

Human *STAT1* gain-of-function heterozygous mutations: chronic mucocutaneous candidiasis and type I interferonopathy

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Abstract

Heterozygous gain-of-function (GOF) mutations in *STAT1* in patients with chronic mucocutaneous candidiasis (CMC) and hypothyroidism were discovered in 2011. CMC is the recurrent or persistent mucocutaneous infection by *Candida* fungi, and hypothyroidism results from autoimmune thyroiditis. Patients with these diseases develop other infectious diseases, including viral, bacterial, and fungal diseases, and other autoimmune manifestations, including enterocolitis, immune cytopenia, endocrinopathies, and systemic lupus erythematosus. *STAT1*-GOF mutations are highly penetrant with a median age at onset of 1 year and often underlie an autosomal dominant trait. As many as 105 mutations at 72 residues, including 65 recurrent mutations, have already been reported in more than 400 patients worldwide. The GOF mechanism involves impaired dephosphorylation of STAT1 in the nucleus. Patient cells show enhanced STAT1-dependent responses to type I and II interferons (IFNs) and IL-27. This impairs Th17 cell development, which accounts for CMC. The pathogenesis of autoimmunity likely involves enhanced type I IFN responses, as in other type I interferonopathies. The pathogenesis of other infections, especially those caused by intramacrophagic bacteria and fungi, which are otherwise seen in patients with diminished type II IFN immunity, has remained mysterious. The cumulative survival rates of patients with and without severe disease (invasive infection, cancer, and/or symptomatic aneurysm) at 60 years of age are 31% and 87%, respectively. Severe autoimmunity also worsens the prognosis. The treatment of patients with *STAT1*-GOF mutations who suffer from severe infectious and autoimmune manifestations relies on hematopoietic stem cell transplantation and/or oral JAK inhibitors.

Introduction

Human signal transducer and activator of transcription 1 (STAT1), one of seven members of the STAT family, is a latent cytoplasmic transcription factor that mediates cell signaling in response to multiple stimuli, including type I, II, and III interferons (IFNs) and interleukin (IL)-27 (1-8). When these ligands bind the two chains of their receptors, Janus kinases (JAKs), JAK1 and JAK2, and/or TYK2 that are constitutively associated with the receptor chains are brought together and activated. These tyrosine-protein kinases phosphorylate the receptors, which recruit STAT1, which is then phosphorylated at tyrosine 701 (Y701) by JAKs. Phosphorylated STAT1 can form a homodimer known as gamma-interferon activation factor (GAF), after stimulation by type II IFN and IL-27 and, to a lesser extent, type I and III IFNs. GAF translocates to the nucleus and binds gamma-interferon-activated sites (GAS) to up- or downregulate IFN-stimulated and IFN-regulated genes (ISGs and IRGs, respectively) (9). Phosphorylated STAT1 also forms a heterotrimer known as interferon-stimulated gene factor 3 (ISGF3) with STAT2 and IRF9 after stimulation by type I or III IFNs. ISGF3 then binds the interferon-stimulated response element (ISRE) and induces or regulates gene transcription (9).

In humans, STAT1 plays a nonredundant role in type I, II, and III IFN and IL-27 signaling (3, 7, 8, 10, 11). Type II IFN-induced GAF-mediated signaling is essential to eliminate intramacrophagic pathogens such as mycobacteria, whereas type I IFN-induced ISRE-mediated signaling plays a pivotal role in host immunity against viruses (12). Inborn errors of human STAT1 with loss-of-function (LOF) mutations cause three types of primary immunodeficiencies (PIDs): i) autosomal recessive (AR) complete STAT1 deficiency (13-16), ii) AR partial STAT1 deficiency (17-19), and iii) autosomal dominant

(AD) STAT1 deficiency (20-27). Patients with AR complete STAT1 deficiency have biallelic complete LOF mutations of *STAT1*. These patients develop life-threatening viral infections, especially herpesvirus and mycobacterial infections, reflecting the lack of STAT1-mediated type I, II, and III IFN and IL-27 signaling (13-17). Hematopoietic stem cell transplantation can resolve these life-threatening conditions (12, 16). AR partial STAT1 deficiency, caused by biallelic hypomorphic *STAT1* mutations, is a milder form of disease, as patients with this disorder suffer from mild viral and mycobacterial disease (17-19). The clinical penetrance of AR STAT1 deficiency in patients is complete (14-19). In contrast, patients with AD STAT1 deficiency develop Mendelian susceptibility to mycobacterial diseases (MSMD), a PID characterized by selective predisposition to mycobacteria in the absence of other prominent immunodeficiencies (20-28). These patients carry heterozygous *STAT1*-LOF mutations for both the type I and II IFN pathways; however, paradoxically, heterozygosity selectively impairs type II IFN-induced GAF-mediated signaling without disturbing type I IFN-induced ISGF3-mediated signaling (20). The clinical penetrance of *STAT1* mutations in AD STAT1 deficiency is incomplete (21-23, 27).

***STAT1* gain-of-function mutations as the genetic etiology of syndromic CMC**

Chronic mucocutaneous candidiasis (CMC) is characterized by recurrent or persistent infection of the nails, skin, and oral and genital mucosa by *Candida* species. CMC is among the broad infectious manifestations of severe T cell immunodeficiencies (29-32). Most patients with AD hyper-IgE syndrome (mutations in *STAT3* but not *IL6ST*) (33-36) and most patients with AR ROR γ T (37) or ZNF341 (38, 39) deficiency present CMC with fewer other infections than those presented by patients with severe T cell

immunodeficiencies. All of these conditions exhibit a scarcity of IL-17-producing T cells and are categorized as syndromic CMC (SCMC). AR AIRE deficiency also causes SCMC by the production of neutralizing autoantibodies against IL-17A and/or IL-17F (40, 41).

