the human cell surface, denoted as the type I IFN receptor. This receptor comprises two subunits, IFNAR1 and IFNAR2, associating with TYK2 and JAK1, respectively. Activation of JAKs by type I IFNs leads to tyrosine phosphorylation of STAT1 and STAT2, resulting in the formation of STAT1:STAT2:interferon regulatory factor 9 (IRF9) complexes, collectively referred to as interferon-stimulated gene factor 3 (ISGF3) complexes. These ISGF3 complexes translocate to the nucleus, where they bind to IFN-stimulated response elements (ISREs), initiating the transcription of interferon-stimulated genes (ISGs). Additionally, the activation of type III IFNs also results in the formation of ISGF3 complexes when they bind to the IFNLR receptor complex, consisting of INFLR1 and IL-10Rβ. Type I IFN activation also induces the formation of STAT1 homodimer as well as other various STAT complexes, which migrate to the nucleus and bind GAS elements in the promoters of specific ISGs. (IRF9; Interferon Regulatory Factor 9)
Conversely, IFN-γ binds to a type II IFN receptor comprising two subunits, IFNGR1 and IFNGR2, associated with JAK1 and JAK2, respectively. The activation of type II IFNs leads to the formation of STAT1 homodimers, which similarly migrate to the nucleus and bind GAS elements, thereby regulating gene transcription.

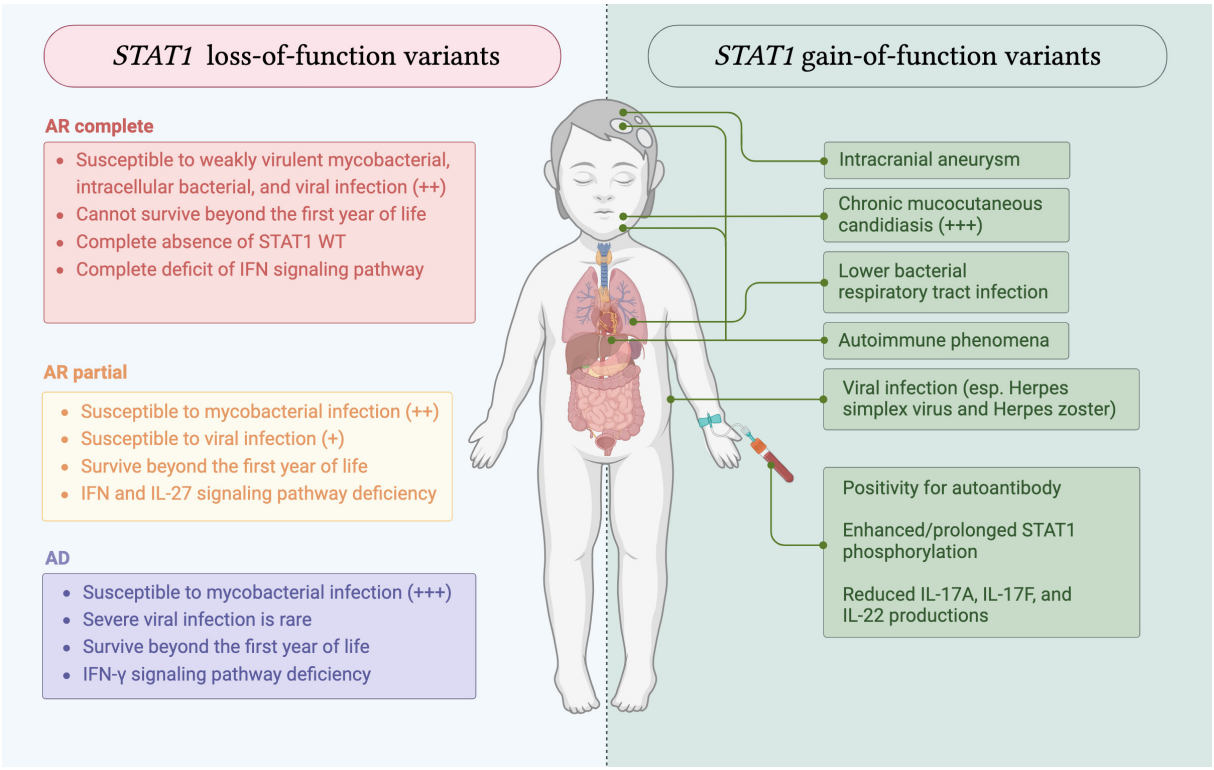

Figure 4. Clinical manifestations of patients with *STAT1* variants. The number of '+' symbols in the bracket indicates the frequency of the manifestation. Created with BioRender.com.

Patients with an AR complete STAT1 deficiency have bi-allelic *STAT1* damaging variants that block STAT1-mediated type I, II, III IFNs, and IL-27 signaling completely. So far, 24 patients with an AR complete STAT1 deficiency have been described.[43,44] They were highly vulnerable to intracellular bacteria including weakly virulent mycobacteria as well as to viruses, especially viruses in the family *Herpesviridae*[44-47] (**Figure 4**). 5 of 24 patients developed severe reactions or infections following live-attenuated viral vaccination including measles, mumps, rubella (MMR), and varicella zoster vaccine. Because of the severe immunodeficiency, the majority of the patients died within the first year of life as a result of the severe infections. To date, 16 mutations have been reported as a cause of AR complete STAT1 deficiency. All the identified variants are associated with complete absent of WT STAT1 expression and seriously compromised IFN-α, IFN-β, and IFN-γ signaling pathways.[46-49] A hematopoietic stem cell transplantation (HSCT) is indicated for these patients.[45]

Patients with AR partial STAT1 deficiency have bi-allelic hypomorphic STAT1 variants. The clinical presentation of this type of STAT1 deficiency is milder than AR complete STAT1 deficiency (**Figure 4**). All the eight patients that have been described so far have milder mycobacterial infections and viral infections which can majorly be controlled by antimicrobial agents. These patients survive longer than patients with AR complete STAT1 deficiency.[45] STAT1-dependent cellular responses to type I, II, III IFNs, and IL-27 signaling in these patients were impaired but not abolished.[44]

