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Clinical aspects of STAT3 gain-of-function germline mutations: A Systematic Review

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Abstract

Background: *STAT3* gain-of-function (GOF) germline mutations have been recently described. A comprehensive overview of this early-onset multiorgan autoimmune and lymphoproliferative disease has not yet been compiled.

Objective: We have conducted a systematic review of published *STAT3* GOF cases in order to describe clinical, diagnostic and therapeutic aspects of the disease.

Methods: A systematic review including articles published before 10/10/2018 in Pubmed, WoS and CENTRAL databases was performed. We described cases of patients with *STAT3* GOF germline mutations with genetic analysis and a concordant phenotype if functional analyses were not performed for the mutation.

Results: The search identified 18 publications describing 42 unique patients. Twenty-eight different mutations were described. Onset of disease was very early with an average age of 3 (0.5-5) years old. The most frequent manifestations were: autoimmune cytopenias (28/42), lymphoproliferation (27/42), enteropathy (24/42), interstitial lung disease (15/42), thyroiditis (13/42), diabetes (10/42), and post-natal growth failure (15/21). Immunodeficiency was not always a predominant feature. Most patients required significant immunosuppressive therapy. Five patients received hematopoietic stem cell transplantation, 4 died from complications. Improvement of symptoms was observed for 8 out of 9 patients that received targeted biotherapies.

69 **Conclusions:** STAT3 GOF syndrome is a new clinical entity to consider when confronted
70 with a patient with early-onset poly-autoimmunity, lymphoproliferation, and growth failure.
71 At this time, precise therapeutic guidelines are lacking, but use of anti-IL-6 receptor and JAK
72 inhibitor biologics are attractive possibilities.
73

Highlights box.

What is already known about this topic?

STAT3 gain-of-function (GOF) germline mutations have been recently described. A comprehensive overview of this early-onset multiorgan autoimmune and lymphoproliferative disease has not yet been compiled.

What does this article add to our knowledge?

We identified 42 unique patients. Typical disease was early-onset with very early endocrine autoimmunity, then pseudo-celiac enteropathy, followed by lymphoproliferation, autoimmune cytopenias and interstitial lung disease in the context of severe growth failure.

How does this study impact current management guidelines ?

STAT3 GOF syndrome is a new entity to consider when confronted with a patient with early onset poly-autoimmunity, lymphoproliferation and/or short stature. There is emerging evidence for the use of targeted biotherapies in this disorder.

Key words: autoimmunity; lymphoproliferative disease; GOF; Evans syndrome; interstitial lung disease; celiac disease; short stature; early onset diabetes; child

98 **Abbreviations:**

- 99 STAT: signal transducer and activator of transcription
- 100 GOF: gain-of-function
- 101 GnomAD: Genome Aggregation Database
- 102 IL: interleukin
- 103 JAK: Janus kinase
- 104 LGL: large granular lymphocytic
- 105 SH2: Src homology 2
- 106 ExAC: Exome Aggregation Consortium
- 107 SOCS: suppressor of cytokine signaling
- 108 EBV: Epstein-Barr virus
- 109 ITP: immune thrombocytopenic purpura
- 110 AIHA: autoimmune hemolytic anemia
- 111 NK: natural killer
- 112 Ig: immunoglobulin
- 113 HLA: human leukocyte antigen
- 114 ILD: interstitial lung disease
- 115 IPF: idiopathic pulmonary fibrosis
- 116 DIP: desquamative interstitial pneumonitis
- 117 LIP: lymphocytic interstitial pneumonitis
- 118 DLCO: diffusing capacity of the lungs for carbon monoxide
- 119 LRTI: lower respiratory tract infections
- 120 TPO: thyroperoxidase
- 121 MRI: magnetic resonance imaging
- 122 GH: growth hormone
- 123 SD: standard deviation

- 124 IGF1: insulin-like growth factor -1
- 125 GVH: graft versus host
- 126 ALPS: autoimmune lymphoproliferative syndrome
- 127 FAS: first apoptosis signal
- 128 IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked
- 129 FOXP3: forkhead box P3
- 130 APDS: activated phosphoinositide 3-kinase delta syndrome
- 131 CHAI: CTLA-4 haploinsufficiency with autoimmune infiltration
- 132 LATAIE: LRBA deficiency with autoantibodies, Treg defects, autoimmune
- 133 infiltration and enteropathy
- 134 IBD: inflammatory bowel disease
- 135 COOP: cryptogenic organizing pneumonia
- 136