The first genetic etiologies of the isolated form of CMC (ICMC), in which CMC is the major clinical manifestation in otherwise healthy individuals, were deciphered with the identification of AR IL-17RA and AD IL-17F deficiencies (42). This discovery was compatible with previous mouse studies showing the essential roles of IL-17 cytokines in the host defense against mucosal immunity to *C. albicans* (43-46). The apparent lack of other infections, in addition to mucocutaneous bacterial infections in patients with AR IL-17RA deficiency, was surprising and indicated the high redundancy of this pathway in humans (47, 48). The identification of these monogenic diseases clearly indicated that inborn errors of human IL-17 immunity underlie ICMC. However, ICMC due to inborn errors of IL-17 cytokines or receptors is relatively rare, with 5 (*IL17F*), 23 (*IL17RA*), and 3 (*IL17RC*) patients reported to date (42, 48-50). Mutations in *TRAF3IP2*, which encodes ACT1, downstream of the IL-17 receptors have been found in only 7 patients (31, 51-53). In addition, very recently, a heterozygous loss-of-expression and LOF mutation in *MAPK8*, which encodes JNK1, was identified in a multiplex family with CMC and connective tissue disorder (54). This discovery indicates that the human JNK1-dependent MAPK signaling pathway is essential for IL-17A- and IL-17F-dependent mucocutaneous immunity to *Candida*.

In 2011, whole-exome sequencing led to the identification of germline *STAT1* mutations as a genetic etiology of CMC in patients with hypothyroidism (55, 56). These *STAT1* mutations were shown to be GOF mutations due to hyperphosphorylation of STAT1 at Y701 in response to stimulation with type I and II IFNs and IL-27 (55). Follow-

up studies found that *STAT1*-GOF mutations can account for more than half of cases of inherited CMC, which in most cases is syndromic (57-60). Indeed, *STAT1*-GOF mutations were shown to cause broader than expected infectious and noninfectious phenotypes in addition to CMC (57, 58). Therefore, *STAT1*-GOF mutations are currently thought to cause SCMC.

Genetics of *STAT1*-GOF mutant alleles

As many as 105 *STAT1*-GOF mutations have been reported (Fig. 1A, Table 1) (55-137). In contrast, fewer *STAT1*-LOF mutations that cause AR complete STAT1 deficiency (4 mutations) (14-16), AR partial STAT1 deficiency (4 mutations) (17-19), and AD STAT1 deficiency (10 mutations) (20-26) have been reported thus far (Fig. 1B, Table 2-4). *STAT1*-GOF mutations were originally identified in the coiled-coil domain (CCD) of STAT1 (55, 56). They were also later found in other domains of STAT1 (58, 103, 105). The majority (87.6%) of these GOF mutations are in the CCD and DNA-binding domain (DBD) of STAT1; GOF mutations in the CCD alone account for half (52.6%) of the cases (58). Four mutations, A267V, R274Q, R274W and T385M, are thought to be recurrent mutations due to hotspot events, but not founder events (Table 1). Moreover, different mutations at the same residue, such as R274Q, R274W, and R274G, have been found for as many as 24 residues. Among 105 mutations, 83 mutations were demonstrated to be GOF mutations by transient gene expression experiments, such as reporter assays (Table 1). The pathogenesis of disease due to the other 22 mutations, excluding four mutations (R70P, T133A, E284K and L354V) (67, 137), has been proven by detecting increased STAT1 phosphorylation or impaired dephosphorylation using patient cells. None of these *STAT1*-GOF mutations, excluding the T133A mutation which

was inherited from an asymptomatic mother (137), can be found in the Genome Aggregation Database (gnomAD), which contains whole-exome sequencing data from 123,136 individuals. The combined annotation-dependent depletion (CADD) score and the minor allele frequency (MAF: based on gnomAD) of all the heterozygous variants of STAT1 found in gnomAD and *STAT1*-GOF mutations were compared as previously described (138) (Fig. 2). Compared with common variants found in gnomAD, *STAT1*-GOF mutations showed lower MAFs and relatively higher CADD scores.

Cellular features

Enhanced STAT1 phosphorylation in response to stimulation with type I and II IFNs and IL-27 was first characterized by immunoblot analysis of Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (EBV-LCLs) derived from patients (55). This finding has been repeatedly confirmed by further studies in which whole peripheral blood mononuclear cells (PBMCs), CD3⁺ or CD4⁺ T cells, NK cells, and monocytes were analyzed upon stimulation with type I and II IFNs or IL-27 (57, 59, 64-66, 74, 77, 80, 82-84, 88-90, 94, 96, 104, 109, 112, 116, 117, 131, 132, 139-141). Fibroblast cell lines from a patient also showed increased STAT1 phosphorylation upon type II IFN stimulation (76). An enhanced response to type III IFN is also expected but has not yet been proven. PBMCs (59, 80) and EBV-LCLs (60, 95, 98) from patients showed persistent STAT1 phosphorylation in the presence of the protein kinase inhibitor staurosporine. On one hand, increased STAT1 protein levels in primary cells from patients with *STAT1*-GOF mutations have been reported in some studies (104, 118, 128, 141).

Molecular and biological features

In patients with AR complete or partial STAT1 deficiency, *STAT1* mutations result in the complete or partial loss of STAT1 protein expression, leading to the impairment of STAT1-mediated type I, II, and III IFN and IL-27 signaling (14-19). MSMD-related *STAT1* mutations do not disturb STAT1 protein expression but exert a dominant-negative effect on WT STAT1-mediated type II IFN signaling (20-27). In contrast, CMC-related *STAT1* mutations are GOF mutations that lead to hyperphosphorylation of STAT1 in response to stimulation with type I and II IFNs and IL-27 (55). The underlying mechanism occurs through enhanced STAT1 phosphorylation due at least in part to impairment of the nuclear dephosphorylation of activated STAT1 (55). Liu et al. first reported that a STAT1-null fibrosarcoma cell line (U3C cells) with transient expression of mutant STAT1 showed persistent STAT1 phosphorylation in the presence of the protein kinase inhibitor staurosporine (55). This observation suggests that the impairment of nuclear dephosphorylation is the molecular mechanism underlying STAT1 hyperphosphorylation. Impaired or delayed dephosphorylation was repeatedly confirmed in several subsequent studies through transient gene expression experiments (97, 114, 130) and the use of patient cells (59, 60, 95, 98, 118).