Currently, the most common form of STAT1 defect is the GOF variants. More than 100 different variants have been described in more than 400 patients.[33,43] *STAT1* GOF variants results in chronic mucocutaneous candidiasis (CMC), a condition characterized by recurring or persistent *Candida* spp. infections affecting the nails, skin, mouth, and genital mucosa.[33,50] The manifestation of CMC is typically observed since early childhood in individuals harboring *STAT1* GOF variants. Significantly, over 95% of these patients experienced the onset of disease before reaching 35 years of age.[51] Notably, CMC is prevalent in more than 60% of individuals with *STAT1* GOF variants. Invasive fungal infections are also reported but with less prevalence. In addition to CMC, more than half of the patients also encountered bacterial respiratory tract infections, with approximately 37% experiencing infections in the lower respiratory tract.[33] Frequently implicated causative agents included *Staphylococcus aureus* and *Streptococcus* spp., followed by *Pseudomonas aeruginosa* and *Haemophilus influenzae*.[33] About half of the patients encountered viral infections, especially with Herpes simplex, Varicella-zoster, CMV, or EBV.[51, 52] Interestingly, more than 60% of the patients also suffered from autoimmunity, for instance, thyroid disease (mostly hypothyroidism), autoimmune cytopenia, autoimmune-related skin diseases (vitiligo, psoriasis, alopecia), diabetes mellitus. Additionally, symptoms resembling systemic lupus erythematosus (SLE) and autoimmune hepatitis were also prevalent among these patients.[33,50-52] Majorly, the patients with autoimmune phenomena are also positive for serological markers

of autoimmune disease, for example antinuclear antibodies (ANA), anti-dsDNA, and anti-thyroglobulin autoantibodies (**Figure 4**).[52]

Cells carrying a *STAT1* GOF variant display increased STAT1 phosphorylation upon activation by cytokines, for example, type I IFN, type II IFN, and IL-27. Variants in the CCD or DBD are associated with prolonged cellular presence of phosphorylated STAT1 because of a defective STAT1 dephosphorylation mechanism. While variants found in the SH2 domain of STAT1 are associated with increased STAT1 phosphorylation without impairment of dephosphorylation mechanism.[50,53,54] A marked decrease in the production of IL-17A, IL-17F, and/or IL-22 from the immune cells of individuals with a *STAT1* GOF variant has been demonstrated, underscoring the susceptibility of the distinct manifestation of CMC in these patients. Besides, depletion of peripheral Th17 and Th22 lymphocyte numbers are also reported in some cases.[55]

The mechanism of autoimmune complications in a patient with a *STAT1* GOF variant is still unclear. Enhanced responses to IFNs activation and HLA expression, as well as suppression of Treg, are proposed to precipitate autoimmune phenomena.[51,56-58] Remarkably, over 10% of individuals with *STAT1* GOF variants encounter intracranial aneurysms at a young age.[51] Additionally, these patients are likely to develop malignancies including cutaneous, oral, laryngeal, esophageal, and gastrointestinal carcinoma.[33] Several patients also exhibit diverse clinical characteristics, for instance, persistence of primary teeth, hypermobility of the joints, osteopenia, and enteropathy.[56,59] These features resemble those found in other immune diseases, such as AD-HIES, IPEX syndrome, APECED syndrome, and nuclear factor κB essential modulator (NEMO) deficiency.[60]

STAT2

STAT2 is a well-known transcription factor in the IFN type I and type III signaling pathway-mediated host antiviral response. Following type I IFN activation, STAT2 may form homodimers or heterodimerizes with STAT1 in combination with IFN regulatory factor 9 (IRF9) which then translocate into the nucleus where it specifically binds the IFN-stimulated response element (ISRE).[61] STAT2 also plays a crucial role in downregulating type I IFN responses via ubiquitin-specific protease (USP18) which is an ISG that is recruited by STAT2 to prevent the binding of JAK1 to IFNAR2. Apart from IFN-α/β/λ, the presence of additional ligands, such as TNF-α, might have a synergistic effect on STAT2 activation.[62,63] Although TNF-α alone has no effect on the phosphorylation of STAT2, synergistic activation by IFN-β and TNF-α of STAT2 is considered to be involved in delayed antiviral responses by regulating alternate genes compared to those induced by type I IFN alone.[63-65]

So far, AR LOF and GOF variants have been described in *STAT2*. AR LOF variants in STAT2 have been identified in 23 patients and results in susceptibility for severe viral infections.[66] A remarkable feature of this disease is the vulnerability to diseases caused by live-attenuated viral vaccines, (such as MMR vaccine, or varicella zoster vaccine) resembling patients with AR complete *STAT1* deficiency.[66,67] Other severe viral infections include influenza A, SARS-CoV-2, HSV-1, enterovirus, Epstein-Barr virus, and adenovirus. 9 patients with *STAT2* LOF variants suffered from systemic hyperinflammation, varying between extended fever requiring hospitalization following a viral infection, sepsis-like presentation, Kawasaki disease, or hemophagocytic lymphohistiocytosis (HLH) (**Figure 5**).[68-71] Transient transfection of *STAT2* LOF variant alleles into HEK293T cells or the use of EBV-transformed cell lines from patients with *STAT2* LOF revealed either a complete absence or truncation of STAT2 protein.[66] STAT2-deficient leukocytes exhibited downregulation of all ISGs. Notably, there was also impaired upregulation of *USP18* following induction with IFN-α2A (**Figure 5**).[66] However, an elevation in the expression of genes associated with TNF/NF-κB signaling and the IL-6/JAK/STAT3 pathway was observed, providing a plausible explanation for the systemic inflammation observed in these patients.[66] A study on dermal fibroblasts from individuals with homozygous STAT2 deficiency revealed a reduction in STAT1 phosphorylation in response to type I IFN, while maintaining intact phosphorylation of STAT1 in response to type II IFN.[71] This finding provides a potential rationale why these patients do not experience susceptibility to mycobacterial infection. Interestingly, a study in three patients with STAT2 deficiency revealed that absence of the STAT2 protein was associated with elongation of mitochondria and a decrease in phosphorylation of the mitochondrial fusion protein, dynamin related protein 1 (DRP1).[68] Morphologic remodeling of mitochondria was reported to affect cell viability and also relates to neurodegenerative disorders.[68,72]