Introduction

The transcription factor signal transducer and activator of transcription 3 (STAT3), one of 7 members of the STAT family of transcription factors, is a critical regulator of multiple processes, including cellular proliferation, survival, differentiation, and regulation of autoimmunity and inflammation¹. Therefore, STAT3 mutations (gain-of-function (GOF) or dominant negative) can result in immunodeficiency, autoimmunity or malignancy. *STAT3* dominant negative germline mutations are well known, and lead to a primary immune deficiency syndrome (known as Buckley or Job Syndrome, or autosomal dominant hyper IgE syndrome OMIM: 147060)². *STAT3* GOF somatic mutations have been reported in malignancies such as LGL leukemia and hepatocellular adenoma³. *STAT3* GOF germline mutations were discovered recently and lead to early-onset multiorgan dysfunction (OMIM: 615952), including autoimmune disorders, lymphoproliferation, susceptibility to infection and severe growth failure^{1,3-5}.

In 2014 and 2015, 3 major studies reported the first 19 patients with STAT3 GOF, describing genetics, clinical features and basic immunophenotyping^{1,4,6}. Only case reports have been published thereafter without a comprehensive overview of clinical aspects and natural history of the disease. Recently a study on management with targeted biotherapies reported 4 additional patients⁷. The phenotype of STAT3 GOF is diverse, which makes its clinical definition and diagnosis difficult. The first manifestations of STAT3 GOF can be nonspecific inflammation and/or lymphoproliferation involving varied organ systems and, therefore, a STAT3 GOF patient could be first seen by a clinician of any subspecialty not yet familiar with this new immune dysregulation syndrome. Herein we have compiled a systematic review of published STAT3 GOF cases in order to describe the genetics, functional studies and clinical aspects of the disease.

Methods

Patients

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. A systematic search was conducted (10/10/2018) to identify studies reporting cases of patients with STAT3 GOF germline mutations. The literature search and evaluation were performed in 3 databases, MEDLINE (Pubmed), Web of Science (WoS) and CENTRAL (Cochrane Central Register of Controlled Trials), for articles published since 01/01/2014 (the first report of STAT3 GOF germline mutation is Flannagan *et al.*, 2014). The following research keywords in English were used: (STAT3 AND "gain of function" or "signal transducer and activator of transcription 3" and "gain of function"), (STAT3 AND "Activating germline mutations" or "signal transducer and activator of transcription 3" and "Activating germline mutations") and (STAT3 AND "activating mutation" or "signal transducer and activator of transcription 3" and "activating mutation"). Another search was manually performed in Google scholar using the function cited by for Flannagan *et al.*, 2014, Haapaniemi *et al.*, 2015 and Milner *et al.*, 2015. Abstracts and full-length text were screened for all suitable articles. We excluded duplicate articles or congress abstracts. To be eligible for inclusion, studies and case reports were required to describe cases of patients with *STAT3* GOF germline mutations with genetic analysis and a concordant phenotype if functional analyses were not performed for the mutation. All types of publications, such as articles, reviews, editorials, letters, and correspondence, reported in English or in French, published online between January 1, 2014 and October 10, 2018 were included.

Genetic evaluation

Frequency of variants was determined using the gnomAD database (<http://gnomad.broadinstitute.org/>). Prediction of pathogenicity was performed in UMD predictor (<http://umd-predictor.eu/>) and Mutation Taster (<http://www.mutationtaster.org/>).

Statistical analysis

Data were collected and organized using Microsoft Excel (Microsoft, Redmond, WA, USA) and statistical analysis was performed using R. Data were presented as median and (first, third) quartiles.

Results

The search identified 18 publications describing 42 unique patients. The list of included articles is presented in **Table E1**, sorted by date of publication. **Figure 1** summarizes the PRISMA diagram.