STAT1 forms two types of homodimers depending on its phosphorylation status, parallel dimers and antiparallel dimers (142, 143). Phosphorylated STAT1 forms parallel dimers, whereas unphosphorylated STAT1 preferentially forms antiparallel dimers with the reciprocal binding of CCD and DBD (CCD/DBD) (144). Kagawa et al. hypothesized that CCD/DBD play an important role in controlling STAT1 activity (23). They mutagenized 342 individual wild-type amino acids in the CCD and DBD to alanine and functionally investigated the effects of those alanine substitutions by measuring type II IFN-induced GAS transcriptional activity. This assay, called systemic alanine scanning,

correctly predicted 100% of previously reported LOF mutations and 78.1% of known GOF mutations in the CCD/DBD of STAT1. The majority of the GOF alanine substituents were located at the interface of the antiparallel STAT1 dimer, suggesting that GOF mutations disrupt dimerization for the formation of antiparallel STAT1 structures. Formation of the antiparallel STAT1 dimer facilitates phosphatase access by presenting phosphorylated Y701 at both ends of the antiparallel dimer for its ready dephosphorylation (142, 144). Therefore, impairment of the dimerization of STAT1 to form antiparallel dimers may lead to resistance against dephosphorylation by phosphatase enzymes. Known and candidate phosphatases that dephosphorylate STAT1 include TCPTP (PTPN2) and SHP-2 (PTPN11) (145). Whether their activities against STAT1-GOF mutant proteins are decreased remains to be tested.

Recently, other possible mechanisms to explain the increased STAT1 phosphorylation observed in patients with *STAT1*-GOF mutations have been suggested (141). Bernasconi et al. identified increased STAT1 protein levels in patient cells (128). Based on this discovery, they reported that increased STAT1 phosphorylation in patients is the consequence of not only impaired dephosphorylation but also increased amounts of total STAT1. Moreover, Zimmerman et al. reported that *STAT1*-GOF mutations cause increased STAT1 protein levels, leading to high levels of STAT1 phosphorylation with normal levels of STAT1 dephosphorylation. Peterson et al. also reported that *STAT1*-GOF mutations cause the premature nuclear import of phosphorylated STAT1 without altering the phosphorylation or dephosphorylation rate (133). The mechanism underlying this increase in the amount of STAT1 protein remains to be characterized, but increased expression of *STAT1* mRNA was found in patients with *STAT1*-GOF mutations (104, 141). In addition, increased STAT1 protein levels with high *STAT1* mRNA levels were

confirmed in a knock-in mouse model with a mutation equivalent to the R274Q mutation in humans (146). Further evidence is required to conclude the molecular mechanisms underlying the hyperactivation of STAT1 in patients with *STAT1*-GOF mutations.

Immunological features

A study of a large cohort of patients with *STAT1*-GOF mutations ($n=274$) revealed the reduced frequency of CD4⁺ T cells (in 28% of the patients studied), CD8⁺ T cells (in 16% of the patients studied), CD19⁺ B cells (in 19% of the patients studied), CD19⁺CD27⁺ memory B cells (in 49% of the patients studied), and CD16⁺CD56⁺ NK cells (in 25% of the patients studied) (58). Impairment of the terminal maturation of NK cells, as shown by the decreased proportions of CD56^{dim} NK cell subsets, with decreased NK cell cytotoxic function was noted (104, 109). However, these findings are not diagnostically relevant; therefore, it is difficult to speculate on this congenital disorder based on the results of general immunological tests. Some patients display dysgammaglobulinemia involving high serum levels of total IgG (in 20% of the patients tested) or low serum levels of total IgG (in 3% of the patients tested), IgG2 (in 38% of the patients tested) or IgG4 (in 50% of the patients tested). The impairment of antigen-specific antibodies against tetanus, diphtheria toxoid, or poliovirus was found in 23% of the patients tested.

A lower proportion of Th17 cells in the peripheral blood, which can explain at least in part the cause of CMC, is frequently, but not always, observed in patients with *STAT1*-GOF mutations (55). However, the molecular mechanisms accounting for the decrease in Th17 cells remain to be elucidated (31). Patient cells showed enhanced STAT1-dependent responses to type I and II IFNs and IL-27 (55). Type I and II IFNs and IL-27, which

predominantly signal via STAT1, are known to inhibit IL-17 T cell development in mice and humans (147). Therefore, the enhancement of type I and II IFN- and/or IL-27-induced STAT1 signaling might explain the inhibition of Th17 cells in those patients. On one hand, IL-6, IL-21 and IL-23 promote Th17 development mainly via the activation of STAT3-mediated signaling (12, 55). Patients with impaired STAT3 signaling due to a dominant negative mutation in *STAT3* (*STAT3*-DN) develop CMC with a decreased frequency of Th17 cells (148). Zhang et al. investigated the mechanism underlying this decrease in Th17 cells by focusing on the clinical and cellular similarities between *STAT1*-GOF and *STAT3*-DN patients (116). They found that naïve T cells upregulate PD-L1 after IL-27 stimulation, leading to the inhibition of Th17 differentiation, in both disorders. The upregulation of PD-L1 was also observed at the basal level in naïve CD4⁺ T cells from patients with *STAT1*-GOF mutations and is thought to be correlated with the inhibition of Th17 differentiation (96).

Ma et al. intensively investigated CD4⁺ helper T cells and identified that patients with *STAT1*-GOF mutations show increased Th1 cell numbers, decreased Th17 cell numbers, and almost normal Th2 and Tfh cell numbers (149). They also found altered proportions of circulating follicular helper (cTfh) cells, which regulate the development of antigen-specific B cell immunity, in patients with *STAT1*-GOF mutations, similar to patients with *STAT3*-DN mutations. Both types of patients presented significant reductions in the number of CCR6⁺CXCR3⁻ cTfh cells, the most proficient B-helper cTfh cell population. They also showed that cTfh cells from these patients produced high levels of IFN- γ , resulting in the inhibition of Tfh-induced B-cell differentiation (149). The strong similarities in the effects of *STAT1*-GOF and *STAT3*-DN mutations on CD4⁺ T cell differentiation may indicate a putative inhibitory effect of hypermorphic *STAT1*

mutations on activity of STAT3 (139, 150, 151). The altered naïve CD4⁺ T cell differentiation for STAT1-GOF suggest the humoral immune defects are CD4⁺ T cell intrinsic. However, intrinsic B cell defects also contributes to poor antibody responses in STAT1-GOF (150). IL-21 plays an important role in isotype switching, affinity maturation, antibody production, and differentiation of B cells (152-154). But, the naïve B cells from patient with STAT1-GOF show poor response to IL-21, resulting in reduced secretion of IgM, IgG, and IgA (150). The increased B-cell apoptosis is also pointed out in patients with STAT1-GOF (96).