GOF variants in *STAT2* have only been described in four patients.[67,73,74] All patients had a variant locating in CCD domain of *STAT2*. These patients manifested symptoms of type I interferonopathy, characterized by sterile systemic inflammation and neurological abnormalities. Notably, unlike patients with LOF variants in *STAT2*, these individuals did not experience severe viral infections. The clinical phenotype associated with *STAT2* GOF variants closely resembles that observed in patients with USP18 deficiency.[75,76] In contrast to the *STAT2* LOF variant, cells carrying *STAT2* GOF variant exhibit STAT2 expression with prolonged phosphorylation following IFN-α stimulation. Notably, all documented *STAT2* GOF variants exhibited either impaired binding with USP18 or disrupted STAT2:USP18 heterodimer trafficking, leading to the failure of STAT2:USP18-mediated negative regulatory control (**Figure 5**).[74,77] Taken together, this suggests that these *STAT2* variants maintain the capacity to transduce IFN-I signaling. Nevertheless, their negative regulatory function is compromised due to the defective interaction with USP18.[67,73,74,77]

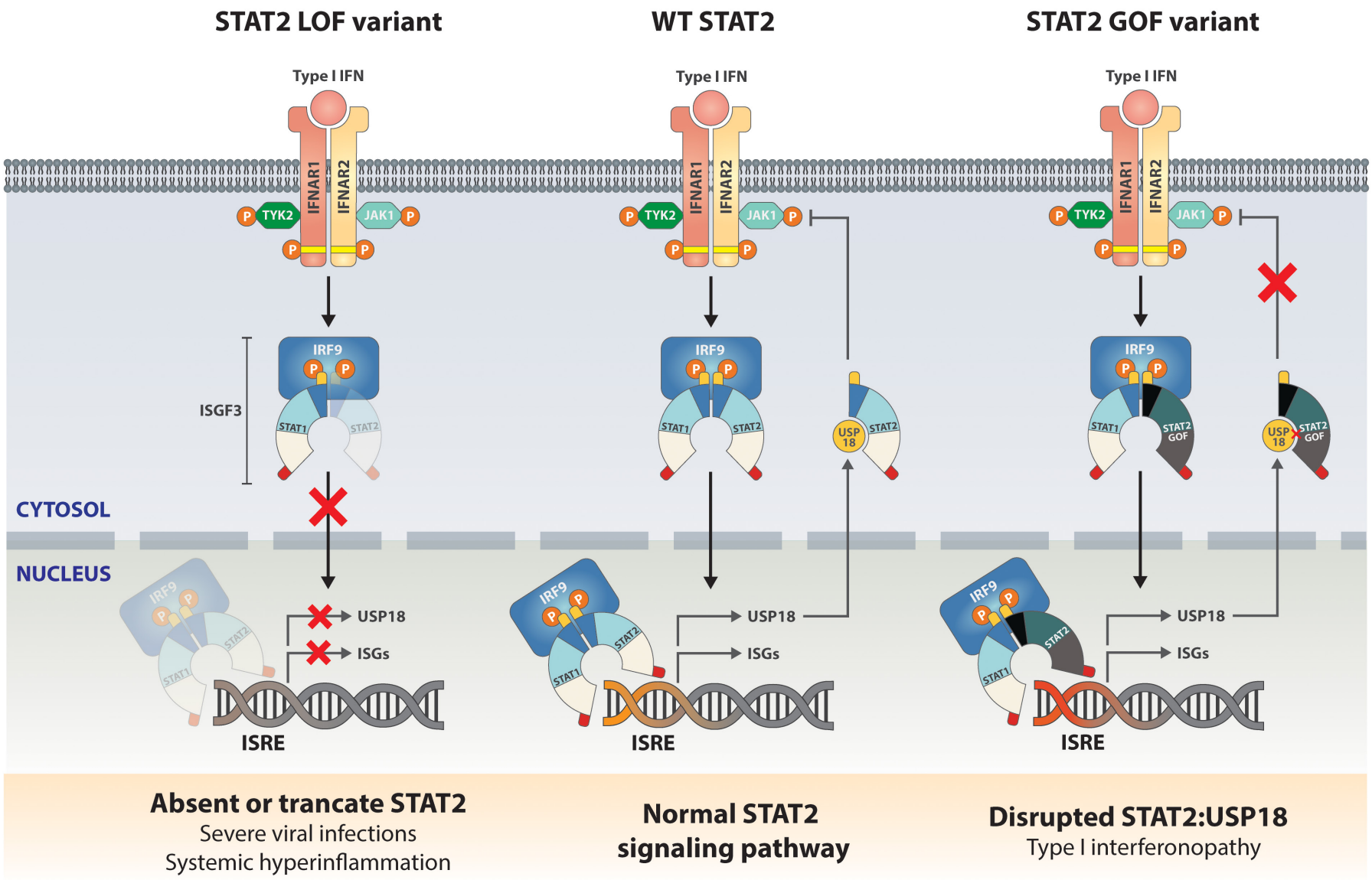

Figure 5. STAT2 signaling pathway. Following stimulation with type I or type III interferon, IFNAR1 and IFNAR2 activation triggers phosphorylation of TYK2 and JAK1. This leads to the phosphorylation of STAT1 and STAT2. The phosphorylated STAT1 and STAT2, along with interferon regulatory factor 9 (IRF9), form the interferon-stimulated gene factor 3 (ISGF3) complex, which translocate to the nucleus and binds IFN-stimulated response elements (ISREs). This initiates a transcription for ISGs. In the later stages of the IFN response, USP18 dimerizes to STAT2 and displaces JAK1 from IFNAR2, thereby negatively regulates IFN signaling (middle). In the case of a *STAT2* LOF variant, the STAT2 protein is either absent or truncated, resulting in the absence of transcription for ISGs and USP18. This absence leads to severe viral infections, particularly in response to live attenuated vaccines, accompanied by hyperinflammation. Conversely, in the case of a *STAT2* GOF variant, defects in STAT2:USP18 dimerization or trafficking are observed. As a consequence, this complex fails to displace JAK1 from IFNAR2, leading to an overregulated IFN signaling pathway. Patients with STAT2 GOF variants typically exhibit characteristics of type I interferonopathy.