Genetics and functional analysis

At this time, 28 different mutations from 42 cases have been described, located mostly in the DNA binding domain (**Figure 2 and Table E2**). All patients were heterozygous for the mutation, 21 were *de novo* mutations and 7 were inherited in an autosomal dominant manner within 5 different families. Six unaffected members were described within 4 out of these 5 families. Penetrance is thus incomplete. Analysis of described STAT3 variants in the gnomAD database showed only one mutation as already reported (p.R246Q with a frequency of 1/246408 alleles), all the others are not reported. All mutations reported but one (an in frame amino acid deletion) are missense. All variants were predicted pathogenic or probably pathogenic by UMD-predictor and disease causing by Mutation Taster. According to ExAC data, *STAT3* is a constrained gene with a pLI (a indicator of intolerance to loss of function) of

1 (the least tolerant),⁸ and indeed all the mutations reported are very rare. These features support autosomal dominant inheritance⁹.

Functional studies were reported for all mutations except 4^{10,11}, and confirmed gain of function of STAT3 (**Table E2**). The functional tests developed were (i) an *in vitro* STAT3 reporter luciferase assay under basal or stimulated conditions (IL-6/growth hormone) in A4, HEK293 or INS-1E cells, and (ii) SOCS3 expression levels under basal or stimulated (IL-21) conditions in EBV-transformed patient cell-lines or primary cells. Of note, under basal conditions, luciferase reporter assay was normal for two patients (but abnormal in stimulated conditions) and under stimulated conditions the assay was normal for two other patients (but abnormal in basal conditions). GOF mutations resulted in at least ten-fold increase of luciferase reporter assays for seven patients. The absence of obvious genotype-phenotype correlations and the fact that STAT3 hyperactivity does not appear to correlate with the severity of the phenotype suggest that environmental and other genetics factors may contribute to this syndrome.¹²

Clinical aspects

Clinical expression phenotypes of STAT3 GOF syndrome are very diverse. Among the 42 cases (sex ratio (M/F) = 18/22 = 0.82)^{1,4,6,7,13–18,10,19–22,11,23–25}, the most frequent features were hematologic disease, failure to thrive/short stature and multi-organ autoimmune disorders. Onset of disease was very early with an average age of onset of 3 (0.5-5) years old (**Figure 3**), including 17 cases occurring before one year. Eight patients died, at a median age of 12.5 years (5.5-18.25), including 4 from complications of stem cell transplantation.

The following description is based on the 42 reported cases.

236 *Hematologic manifestations*

237 Hematologic disease was the most frequent finding (35/42), especially autoimmune
 238 cytopenias (28/42), including immune thrombocytopenia (ITP)^{4,6,11,15–17,19,22,23} (24/28),
 239 autoimmune hemolytic anemia (AIHA)^{1,4,11,15–17,19,22,23} (19/28) and neutropenia^{4,16,17} (9/28)
 240 (**Table E3**). AIHA and ITP were observed concurrently in 15 cases^{4,7,11,15–17,19,22,23}. There was
 241 an autoimmune pancytopenia in 4 cases⁴. Age of onset was specified in 8 cases; median age
 242 of onset was 3.5 years old (**Figure 3**).

243 Immunodeficiency (hypogammaglobulinemia or lymphopenia or recurrent infections)
 244 was not as severe as could be expected from the phenotype of *STAT3* dominant negative
 245 mutations, but it was described in many cases (28/42). Infection susceptibility was described
 246 in most *STAT3* GOF patients (23/28), mainly recurrent upper and lower respiratory tract
 247 infections (11/23)^{1,4,6,14,16,19,21,24} leading to bronchiectasis in 2 cases.
 248 Hypogammaglobulinemia was reported in about half of patients^{1,4,6,11,14,15,17,21,22,24} (18/28); it
 249 was associated with clinical infection in 16 cases. A decrease of immune cells to varying
 250 degrees was noticed for T-cells^{4,6,11,19,20} (8/28), NK-cells^{1,6,11,16,19,24} (9/28), and B-cells,
 251 particularly memory B-cells^{4,19,20} (4/28). Further, T regulatory cell levels were reported in 11
 252 patients, and were decreased in 10/11 cases, all with multi-organ autoimmunity. Th17 cells
 253 levels were also reported in 4 cases, but were high in only one case.