Inborn error of immunity due to upregulated type I IFN signaling, called type I interferonopathy, causes severe inflammatory phenotypes and autoimmunity (155-157). Type I interferonopathies, first proposed in 2011, are defined as Mendelian disorders associated with upregulated type I IFN signaling due to inappropriate stimulation of the type I IFN response pathway or defective negative regulation of the type I IFN system (156). In addition, PBMCs from patients with *STAT1*-GOF mutations also show an enhanced response to type I IFNs associated with the hyperphosphorylation of STAT1 (125, 158). Whether diseases caused by *STAT1*-GOF mutations should be included among type I interferonopathies is under discussion. As of 2016, diseases caused by *STAT1*-GOF mutations were not included in this disease category because of insufficient evidence of a functional relationship between ISG production and clinical phenotype (155).

Inappropriate exposure to type I IFN is detrimental to mammals (159). Indeed, patients treated with recombinant type I IFNs sometimes present autoimmune conditions, including SLE, autoimmune thyroid disease, type 1 diabetes mellitus, Sjögren's syndrome, hemolytic anemia, thrombocytopenia, hypothyroidism, inflammatory myositis,

Raynaud's disease and vitiligo (160, 161). Patients with type I interferonopathies, monogenic diseases in which type I IFN production is constitutively upregulated, develop various developmental manifestations, as well as signs of immunodeficiency and autoinflammation (162). They also develop several autoimmune manifestations with neurological and dermatological phenotypes, particularly SLE (155, 157, 159). In general, patients with *STAT1*-GOF mutations do not present the typical neurological and/or dermatological phenotypes associated with type I interferonopathies. However, SLE is a representative complication in patients with *STAT1*-GOF mutations. In addition, autoimmunity is a common feature of patients with *STAT1*-GOF mutations. Indeed, this autoimmunity can be early and severe and occasionally resembles the series of symptoms in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (108). Autoimmunity in patients with IPEX syndrome results from defects in regulatory T cells (Tregs) due to mutations in *FOXP3*, a master transcriptional factor in Tregs (163). In contrast, Treg numbers and function were found to be intact in *STAT1*-GOF patients with severe autoimmunity that resembled IPEX syndrome (these patients were diagnosed with an IPEX-like syndrome) (108). Kaleviste et al. focused on this clinical observation and investigated type I IFN signaling in *STAT1*-GOF patients (132). These *STAT1*-GOF patients showed the clear expression of genes with an interferon signature, whereas circulating levels of IFN- α were not persistently elevated. The authors also examined enrichment in trimethylation of the lysine 4 residue of histone 3 (H3K4me3), an epigenetic modification associated with the activation of transcription, in areas associated with ISGs in *STAT1*-GOF patients. Together with calcification of the blood vessels and SLE-like disease, which are characteristics shared between type I interferonopathies and *STAT1*-GOF, this suggests that *STAT1*-GOF patients are predisposed to IFN-related

autoimmunity. Currently, STAT1-GOF are not included among type I interferonopathies, but we see no reason why they should not be, given that *STAT1*-GOF mutations have been shown to result in increased type I IFN responses in various cell types (55, 57, 83, 84, 90, 131, 132, 139, 155).

Clinical manifestations

The largest systematic study to investigate the clinical manifestations of patients with *STAT1*-GOF mutations was reported in 2016 (58). This study investigated 274 patients proven to have *STAT1*-GOF mutations from 167 families originating from 40 countries (58). Among these 274 cases, 167 (61%) were familial cases (60 families). The penetrance of *STAT1*-GOF mutations was complete, with 98% of patients who developed CMC showing a median age at onset of one year. None of the remaining 2% of cases (6 patients) were totally asymptomatic. Indeed, five had an invasive bacterial infection, one had an invasive fungal infection, 4 had hypothyroidism, and 1 had cerebral aneurysm. The global penetrance of STAT-GOF is therefore considered to be almost complete. The male/female ratio among the patients was 1.03, suggesting that this disorder is distributed equally among sexes. This large cohort study revealed detailed infectious and noninfectious manifestations of *STAT1*-GOF mutations in patients.

A) Infectious manifestations

The majority of the patients developed CMC (98%: n=268), with a median age at onset of one year (58). Mucocutaneous fungal infections, mostly caused by *Candida albicans*, affected the oral mucosa (93%: n=254), skin (57%: n=155), esophageal/genital mucosa (56%: n=153), nails (56%: n=56), and/or scalp (20%: n=55). Superficial

dermatophytic infection of the scalp, skin or nails was suspected in 16% of patients with microbiological confirmation (*Trichophyton* spp. or *Microsporon* spp. isolated in 52% of those patients). Twenty-eight patients (10%) developed an invasive fungal infection, including invasive candidiasis (4%: n=10), fungal pneumonia (6%: n=17), or cryptococcal meningitis (1%: n=3). The causative agents of these invasive fungal infections were *Candida* spp. (29% of the isolated fungi), *Pneumocystis jirovecii* (18%), *Cryptococcus* spp. (18%), *Aspergillus* spp. (15%), and others (20%).

Many of the patients in this cohort study (74%: n=202) also developed bacterial infections, including lower respiratory infections (47%: n=129), ENT infections (44%: n=121), and/or skin infections (28%: n=77) (58). Recurrent or chronic sinusitis or otitis media account for most of the ear, nose, and throat infections, and folliculitis was the main cause of the skin infections. Bacteria were isolated from 99 patients (36%), and the causative agents were found to be *Staphylococcus aureus* (36% of the isolated bacteria), *Streptococcus* spp. (20% of the isolated bacteria), *Pseudomonas aeruginosa* (13% of the isolated bacteria), *Haemophilus influenzae* (9% of the isolated bacteria)), and other bacteria (21%). Mycobacterial infections were found in 6% of patients with *STAT1*-GOF mutations, and these infections were mostly caused by *Mycobacterium tuberculosis* (35% of the mycobacteria isolated), Bacille Calmette-Guérin (BCG) (30% of the mycobacteria isolated), or other mycobacteria (35% of the mycobacteria isolated), suggesting that patients with *STAT1*-GOF mutations are at risk for mycobacterial infection.