STAT3

STAT3 was initially named as acute phase response factor (APRF) for its ability to interact with the promoter region of acute-phase genes and is also reported as a transcription factor related to several inflammatory cytokine and peptide hormones, such as IFNs, IL-6, IL-10, IL-21, platelet-derived growth factor, leptin, and growth hormone (GH).[13,78-83] STAT3 is recognized as a transcription factor involved in many diverse physiological and pathological cellular processes, including cellular proliferation, differentiation, metabolism, inflammation, angiogenesis, and cancer metastasis.[80,82,83]

STAT3 is essential for the differentiation and maintenance of human Th17 via TGF-β1, IL-6, IL-21, and IL-23.[84,85] Deletion of STAT3 in T lymphocytes obliterates Th17 generation and autoimmune features in many autoimmune disease models.[86] On the other hand, deficiency of suppressor of cytokine signaling 3 (SOCS3), which is the negative regulator of STAT3, leads to an increase in Th17 development. Furthermore, mice lacking *Socs3* in myeloid cells aggravate autoimmune phenomena such as experimental autoimmune encephalomyelitis and colitis.[87-91]

Missense variants are predominant in STAT3, with only AD variant have been reported. These variants result in either a LOF or a GOF of the STAT3 protein. Notably, LOF missense variants within *STAT3* may harbor at the same locations as GOF variants in the *STAT3* gene. Therefore, the impact on STAT3 function is independent of the variant's domain location but appears to be primarily influenced by the nature and charge of the altered amino acid.[92,93]

STAT3 LOF variants have a dominant negative effect and lead to the clinical presentation of dominant-negative hyper IgE syndrome (HIES), a disease also known as Job's syndrome.[78,94,95] HIES is classified as inborn error of immunity (IEI) with multiorgan involvement and is traditionally defined with triad of; high level of IgE, dermatitis, and recurrent skin and lung infections.[96,97] Not only damaging variants in *STAT3* but also in *TYK2*, *PGM3*, *DOCK8*, *ZNF341*, *CARD11*, *ERBIN*, *TGFBR1*, *TGFBR2*, and *IL6ST* are causing HIES.[98] Most patients with *STAT3* LOF related AD-HIES experience the first symptoms of eczema within the first year of life.[79,97,99]

Typical infections associated with AD-HIES involve the skin and lungs.[79,96,99] Bacterial skin abscesses are common and *Staphylococcus aureus* is documented as the main pathogen.[100] The majority of AD-HIES patients suffer from recurrent upper and lower respiratory tract infections, predominantly caused by *Staphylococcus aureus* or *Streptococcus pneumoniae*.[100] Pneumatoceles are the most common complication in these patients and likely are the result of severe parenchymal lung damage caused by recurrent bacterial infections.[96,97,100] More than half of the patients also experience CMC, while invasive fungal infections are less common. Herpes skin infection is diagnosed in approximately 10% of AD-HIES patients.[79,96,99] A characteristic infection feature of HIES is diminished inflammatory activity, manifesting as an insufficient cutaneous inflammatory response that leads to the formation of 'cold' skin abscesses (**Figure 6**).[101]