254 Lymphoproliferation (27/42) was described mainly with adenopathy^{1,4,7,11,15–17,19,20}
 255 (23/27) and hepatosplenomegaly^{1,4,7,14–17,19,20,23} (23/27), which was concomitant in 19 cases.
 256 There was mediastinal adenopathy in 3 cases. Age of onset of lymphoproliferation was
 257 specified in 8 cases, the median age was 2.5 (1.9–3.5) years old for adenopathy and 3 (1.9–8)
 258 years old for hepatosplenomegaly (**Figure 3**). Hemophagocytic lymphohistiocytosis was
 259 described in 1 case⁷. Malignancy was described in 2 cases. One adult patient had Hodgkin
 260 lymphoma⁴ and 1 patient developed LGL leukemia¹ at the age of 14. IgA level was reported

in 16 cases, being elevated in 5 cases^{10,14,20,21} and low in 8 cases^{1,11,15,17,22}. Hyper IgA was invariantly associated with enteropathy.

Gastrointestinal manifestations

Hepatic and digestive diseases were described in 26 cases (**Table E4**). Enteropathy was found in 24/42 patients (**Table E5**). Average age of onset, reported for 11 patients, was 1 (0.5-1.02) year, and most (9/11) before the second year (**Figure 3**). The clinical signs were diarrhea (17 patients), abdominal pain (4) and vomiting (2), associated with growth failure in 12 cases. The disease was initially classified as celiac disease in 9 cases. Response to gluten free diet was reported for 7 patients: 1 patient responded¹, 1 patient responded partially¹, 1 patient responded in combination with azathioprine and tacrolimus¹⁰, and there was no response for 4 patients^{1,14,20,24}. For the 5 patients with reported HLA, 3 were DQ2 or DQ8 positive. Both DQ2 positive patients were responsive to a gluten free diet. Of the 11/42 patients for which celiac antibodies (anti-gliadin (G), anti-tissue transglutaminase (TTG) or anti-endomysium (E)) were tested^{1,6,10,18,20}, 9 were positive and 2 were negative¹. The 2 patients with negative celiac antibodies had no clinical signs of enteropathy. Among the 9 patients with positive celiac antibodies, 7 had positive anti-G (5 isolated anti-G, 1 anti-G/E, 1 anti-G/TTG) and 1 had isolated anti-TTG. Antibody specificity was not reported for the last patient.

Gastrointestinal biopsy results were reported in multiple STAT3 GOF patients. Pathology of the esophagus showed normal¹⁸ or mild changes, such as basal cell hyperplasia^{4,21}. Pathology of the stomach showed moderate gastritis in 9 patients^{1,4,14,18,20,21,24}, with lymphocytic infiltration noted in 4. In 14 patients^{1,4,6,10,14,18,20–22,24}, abnormalities of the duodenum were reported. This was predominantly villous atrophy (11 patients^{1,4,10,14,18,20}) as well as inflammation (12 patients^{1,4,6,10,14,18,20,21,24}) with intraepithelial lymphocyte infiltration

in 9 patients^{1,6,10,14,18,20,21,24}. For 5 patients with sequential biopsies, 2 had a normalization of the biopsy^{1,18}, 1 patient's was initially normal followed by a second abnormal one¹, and 2 no improvement^{20,24}. Colonic biopsies were normal in only 1 case¹⁴ and showed colitis in 5 cases^{4,6,11,18}.

Additionally, 9 patients were reported with liver disease^{4,7,10,11,20,22}, 4 with autoimmune hepatitis (with anti-DNA and anti-smooth muscle antibodies) requiring liver transplant in 2 of them. One patient had achalasia⁴. Three patients developed pancreatic exocrine insufficiency^{1,20}, 2 of them also had diabetes¹.

Seven patients affected by enteropathy were treated with targeted biotherapies (tocilizumab and ruxolitinib or tofacitinib) that improved their symptoms, need for TPN or NG tube feeds and nutrition status. One patient that benefited from stem cell transplantation had improvement in his gastrointestinal symptoms, especially diarrhea⁴. One patient gained control of her diarrhea with immunosuppressive treatment (azathioprine and tacrolimus) in association with a gluten free diet¹⁰.