The molecular mechanism underlying mycobacterial disease in patients with *STAT1*-GOF mutations has remained elusive. IFN- γ -induced GAF-mediated signaling is essential to eliminate mycobacteria. IFN- γ production in *STAT1*-GOF patients has been investigated in several studies. Some studies showed that IFN- γ production in response

to cytokine or polyclonal stimulation was normal upon the stimulation of PBMCs (73, 78, 89, 100, 114) or CD4⁺ T cells (65, 88, 96, 98, 105, 114, 150) with PMA and ionomycin, whereas other studies found decreased IFN- γ production by PBMCs (56, 93, 100, 119-123, 129) or CD4⁺ T cells (66, 101, 118-123, 149). In addition, some studies detected increased type II IFN production by PBMCs (94), CD4⁺ T cells (64, 74, 82, 112), or CD8⁺ T cells (82, 112). These results suggest that IFN- γ production in response to cytokine or polyclonal stimulation is highly variable among patients with *STAT1*-GOF mutations. IFN- γ production by PBMCs (60, 89, 114) and CD4⁺ T cells (114) in response to *Candida* antigen was found to be normal, but IFN- γ was barely detected upon PBMC stimulation in other studies (73, 100, 129). Weak IFN- γ production by patient PBMCs upon stimulation with heat-killed *S. aureus* (73), β -glucan (78), and *Penicillium marneffei* (129) was also reported. In our experience, the purified protein derivatives of tuberculin (PPD) skin test in a patient with mycobacterial infections was negative at 3 and 6 years of age, indicating a defective T cell response (95). Kataoka et al. identified a patient with an R274G *STAT1*-GOF mutation and disseminated *M. tuberculosis* infection despite the negative results of QuantiFERON-TB Gold Plus and PPD skin tests. They hypothesized that the pathogenesis of the infection was the exhaustion of specific immune subsets sensitive to the aberrant activation of STAT1 (80). In addition, an impaired response to type II IFN restimulation is thought to be responsible for mycobacterial susceptibility (98). Additional cases and experimental verification are required to explain the host susceptibility to mycobacteria observed in STAT1-GOF patients.

Approximately 38% of STAT1-GOF patients showed susceptibility to viral infections, developing recurrent mucocutaneous viral infections (32%: n=88) or at least one systemic or atypical viral infection (8%: n=18) (58). The main causes of

mucocutaneous viral infections were herpes simplex virus (HSV) (27% of the viruses isolated) and varicella-zoster virus (VZV) (31%), which causes severe chickenpox or shingles in childhood. Recurrent molluscum contagiosum or warts were also frequent and found in 12% (n=32) of patients with *STAT1*-GOF mutations. Systemic viral infection was mainly associated with cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infection. Uncontrollable CMV infection requiring antiviral treatment occurred in 8 patients (3%). In contrast, 10 patients (4%) developed a chronic active EBV infection that was not severe and did not require specific treatment. Although the frequency was low, systemic or atypical viral infections associated with human herpesvirus 6 (HHV6), parvovirus, BK virus, and hepatitis C virus (HCV) were also reported. Furthermore, two patients developed live vaccine (smallpox and measles)-induced severe disease.

In addition to the infections reported in this large cohort study (58), several individual case reports or small studies on a series of patients with rare infections have been reported. In terms of viral infections, progressive multifocal leukoencephalopathy caused by the polyomavirus JC virus (n=4) (115) and Orf infection, a zoonotic infection caused by a dermatotropic parapoxvirus (n=1) (81), have been described. In terms of fungal infections, cutaneous infection caused by *Fusarium solani* (fusariosis) (110) or *Demodex* spp. (demodicidosis) (99, 126) and disseminated infection caused by *Coccidioides immitis* (coccidioidomycosis) (58, 98), *Histoplasma capsulatum* (histoplasmosis) (98), *Penicillium marneffei* (124, 129), or *Apophysomyces trapeziformis* (mucormycosis) (58, 85) have been reported. Both coccidioidomycosis and histoplasmosis have been reported in patients with autosomal dominant partial IFN- γ R1 deficiency (164, 165). In addition, *Penicillium marneffei* infections, coccidioidomycosis, and histoplasmosis have been reported in patients with anti-type II IFN antibody (166,

167). These observations, together with their susceptibility to mycobacteria, suggest that patients with *STAT1*-GOF mutations may disturb type II IFN induced host immune response.

B) Autoimmunity

More than one third of patients with *STAT1*-GOF mutations presented with autoimmune manifestations (37%: n=60) in the large cohort study (58). The male/female ratio was 0.79, suggesting that affected women have higher risk of autoimmunity. Endocrine organs were main target of autoimmunity, including hypothyroidism (22%: n=60), type 1 diabetes mellitus (4%: n=11), and hyperthyroidism (n=1). Some of the patients developed cutaneous diseases (10%: n=28), including vitiligo, alopecia, or psoriasis. Five female patients developed SLE and one patient had scleroderma. Some of the patients developed autoimmune hepatitis (2%: n=6), hematological autoimmunity (4%: n=11) which cause hemolytic anemia or autoimmune thrombocytopenia, and autoantibody positive pernicious anemia (n=1) or celiac disease (n=4). Most patients with autoimmune manifestations were positive for autoantibodies (65% of the patients tested for the presence of autoantibodies). Inflammatory bowel disease was found in 6 patients, including Crohn's disease (n=2), ulcerative colitis (n=2), and enteropathy with lymphocytic infiltration (n=2).

In addition to this study, Uzel *et al.* reported 5 children who presented severe autoimmune symptoms, resembling those found in patients with IPEX syndrome (108). These patients presented severe autoimmunity from infancy (one from toddler), such as protein losing enteropathy (n=2), villous blunting or atrophy (n=3), hematological autoimmunity (n=2), and type 1 diabetes mellitus (n=3). These patients also presented

CMC (n=4) together with bacterial (n=5) and/or viral (n=3) infections. Leiding *et al.* also described 5 patients with IPEX-like syndrome, who were treated with hematopoietic stem cell transplantation (86). Interestingly, 5 of these 10 unrelated patients with IPEX-like syndrome carried the T385M *STAT1* mutation, suggesting that specific GOF mutations may be related to the development of severe autoimmunity

C) Others

Patients with *STAT1*-GOF mutations present a broad clinical spectrum of variable severity. Some of the patients suffer from life-threatening infections (64, 86, 100). Persistent or recurrent lower respiratory tract infections result in bronchiectasis (14-21%) (57, 58, 66, 108). Persistent or recurrent mucocutaneous fungal infections may lead to the development of squamous cell carcinoma. Indeed, squamous cell carcinomas which affect cutaneous, gastrointestinal, or laryngeal regions, were found in 4% of the patients with *STAT1*-GOF mutations (58). As for the other rare symptoms, a case with enamel defect and delayed dental shedding (n=1), and a case with psoriasiform hyperkeratosis (n=1) have also been reported (73, 93).