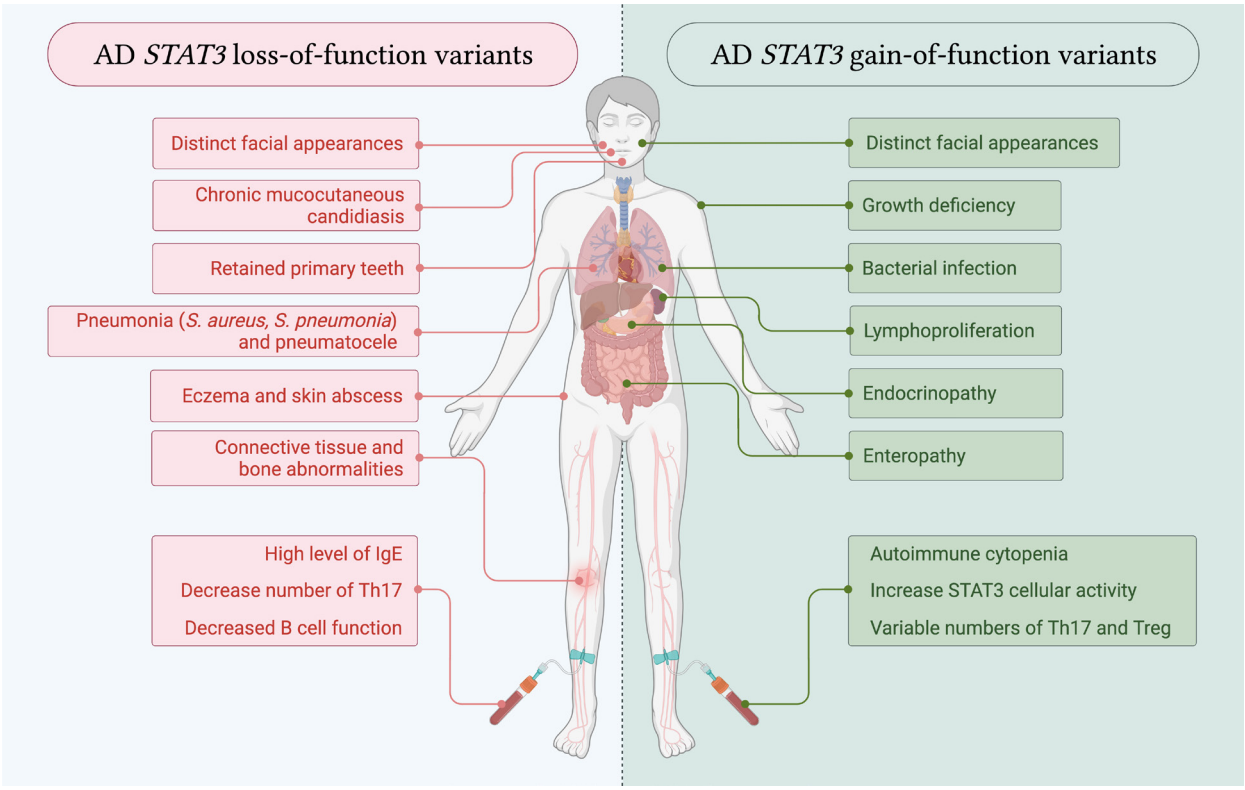

Figure 6. Clinical manifestations of patients with *STAT3* variants. Created with BioRender.com.

STAT3 is crucial for Th17 differentiation and development, therefore dominant-negative variants in *STAT3* are associated with a pronounced depletion in Th17 response resulting in the clinical phenotype of CMC.[102-104] IL-17 signaling is inducing chemotaxis and proliferation of neutrophils. In the patients with AD-HIES reduced neutrophil chemotaxis and functional disabilities are well recognized.[101] Impairment in IL-17 signaling mechanism could thereby explain depletion of neutrophil responses and the recurrent staphylococcal infections.[105] The reduction in STAT3 expression and phosphorylation may not consistently detected in cells carrying a *STAT3* LOF variant, particularly when the variant is located away from the STAT3 phosphorylation site at Y705.[102,106] However, the majority of *STAT3* LOF variant cells have impaired nuclear translocation and DNA-binding mechanism.[106] Thus, induction of STAT3-regulated genes (e.g., *SOCS3*, *PRDM1*, and *IL2RA*) are diminished in these cells.[102] Interestingly, an increased level of STAT1 phosphorylation was observed in cells from patients with AD-HIES, along with heightened expression of STAT1-targeted genes upon stimulation with IFN.[107] These findings may provide a potential explanation for the overlapping clinical manifestations observed in patients with *STAT1* GOF variants and AD-HIES.

Clinical features of HIES also include skeletal and connective tissue defects. Dysmorphic features were reported with distinct facial appearance, including, prominent forehead, prognathism, cathedral palate, broad nasal bridge, and rough facial skin.[96,97,99] Retention of primary teeth is also reported in more than 60% of the patients. Skeletal abnormalities further include abnormal bone fractures, osteopenia, joint hyperextensibility, atypical joint dislocation, and scoliosis (**Figure 6**).[97,99] STAT3 signaling pathway is essential for bone formation and homeostasis and IL-6-induced STAT3 signaling plays an important role in the differentiation of osteoblasts and osteoclasts. Osteoclasts from HIES patients showed increased bone resorption activity than that of healthy control.[106,108] Experiments in osteoblasts from mice deficient for STAT3 also show an osteoporotic phenotype due to a reduction of bone formation rate.[108,109] However, the molecular pathogenesis for other connective tissue defects in AD-HIES still remains unresolved.

Patients with germline heterozygous *STAT3* GOF variants were first reported in 2014. The clinical manifestations of patients with *STAT3* GOF variant are heterogeneous. Until now, more than seventy *STAT3* GOF variants have been described.[110] The common clinical features of these patients include lymphoproliferation, autoimmune cytopenia, growth delay, skin disease, interstitial lung disease (ILD), endocrinopathies, such as hypothyroidism and DM type I.[78,110-112] Severe and recurrent infections are reported in several organ systems particularly in respiratory tracts which may lead to development of bronchiectasis. A variety of pathogens including varicella-zoster virus, *Pseudomonas* spp., *Mycobacterium avium*, and *Candida* spp. are causative agents of infections found in these patients (**Figure 6**).[113,114] However, the overall clinical features of immunodeficiency are less severe in patients with *STAT3* GOF variants than those with *STAT3* LOF variants.[112]

More than half of patients experience growth failure.[115] Available data in the patients with the *STAT3* GOF variants were reported from -2 to -6.7 SDS.[115] One third of patients were born under 2 standard deviations in height and/or weight below the normal range.[112] Around 70% of patients with physical growth retardation were detected with low level of serum Insulin-like growth factor 1 (IGF-1).[112,113,116] Three of the four patients who underwent GH stimulation tests showed a positive result.[113,116,117] Two cases were evaluated in IGF-1 generation test and revealed partial GH insensitivity.[116,118]

In contrast to *STAT1* GOF variant, hyperphosphorylation or prolonged STAT3 phosphorylation is not always present in cells from patients with a *STAT3* GOF variant.[113,118,119] Therefore, STAT3 phosphorylation is not the suggested diagnosis test for this disease. However, increased endogenous STAT3 transcriptional activity was always detected in the cells carrying *STAT3* GOF variant either by luciferase reporter assay or expression of STAT3-controlled genes (e.g. *SOCS3*).[113,114,117,119] Interestingly, some studies also noticed that STAT1 and STAT5 phosphorylation level in these cells were decreased after cytokine activation when compared to control cells.[113,116]

STAT4

STAT4 plays a role in human innate and adaptive immune responses especially in development of Th1 lineage upon IL-12 activation.[120] IL-12, the primary activator of STAT4, is majorly produced by macrophages and dendritic cells.[120] Following pathogen detection, antigen-presenting cells secrete IL-12 to activate target cells including T lymphocytes to differentiate into IFN-γ–producing Th1.[121] When IL-12 binds to the IL-12R, it activates JAK2 and TYK2, which induces phosphorylation of the IL12R.[122] This process recruits STAT4 proteins, which then bind to the activated receptor, and phosphorylation of STAT4 subsequently takes place.[122] Phosphorylated STAT4 proteins are then dimerized and migrate to the nucleus, where they bind to specific DNA sequences and regulate several gene transcription, including *IFNG*, *CXCL9*, and *LY6A*.[123] The phosphorylation of STAT4 on both tyrosine 693 and serine 721 residues is crucial for Th1 differentiation and IFN-γ secretion.[124]