Pulmonary manifestations

Twenty-two patients developed lung disease with a predominance of interstitial lung disease and recurrent lower respiratory tract infection (**Table E6**). 15/42 patients developed interstitial lung disease^{1,4,7,10,11,15,20,21,24}. The median age of onset, specified in 7 cases, was 16 (9-18) years old and was substantially delayed in regards to hematologic, endocrine and gastrointestinal features (**Figure 3**). Among these 15 patients, 7 had lymphocytic interstitial pneumonitis (LIP)^{4,7,15,20,21}, 2 granulomatous lung disease^{11,21}, 2 desquamative interstitial pneumonitis (DIP)^{1,24}, 1 idiopathic pulmonary fibrosis (IPF)⁴ and 1 cryptogenic organizing pneumonia (COOP)¹. With regard to the clinical presentation, asthma-like symptoms in early infancy were described in 3 patients who presented later with ILD. A lung biopsy was

performed in 3 cases^{1,4,15}. The first case showed diffuse interstitial fibrosis (IPF)⁴. The second displayed a diffuse interstitial fibrosis that was associated with alveolar thickening and macrophages (DIP)¹. The third case displayed atypical interstitial lymphoblastic infiltrate (LIP)¹⁵.

Three patients required long-term oxygen therapy and 1 invasive ventilation. Immunosuppressive treatment of pulmonary manifestations was detailed in 8 cases (steroids in 6 cases, tacrolimus in 2 cases, mycophenolate mofetil in 2 cases, azathioprine in 1 case, sirolimus in 1 case, cyclosporine in 1 case, and tocilizumab combined with ruxolitinib in 2 cases, tocilizumab combined with tofacitinib in one case). Immune suppression had a positive effect in 2 cases: 1 patient who benefited from tocilizumab associated with ruxolitinib required less oxygen²⁰; 1 patient who was treated with mycophenolate mofetil had improvement in DLCO⁴. One pulmonary patient had a bone marrow transplant, but died from multiple organ failure²¹.

11/42 patients had recurrent lower respiratory tract infections^{1,4,6,14,16,19,21,22,24} (LRTI). There was 1 case of *Mycobacterium avium* lung infection⁶. Among patients with LRTIs, 6 had hypogammaglobulinemia, 4 T-cell lymphopenia, 4 decreased NK-cells and 1 pan-lymphopenia. Seven of these patients were treated with immunosuppressive treatment and the chronology of lung infection with regard to treatment onset was not detailed.

Endocrinopathies

Endocrine diseases were also a frequent feature, reported in 21/42 patients (**Table E7**). Type I diabetes was the earliest endocrine manifestation in STAT3 GOF syndrome (10/42)^{1,4,11,16-18}. It appeared before 1 month of life in 3 cases and between 1 month and 12 months in 3 cases. The median age of onset of type I diabetes was 8 weeks (2.3-35.5) (**Figure 3**). HLA genotyping was high risk in 3 cases^{1,16} and low risk in 2 cases.

Hypothyroidism was found in 13/42^{1,4,7,10,11,16-18,21} STAT3 GOF patients. It appeared before 1 month of life in 2 cases. The median age of onset of thyroid disease was 2 (0-2.5) years old. Hypothyroidism and diabetes were co-morbid in 4 cases^{1,11,16,18}.

To a lesser extent, the pituitary gland was affected. One patient was described with isolated central corticotropin deficiency²⁰. There was 1 case of presumed pituitary dysfunction that responded well to GH treatment⁴. Finally, 1 patient was reported with GH insensitivity¹⁶.

Growth failure

Weight and/or height at birth were recorded in 14 cases, intrauterine growth restriction (weight and/or height at birth < -2SD) was present in 5 cases (35%)^{1,16,20,21} (**Figure 4**). Post-natal growth failure (height and/or weight < -2SD) was presented in 15 cases among 21 where it was reported (75%)^{1,4,7,10,16,18,20,21,24}. Height was less than or equal to -4SD in 5 cases^{1,4,16,20}. Growth failure was associated with intrauterine growth restriction in 3 cases. IGF1 was low in 7 of 10 patients tested, however, 5 also had enteropathy which could have affected nutritional status and so the IGF1 level. GH stimulation was performed in 4 cases^{1,4,16}, there was a positive response in 3 cases^{1,4,16}. 7 patients benefited from GH treatment, it was effective in 3^{4,21} and showed a partial response in 4^{1,20,21,24}.

Other features

Dysmorphic features are not a predominant feature of STAT3 GOF syndrome (2/37). Cutaneous disease (16/42) was mainly atopic dermatitis (12/16)^{1,4,6,7,10,11,20,21} and alopecia (4/16)^{4,10,14} (**Table E8**). The age of onset of cutaneous disease was reported in 4 cases all under the age of 2 years.