Aneurysm occurs at a higher rate in patients with CMC than in healthy individuals (168-170). The large cohort study revealed that 6% (n=17) of patients with *STAT1*-GOF mutations had aneurysms (58). Most aneurysms were located in the cerebral vascular system and were found to be multiple aneurysms (55, 56, 58, 66, 106). In contrast, extracerebral aneurysms were less common (68). Indeed, only one genetically confirmed patient who developed recurrent abdominal and thoracic aortic aneurysm with signs of vasculitis has been reported (106). In terms of extracerebral vascular disease, a patient with systemic, inflammatory large-vessel vasculitis (87) and a patient with aortic

calcification (102) have been reported. The cause of aneurysm in these patients remains unclear. In some cases, *Candida* hyphae were identified in the aneurysm tissue by histology, suggesting that they were mycotic aneurysms (60, 86, 168, 169). On the other hand, aneurysm coexisted with autoimmune symptoms such as atopy or thyroid dysfunction in three-quarters of the patients studied (12 of 16 patients with aneurysm) (58). In addition, the coexistence of severe autoimmunity and aneurysm, or the coexistence of vasculitis and aneurysms, was found in several patients with *STAT1*-GOF mutations (57, 106, 108). Aneurysm was reported in patients with *STAT3*-LOF mutations (171) but not in other patients with SCMC. These clinical observations may reflect the link between aneurysm and autoimmunity in patients with *STAT1*-GOF mutations.

Outcome and treatment

Approximately 12% of patients with *STAT1*-GOF mutations died at a median age of 30 years with severe infection (38%), cancer (24%) and/or cerebral hemorrhage associated with aneurysm (15%) (58). Therefore, invasive infection, cancer and/or symptomatic aneurysm are predictors of a poor outcome. Indeed, the cumulative survival rate at 60 years of age in *STAT1*-GOF patients with these predictors was 31%, whereas that of *STAT1*-GOF patients without these predictors was 87% (58). Failure to thrive was found in 12% of patients. Approximately 21% of patients develop bronchiectasis and cystic pulmonary lesions associated with recurrent pneumonia or bronchitis. Recurrent esophageal candidiasis resulted in secondary gastrointestinal complications, such as dysphagia (6.9%) or esophageal stenosis (4.4%).

Most patients with *STAT1*-GOF mutations required long-term topical and/or systemic antifungal treatment (57, 58). Triazoles are frequently used for topical treatment,

and nystatin is a good alternative (57). A large cohort study provided an overview of the treatments used for STAT1-GOF patients (58). Fluconazole is the main first-line oral therapy, followed by itraconazole and/or posaconazole. Approximately 39% of patients treated with long-term antifungal therapy showed clinical resistance to at least one antifungal agent. These patients required second- or third-line treatments, including voriconazole, echinocandins, terbinafine or liposomal amphotericin B. Antibacterial prophylaxis, mainly co-trimoxazole, against recurrent lower respiratory infection was used in 24% of patients. Polyvalent immunoglobulins were used in 13% of patients who suffered from recurrent pneumonia. In terms of noninfectious manifestations, the patients occasionally developed severe autoimmune disorder and were treated with immunosuppressive agents (58, 87, 108).

Hematopoietic stem cell transplantation (HSCT) can be a curable treatment in patients with PIDs and has also been applied in patients who suffer from severe infectious and/or autoimmune manifestations (63, 75, 82, 86). Leiding et al. investigated 15 patients with *STAT1*-GOF mutations treated with HSCT (86). All patients in this cohort suffered from severe infection, and five suffered from severe autoimmunity with a diagnosis of IPEX-like syndrome. The symptoms associated with *STAT1*-GOF mutations disappeared after HSCT, suggesting that HSCT can be a curative treatment for this congenital disorder. However, HSCT simultaneously significantly increased the risk of secondary graft failure, which was found in 50% of patients with primary engraftment, and transplant-related mortality. Indeed, the three-year overall survival after transplantation was only 40%. This study also revealed that GOF mutations in the STAT1 DBD might be associated with more severe clinical manifestations. Indeed, the GOF mutations in 10 of the 15 patients were in the DBD (86), whereas approximately two-thirds of the mutations were identified

in the CCD (58). Among the 15 patients treated with HSCT, 5 patients carried the T385M GOF mutation, which is frequently found in patients with IPEX-like syndrome (86, 108) and/or combined immunodeficiency (64, 100). In addition, some studies have suggested that severe clinical manifestations are correlated with specific *STAT1*-GOF mutations, such as C284R, I294T, C324R, C324F, and T385M (64, 87, 100). These observations suggest a genotype-phenotype correlation, at least for some of the *STAT1*-GOF mutations identified.

Several patients were administered the Janus kinase (JAK) inhibitor ruxolitinib for the treatment of severe clinical manifestations (65, 77, 109, 112, 117, 172, 173). Ruxolitinib seemed to improve CMC and autoimmune manifestations in the majority of the patients treated (65, 77, 109, 112, 172, 173). Forbes et al. investigated 11 patients administered ruxolitinib for the treatment of autoimmunity or immune dysregulation not controlled with other therapies (172). Ten of these patients showed significant clinical improvement due to oral ruxolitinib, suggesting ruxolitinib as a possible therapeutic choice in most patients. In contrast, the therapeutic failure of ruxolitinib with worsening fungal infections, such as CMC and coccidioidomycosis, or herpes zoster infection have also been reported (117, 172). Thus, acyclovir prophylaxis has been used to prevent herpes virus infection during ruxolitinib treatment (172). Ruxolitinib suppresses the hyperresponsiveness of STAT1 to ligand stimulation, leading to the normalization of Th1 and follicular T helper cell responses (112), and the partial rescue of NK cell differentiation and function (109). The effect of ruxolitinib on Th17 cell differentiation and/or IL-17 cytokine production is controversial. Some reports describe a positive effect (112, 173), whereas the frequency of circulating Th17 cells in some patients did not change following treatment (65, 117). Another JAK inhibitor, baricitinib, was used in one

patient (90). The patient showed remarkable clinical improvement in response to oral baricitinib with partial restoration of IL-17A production by PBMCs. A few patients were treated with GM-CSF or G-CSF; however, the effectiveness of these treatments is controversial (113, 174, 175).