Defects in STAT4 were not reported in 2022 update on the classification from the international union of immunological societies expert committee (IUIS).[125] However, two families have been reported to harbor damaging LOF variants in *STAT4*.[126,127] Two family members from the first family had a heterozygous STAT4 p.E651V variant. One family member presented with a chronic gastrointestinal *Paracoccidioides brasiliensis* infection while the other patient had *P. brasiliensis* skin infection.[126] Three family members from the second family had the heterozygous *STAT4* p.E626G variant and presented with disseminated *Coccidioides* fungal infection.[127] Interestingly, in both families, no increased sensitivity for viral or

bacterial infections were described. Reduction of STAT4 phosphorylation was detected in T lymphocytes and impaired IFN-γ production was measured in these patients PBMCs.[126] A murine model with p.E626G heterozygous *STAT4* variant also failed to produce IFN-γ upon infection with *Coccidioides* spp.[127]

Several single-nucleotide polymorphisms located in *STAT4* were reported and associated with asthma, Sjögren's syndrome, rheumatoid arthritis (RA), and SLE.[128-130] Recently, three heterozygous GOF variants in *STAT4* have been described in patients with disabling pansclerotic morphea, a rare systemic inflammatory disorder, characterized by poor wound healing, extensive fibrosis, cytopenias, hypogammaglobulinemia, and the potential development of squamous cell carcinoma.[131] Primary skin fibroblasts from the patients had enhanced interleukin-6 secretion in the absence of stimulation. Enhanced STAT4 phosphorylation was detected in unstimulated U3A cells carrying *STAT4* GOF variants. Following IFN-α stimulation, levels of phosphorylated STAT4 were prolonged in cells containing *STAT4* GOF variants. Transcriptional activity and accumulation of STAT4 in the nucleus were also enhanced in the mutant cell lines.[131]

STAT5

Human STAT5 is comprised of 2 related proteins, STAT5A and STAT5B which are encoded by the *STAT5A* and *STAT5B* genes located on chromosome 17.[5,132] These proteins are both involved in several critical cellular functions, including proliferation and differentiation.[133] Although *STAT5A* and *STAT5B* have a relatively homologous protein sequence (93.6%),[134-137] STAT5A and STAT5B are not interchangeable and each of these proteins has unique biological functions which could be the results of differences in expression levels, DNA binding affinities, or kinetics.[138,139] While STAT5A is involved in human nervous system development including neuronal process extension, recycling of synaptic vesicles, and anti-apoptosis, for instance, via regulation of *NDRG1*, *DNAJC6*, *MAP3K5* and *BCL2L1*.[132] STAT5B activity is associated with T lymphocyte development and function, via the regulation of *DOCK8*, *SNX9*, *FOXP3*, and *IL2RA* and also recognized as one of the key pathways involved in growth hormone-induced IGF-1 production.[132,140,141]

Germline *STAT5A* variants have never been reported in humans, while 9 AR and 4 AD *STAT5B* LOF variants have been described.[142-144] All patients with AR *STAT5B* LOF mutations display a severe postnatal growth defect without intellectual impairment. These patients' heights ranged from -3.0 to -9.9 SDS.[142] Patients with *STAT5B* LOF variants had similar biochemical and growth characteristics to those with classical growth hormone insensitivity syndrome (Laron syndrome), which is caused by a genetic variation in the *GHR* gene.[142] Low serum IGF-1 levels were reported in relation to bone age and puberty.[142] Normal basal GH levels were found during the endocrine examination of *STAT5B* variant individuals, however blood levels of IGF-1, IGFBP-3, and IGFALS were typically low and cannot be restored by GH treatment. Serum prolactin levels were also found to be exceptionally high in a number of cases. However, the cause of hyperprolactinemia is still unknown, probably as a result of pituitary overproduction of GH.[142] Some individuals had facial dysmorphic characteristics, such as a high-pitched voice, a prominent forehead, and a depressed nasal bridge.[142]

Another clinical manifestation of patients with STAT5B deficiency is immunological dysfunction, which is distinct from patients with *GHR* or *IGFI* LOF variants.[145] Even among siblings sharing the same genetic variant, patients with STAT5B deficiency have a highly variable immunological profile.[134] Susceptibility to viral and bacterial infections are also reported in these patients which might be associated with defective IL-2 STAT5 signaling pathway. Clinical presentations of these patients are also similar to that of IPEX-like syndrome, including eczema, gastrointestinal symptoms, and autoimmune thyroiditis.[146,147] Other autoimmune phenomena in these patients includes juvenile idiopathic arthritis and type 1 diabetes. The common laboratory findings include hypergammaglobulinemia, decreased T lymphocyte and NK cell numbers, and defective T lymphocyte functions.[133,134] Reduction in $CD4^{+}CD25^{high}$ regulatory T lymphocyte numbers and function are also described in combination with diminished expression of FOXP3.[142,148] IL-2-induced upregulation of CD25 on $CD4^{+}$ T lymphocytes carrying homozygous missense *STAT5b* variants was found to be impeded.[148] Furthermore, a rare autoimmune-related pulmonary condition called lymphoid interstitial pneumonia is also reported and results in lung fibrosis and respiratory failure.[149] The pathophysiological mechanisms governing this pulmonary manifestation remain elusive, with emerging evidence suggesting a potential association with the disruption of the GM-CSF signaling pathway, a regulatory cascade implicated in pulmonary alveolar proteinosis.[150] Intriguingly, the administration of corticosteroids proves ineffective in restoring pulmonary function for a substantial number of patients.[142] The long-term prognosis for patients with AR STAT5B LOF variants is poor.

Surprisingly, the first-degree relatives of AR *STAT5B* variant patients that carrying heterozygous *STAT5B* variants had lower height compared to their non-carrier relatives, although their height were still within the normal reference.[151] Additionally, measurement of serum IGF-I and IGF-BP3 in these AR heterozygous *STAT5B* variant relatives revealed lower levels than that found in their non-carrier relatives.[151] These findings demonstrate the negative influence of *STAT5B* LOF variants on growth regulation and height.