Conclusion

STAT1-GOF mutations are the genetic etiology of a unique disorder that combines the manifestations of SCMC and autoimmunity. CMC is caused by impaired IL-17-mediated immunity, but its mechanisms have remained elusive. Thyroiditis is a type I interferonopathy, although the specific nature of this manifestation is also elusive. Most other infectious and noninfectious manifestations are unexplained. Determining the molecular mechanism underlying mycobacterial disease is particularly challenging, as these infections are typically seen in patients with impaired type II IFN immunity. Remarkably, the discovery of *STAT1*-GOF mutations has paved the way for the treatment of patients with JAK inhibitors, illustrating the therapeutic impact of the genetic and immunological dissection of human infectious diseases. Immunosuppression with these inhibitors not only improves autoimmunity but also paradoxically improves host defense. The potential risks and benefits of HSCT should be considered in this context. Further studies are required to better understand the pathogenesis of the diverse manifestations seen in patients with *STAT1*-GOF mutations as a prerequisite to better manage these patients.

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Conflicts of interest

The authors declare that they have no relevant conflicts of interest.

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Figure 1

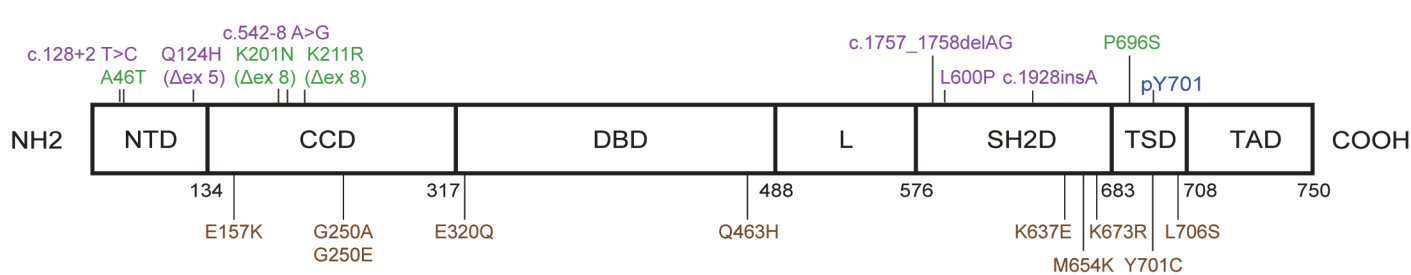
Known GOF and LOF mutations of *STAT1*

GOF mutations are shown in red, while LOF mutations that cause AD-STAT1 deficiency, AR-STAT1 complete deficiency and AR-STAT1 partial deficiency are shown in brown, purple and green, respectively. N-terminal domain (NTD), coiled-coil domain (CCD), DNA-binding domain (DBD), linker (L) domain, SH2 domain (SH2D), tail segment domain (TSD), transactivation domain (TAD).

Figure 2

Population genetics for STAT1. CADD score (y-axis) vs. minor allele frequency (MAF, x-axis) for all the heterozygous variants of *STAT1* found in gnomAD (blue) and *STAT1*-GOF mutations (red).

B



CADD

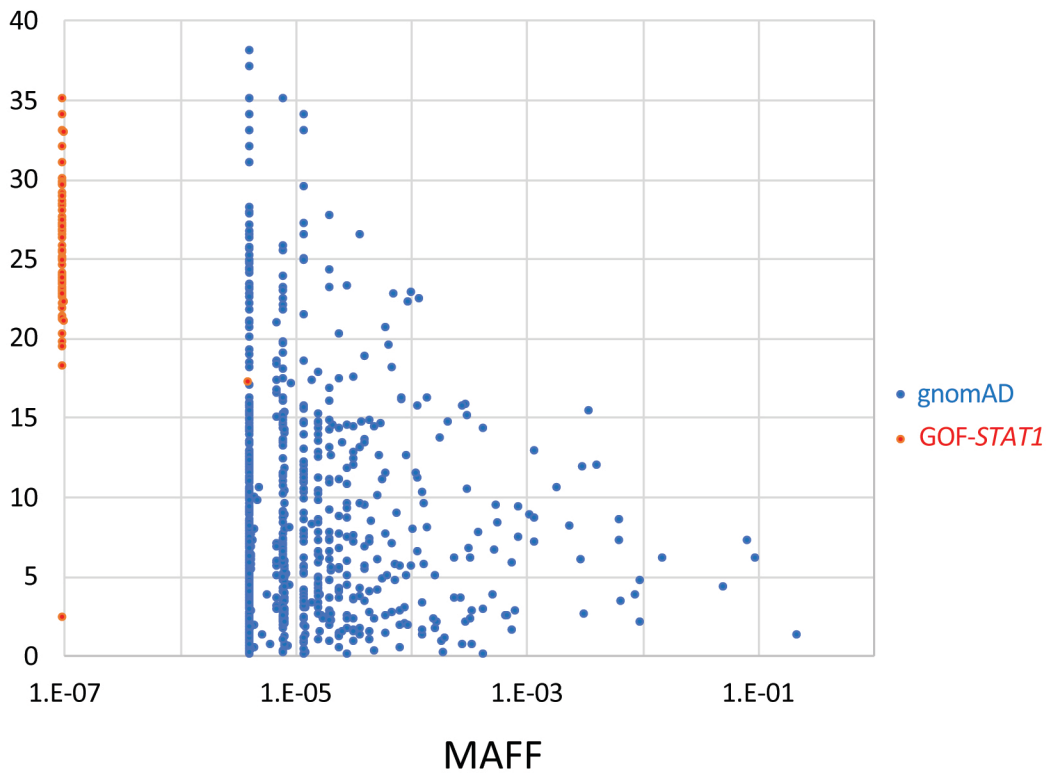


Table 1

Functional domains	GOF mutations	Cellular assay*	Functional test**	References	
N-terminal domain (NTD)	D65N	Proved	n/a	(118-123)	
	R70H	n/a	GOF	(58)	
	R70P	n/a	n/a	(67)	
	N89Y	n/a	GOF	(58)	
	T133A	n/a	n/a	(137)	
Coiled domain (CCD)	coil	D151E	n/a	GOF	(58)
		I156T	n/a	GOF	(58, 96, 116)
		I160F	n/a	GOF	(58)
		L163R	n/a	GOF	(58, 91)
		D165G	Proved	GOF	(55, 58, 60, 86)
		D165H	Proved	GOF	(55, 58, 116, 128)
		Q167E	n/a	GOF	(58)
		Q167H	n/a	GOF	(58)
		Q167P	n/a	GOF	(58)
		D168E	Proved	GOF	(58, 134)
		Y170N	n/a	GOF	(55, 58, 135)
		D171N	n/a	GOF	(58)
		F172L	Proved	GOF	(57, 83, 98)
		C174R	n/a	GOF	(55, 58)
		N179K	n/a	GOF	(58, 60)
		M202I	Proved	GOF	(55, 58, 109)
		M202T	n/a	GOF	(58, 111)
		M202V	Proved	GOF	(55, 57-59, 110)
		L206H	n/a	GOF	(58)
		L206P	Proved	GOF	(65)
		R210G	n/a	GOF	(58)
		R210I	Proved	GOF	(58, 108)
		R210K	n/a	GOF	(58, 69)
		E235A	Proved	n/a	(96, 116)
		A267V	Proved	GOF	(55, 56, 58-60, 66, 68, 70, 79, 84, 93,