Four different germline AD *STAT5B* variants have been reported in humans. These variants were either located in the CCD or the DBD of STAT5B.[143] Following GH stimulation, normal tyrosine phosphorylation and dimerization of STAT5B occurred. However, in the cells carrying variant located in the CCD, impaired STAT5B nuclear migration was observed, while variations located in the DBD were associated with a loss of DNA-binding functions.[143] All patients with AD *STAT5B* variants experienced postnatal growth impairment and GH insensitivity, although the somatic growth defects in these patients were milder than those seen in patients with

homozygous *STAT5B* variants.[152] Most of the patients with the AD *STAT5B* variants had eczema and elevated levels of IgE, but none of them experienced severe immune dysfunction.[143]

STAT6

Human STAT6 is encoded by the STAT6 gene that is located in the vicinity of STAT2 on chromosome 12. In comparison to other STATs, both STAT6 and STAT2 have relatively longer TAD (**Figure 1**).[153] STAT6 is well-known as essential transcription factor for development of Th2 and for the production of IgE and IgG1 in B lymphocytes.[154] The humoral immune responses, such as the immunological response to helminths and the development of allergy disorders, are regulated by the activation of STAT6 by IL-4 and IL-13.[154,155] When IL-4 binds to IL-4Rα, it causes the secondary receptor chains dimerization which result in two types of IL-4 receptor complexes: type I and type II.[153] Type I IL-4 receptor complex consists of IL-4Rα chain and IL-2Rγc (γc), while the type II IL-4 receptor consists of IL-4Rα chain and IL-13Rα1.[153,155] The expression of these two types of IL-4 receptors varies depending on the cell type and the expression of the secondary receptor chains. While IL-13Rα1 is expressed by non-hematopoietic cells, the expression of γc is increased on hematopoietic cells but low to absent in non-hematopoietic cells.[155,156] Type I IL-4 receptor can be activated by IL-4, while the type II IL-4 receptor can be activated by either IL-4 or IL-13.[153]

STAT6 is also downstream of several other ligands, including IL-3, IL-15, and platelet-derived growth factor (PDGF-BB).[157] 80% of IL-4 responsive genes in human T lymphocytes, including *GATA3*, *CRTH2*, and *SOCS1* are modulated via STAT6.[154,158] Moreover, STAT6 is implicated in adaptive immunity to viral infection. Following viral invasion, signaling of STAT6 pathway is different from that of the traditional IL-4/IL-13 signaling pathway in that both stimulator of interferon genes (STING) and STAT6 serine phosphorylation are required.[157] *Stat6*$^{-/-}$ mice are more vulnerable to viral and parasitic infections.[157,159] However, in *Stat6*$^{-/-}$ mice, several allergic asthma features including Th2 aggregation, airway eosinophilia, and mucus production are reduced.[160,161] The first family with a *STAT6* GOF variant (p.Glue377Lys) was described in a family with early-onset allergies.[162] The father had coarse facies, hypotrichosis, a history of food allergy, dry skin, and moderate atopic dermatitis. The index patient experienced severe atopic dermatitis, six episodes of anaphylaxis, allergic eosinophilic gastroenteritis with protein-losing enteropathy, respiratory allergies, and enamel hypoplasia. The index patient's youngest sister has also suffered from severe atopic dermatitis, ascites from allergic eosinophilic gastroenteritis and protein-losing enteropathy.[162] Two other publications have additionally reported 17 patients from 11 different families with GOF AD *STAT6* variants.[163,164] All patients have early-onset allergic immune dysregulation, including treatment-resistant atopic dermatitis, hypereosinophilia, asthma, IgE-mediated food allergies, and anaphylaxis. Eosinophilia and significantly increased serum IgE levels were measured in these patients, suggesting a potential link between *STAT6* GOF variant and HIES.[163,164] Interestingly, half of the patients experienced recurrent skin, respiratory, and viral infections. Seven patients exhibited short stature (below the third percentile for age), while skeletal defects including pathologic fractures and generalized hypermobility were reported in five patients. Two patients succumbed to the disease; one from anaphylaxis at the age of 20 and another from a cerebral aneurysm at the age of 35.[163] Defects in STAT6 phosphorylation are found in most patient lymphocytes. Increased expression of STAT6 target genes was detected in human cell lines transduced with *STAT6* GOF variants.[163,164] A high frequency of IL5, IL13, and IL4 memory CD4 T cells suggested a skewing towards the Th2 pathway in these patients.[163,164]

Laboratory diagnostics for genetic variants in STAT

While an increasing number of variants in the STAT genes are being described and validated, the pathogenicity of new, unknown variants must be confirmed through functional validation. However, the functional laboratory tests for STATs are not always straightforward and available in routine diagnostics in most laboratories. STAT proteins are involved in various immunological processes, and defects may not necessarily completely block the differentiation of specific immune cells but rather result in functional impairments. Genetic variants in the STAT genes can have one or more of the following effects on the function of the STAT protein: 1) changes in protein expression, 2) change in phosphorylation of the STAT protein, 3) changes in the binding capacity to DNA, 4) changes in the activation of STAT-regulated genes. We will discuss several tests that can be used to assess the above-mentioned effects on the function of STAT. Incidentally, most of these tests can also be used to validate other genetic defects in the same STAT signaling pathway.[165]

Immune phenotyping using flow cytometry

Immune phenotypic analysis using flow cytometry is of limited value in the diagnostic work-up for STAT defects, since STAT defects do not necessarily lead to a defect in the absolute number of T-, B- or NK cells. However, in some of the patients, STAT defects can lead to changes in the T-and B-cell subsets.