				98, 104, 120, 124, 128, 132)
	Q271P	n/a	GOF	(55, 58)
	R274G	Proved	GOF	(94, 98)
	R274Q	Proved	GOF	(55, 57-60, 78, 80, 86, 92, 111, 113, 115, 118-123, 126, 128, 132, 133)
	R274W	Proved	GOF	(55-58, 60, 69, 81, 86, 92, 101, 106, 107, 109, 117, 128)
	K278E	Proved	n/a	(114)
	L283F	Proved	n/a	(118-123)
	L283M	Proved	GOF	(70, 104)
	L283S	Proved	n/a	(102, 132)
	E284K	n/a	n/a	(67)
	Q285K	Proved	n/a	(109)
	Q285R	n/a	GOF	(58, 60)
	K286I	n/a	GOF	(55, 58)
	Y287D	Proved	GOF	(57, 58)
	Y287H	n/a	GOF	(58)
	T288A	Proved	GOF	(55, 58, 118-123, 127)
	T288I	Proved	GOF	(58, 124)
	T288P	n/a	GOF	(58)
	Y289C	Proved	GOF	(58, 109)
	Y289H	n/a	GOF	(58)
	D292E	Proved	GOF	(58, 86)
	D292N	n/a	GOF	(58)
	P293L	n/a	GOF	(58, 62)
	P293S	Proved	GOF	(57, 58)
	P293T	n/a	GOF	(58)
	I294T	Proved	GOF	(58, 86, 100)
	K298N	Proved	n/a	(88)
	L301del	n/a	GOF	(58)

DNA binding domain (DBD)	R321G	n/a	GOF	(58)
	R321S	Proved	GOF	(58, 128)
	C324F	Proved	n/a	(64, 82)
	C324R	n/a	GOF	(58, 87, 99, 100)
	M325K	Proved	n/a	(118)
	H328R	Proved	GOF	(86, 109, 116, 128)
	P329L	Proved	GOF	(59, 109, 111)
	Q330K	Proved	n/a	(118-123)
	K344E	n/a	GOF	(58)
	L351F	Proved	GOF	(58, 70, 104, 118-123)
	E353K	Proved	GOF	(58, 96, 98, 109, 116, 117, 128)
	L354M	Proved	GOF	(58, 59)
	L354V	n/a	n/a	(137)
	N355D	n/a	GOF	(58)
	N357D	n/a	GOF	(58, 111)
	L358F	Proved	n/a	(124, 129)
	L358W	Proved	GOF	(58, 108, 116)
	E370D	Proved	GOF	(58, 85)
	G384C	n/a	GOF	(58)
	G384D	Proved	GOF	(58, 114)
	T385K	Proved	n/a	(57)
	T385M	Proved	GOF	(57, 58, 60, 64, 70-73, 75, 82, 86, 95, 98, 104, 105, 108, 109, 115, 116, 118, 121, 122, 128)
	T387A	Proved	GOF	(58, 70, 74, 77, 104)
	K388E	Proved	GOF	(57, 58, 73, 111)
	V389A	n/a	GOF	(58, 132)
	V389L	Proved	GOF	(76)
	M390I	Proved	GOF	(58, 124)
	M390T	Proved	GOF	(58, 59, 86, 132)
	M392T	n/a	GOF	(58)

	N397D	Proved	GOF	(57, 58, 63, 86)
	L400Q	Proved	GOF	(58, 115)
	L400V	Proved	GOF	(58, 70, 104)
	F404Y	Proved	GOF	(57, 58, 61)
	T419R	n/a	GOF	(58)
	T437I	Proved	n/a	(111)
	T437N	Proved	GOF	(131)
	S466R	Proved	GOF	(57, 58, 86, 125)
Linker domain (L)	D517G	n/a	GOF	(58)
	C543R	n/a	GOF	(176)
	E545K	Proved	n/a	(112)
	N574H	Proved	n/a	(136)
	N574I	n/a	GOF	(58)
SH2 domain (SH2D)	H629Y	Proved	n/a	(94, 103, 116, 128)
	V653I	Proved	n/a	(89, 90)
	N658S	n/a	GOF	(58)
Transactivation domain (TAD)	E705V	Proved	GOF	(97)
	S708F	Proved	n/a	(118-123)
	E711Q	n/a	GOF	(58, 123)
	T720I	n/a	GOF	(58, 67)

Known *STAT1*-GOF mutations

* Increased STAT1 phosphorylation or impaired dephosphorylation was demonstrated with patient cells.

** GOF was confirmed by gene expression assays, including reporter assays.

n/a: data not available

Table 2

Functional domains	LOF mutations	References
N-terminal domain (NTD)	Q124H (Δ exon 5)	(16)
	c.128+2 T>G	(177)
Coiled coil domain (CCD)	c.542-8 A>G	(177)
SH2 domain (SH2D)	c.1757_1758delAG	(14)
	L600P	(14)
	c.1928insA	(15)

Known LOF mutations that cause AR complete STAT1 deficiency.

Table 3

Functional domains	LOF mutations	References
N-terminal domain (NTD)	A46T*	(19)
Coiled coil domain (CCD)	K201N (Δ exon 8)	(18)
	K211R* (Δ exon 8)	(19)
Tail segment domain (TSD)	P696S	(17)

Known LOF mutations that cause AR partial STAT1 deficiency.

* A46T and K211R were identified as compound heterozygous mutations.

Table 4

Functional domains	LOF mutations	References
Coiled coil domain (CCD)	E157K	(23)
	G250A	(26)
	G250E	(23)
DNA binding domain (DBD)	E320Q	(21)
	Q463H	(21)
SH2 domain (SH2D)	K637E	(22)
	M654K	(25)
	K673R	(22)
Tail segment domain (TSD)	Y701C	(24)
	L706S	(20)

Known LOF mutations that cause AD STAT1 deficiency.