For *STAT1* LOF variants, available articles reported varying data sets. In general, most patients had normal immune cell (sub) populations. However, these individuals tend to have compromised antibody production when exposed to protein antigens. Approximately one-fourth showed high white blood cell counts, and one-third had eosinophilia. Further analysis of lymphocyte subsets revealed that the proliferation of $CD4^+$ T lymphocytes, $CD19^+$ or $CD20^+$ B lymphocytes, and $CD27^+CD19^+$ memory B lymphocyte populations remained within normal ranges and around 20% showed elevated NK cell counts.[33]

In the context of *STAT1* GOF variants, approximately one-third of the patients exhibit diminished $CD4^+$ T cell counts and impaired T cell proliferation responses to mitogens or antigens. Additionally, more than 80% of individuals with STAT1 GOF variants experience a reduction in circulating Th17 cell numbers.[55,166] Roughly half of the patients display decreased memory B lymphocyte levels, while approximately one-third exhibit reduced NK cell counts.[166]

Approximately 90% of the patients with *STAT3* LOF variants are deficient for IL-17-producing Th17 lymphocytes or exhibit diminished proportions of Th17.[103,104] Consequently, the consideration of specialized assessments, such as *ex vivo* staining for IL-17A following induction of naïve $CD4^+$ T-lymphocytes, becomes an available option, for example, following stimulation with CD2/CD3/CD28 microbeads or PMA (phorbol-12-myristate-13-acetate), ionomycin with Brefeldin A.[103,167] However, assessing the Th17 population may pose challenges within the scope of routine laboratory investigations. Alternatively, the feasibility of assessing a reduction in circulating $CD19^+CD27^+$ memory B lymphocytes which is observed in over 90% of patients, may be more pragmatic.[97,99,168-170]

The majority of the patients with *STAT3* GOF variants have aberrancies in their immune phenotype. Decrease in Treg cells and an increase in Th17 cells were expected but the number of subjects in which Treg and Th17 cells were quantified are still inadequate to show a consistency of phenotype in both subsets. Elevated levels of double negative $CD4^-/CD8^-$ T cells were measured in *STAT3* GOF patients which is comparable with autoimmune lymphoproliferative syndrome (ALPS). Decreased memory B cells and decreased NK cells were also reported in some patients.[171] In patients with *STAT5B* variants the immune phenotype is variable; however, reduced number of NK-cells, T cells and regulatory T cells have been reported.[110,172]

Expression and phosphorylation of STAT proteins

The expression and phosphorylation of the STAT proteins is essential for the function of STAT and can be measured using flow cytometry or western blot. Since the different STAT proteins signal downstream of different receptors and are expressed in different cell types, it is important to analyze the appropriate cell type and stimulate the cells with a ligand specific for the STAT protein (**Table 1**). *STAT1* LOF variants can lead to reduced expression or phosphorylation of the STAT protein, while GOF variants may lead to increased phosphorylation of the STAT protein, which may be detected at baseline (unstimulated conditions) or following cytokine induction. Occasionally, the level of phosphorylation is not changed, yet the persistence of phosphorylation is prolonged compared to controls. Thus, when there is suspicion of a GOF, it becomes crucial to assess STAT phosphorylation at multiple time points post-stimulation. Notably, GOF variants in STAT do not necessarily induce alterations in phosphorylation. While STAT1 GOF variants commonly result in prolonged or hyperphosphorylation, this pattern does not apply to STAT3 GOF variants.[113,118,119] Therefore, if a GOF variant is predicted but no apparent changes in phosphorylation are observed, additional functional assays are required to confirm or rule out a GOF variant.

DNA binding capacity of the STAT proteins

A commonly used method to assess the binding of transcription factors like STAT to DNA is to use a electrophoretic mobility shift assay (EMSA). In this assay, after cell stimulation and protein isolation, the STAT protein is incubated with a labeled probe containing a GAS or ISRE element specific to that particular STAT protein. Following incubation, this probe/protein mixture is separated by size on a gel. Test specificity is enhanced by using larger probes that bind to STAT proteins, resulting in a noticeable shift in size compared to unbound probes (referred to as naked probes). Particularly, genetic variants in the DNA binding domain may lead to either diminished or enhanced binding to the probe. However, it is important to note that this technique is labor-intensive and technically challenging, rendering it less suitable for routine use in diagnostic laboratories.

Induction of STAT regulated target genes and proteins

The induction of STAT regulated target genes or proteins can be measured using different techniques, including a luciferase reporter assay, real-time quantitative PCR, or an immunoassay for downstream STAT inducible targets. In many publications, luciferase assay is used to measure STAT transcriptional activity. For this assay an expression plasmid with the STAT variant has to be made and transfected in a cell line together with the specific GAS or ISRE luciferase plasmid. The cloning and transfection of the cell line is labor-intensive and can take quite some time, making this assay also not suitable for routine diagnostics. An alternative for this assay is to stimulate mononuclear cells form the patient and measure the induction of expression of STAT regulated genes by real-time quantitative PCR or immunoassays. The induction of RNA expression can already be measured after a few hours of stimulation, and to measure (secreted) proteins usually 24-48 hours is sufficient. The above-described assays are an indirect measure for STAT function, but the advantage is that these tests can be performed with techniques that are available in most immunological laboratories.

Conclusions

A large number of patients with genetic defects in STAT genes have been documented. The clinical manifestations are intricately linked to the specific affected STAT gene and the nature of the variant; gain-of-function (GOF) or loss-of-function (LOF). The clinical outcomes of these patients vary with each specific defect, emphasize the complexity of the STAT signaling pathway, necessitating tailored approaches to patient management.

With the increasing availability of genetic testing, a growing number of variants are being discovered. Some of these variants have known clinical significance, while others pose unknown implications. This expanding landscape underscores the importance of robust functional testing for STAT variants, despite the technical challenges and the time-intensive nature of such testing. Therefore, it is imperative to publish newly identified functional variants to genetic databases, alongside detailed phenotypic information. This will contribute to a collective understanding that could enhance our insight into the clinical implications and therapeutic strategies for individuals with STAT deficiencies.

Conflicts of Interest

The authors declare no conflict of interest.

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- Figures, K.M.
- Supervision, N.H. and P.M.H.
- All authors have read and agreed to the published version of the manuscript.

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