Arthritis was described in 9 cases^{1,4,6,11,20,22,24}, including 3 cases of polyarthritis^{4,24}, 1 severe arthritis¹¹ and 1 case of oligoarthritis²⁰. In 1 case, polyarthritis regressed with systemic

treatment with tocilizumab⁴. The age of onset was specified in 4 cases, with a median of 11 (9-13.25) years of age.

Six patients suffered from osteoporosis^{1,4,14,20,24} and 2 from osteopenia⁴; among them 2 had received corticosteroid treatment. There was 1 case of acroosteolysis⁴, resorption of the distal bony phalanges. Delayed bone age was noted in 6 cases^{4,16,24}. Two patients presented with joint laxity⁴. Dental abnormalities such as decay or delayed teeth eruption (a common feature of STAT3 dominant negative mutations) were noted in 5 cases^{1,4,16,20}.

Renal disease was reported in 3 cases^{14,20,22}. One patient presented with nephrolithiasis at an adult age. There was 1 case of proximal tubulopathy with severe hypercalciuria at 5 years old, associated with cortical renal hyperechogenicities compatible with microcysts, but without renal impairment. Finally, one patient had a chronic renal impairment, and died from end stage renal failure.

Ocular affects were reported in 5/42 patients^{1,19,20,22}, including 2 patients with uveitis and keratoconjunctivitis sicca in 2 cases. Neurological disease was not predominant, with 1 case of ocular myasthenia gravis⁴, 1 case of cerebral atrophy that led to abnormal psychomotor development⁴, and 1 patient with cerebral palsy⁴. Three patients^{1,4} developed thrombosis (1 internal carotid and 2 vena cava), but additional details were not provided. Finally 1 patient developed disseminated intra-vascular coagulation⁷.

Clinical Progression

STAT3 GOF syndrome encompasses many target organ diseases with a sequential median age of onset (**Figure 3 and Figure 5**). Endocrine and gastrointestinal disease is very early-onset and appears clearly before the onset of hematologic disease. Poly-autoimmunity with ≥ 2 autoimmune diseases was frequent (29/42); ≥ 3 autoimmune diseases was reported in 16/42 patients. Single organ autoimmune disease was reported in 10 patients and 3 patients had no

obvious autoimmunity. Although hematologic disease was also predominant, it was not invariant as 7 patients did not have any hematologic disease (**Figure 6**). Finally, 4 patients had neither poly-autoimmunity nor hematologic disease.

Therapeutic options

Most of the symptomatic patients with STAT3 GOF syndrome required significant immunosuppressive therapy. Treatment was outlined in 23 cases and is presented in **Table 1**. Non targeted immunosuppressive therapies were overall ineffective.

Five patients^{4,7,16,21} received hematopoietic stem cell transplantation, 4 died from complications (GVH disease and systemic adenovirus in 1 case, multiple organ failure in 1 case, systemic adenovirus infection in 1 case and adenovirus pneumonia and post-transplant hemophagocytic lymphohistiocytosis in 1 case); the other had improved growth and a complete remission of his autoimmune disease⁴.

Other therapeutic options consisted of targeting molecules which play an important role in the STAT3 pathway. Nine patients^{4,7,14,20,24} received anti-IL6 receptor monoclonal antibody therapy (tocilizumab), with a positive effect on autoimmune disease except in 1 patient⁷. The effect nevertheless seems to plateau. In 1 case it was used in monotherapy⁴ and it resulted in improvement of the general condition, alleviation of diarrhea and increased Treg cells¹⁴. In the 8 other patients tocilizumab was given with a Jak inhibitor (ruxolitinib or tofacitinib) and this showed clear improvement in 5 patients, most notably for their enteropathy and oxygen support.

Discussion

In recent years, multiple monogenic causes of early-onset autoimmunity and lymphoproliferation have been identified, now including STAT3 GOF syndrome^{3,4,7,26–32}.

These disorders have overlapping clinical and immunologic features (**Table 2**). In addition to these early-onset, monogenic autoimmune syndromes, the differential diagnoses for the symptoms of STAT3 GOF syndrome are plentiful due to multiple features within each organ system. Therefore, several differential diagnoses can be considered. Our systematic review summarizes current knowledge about specific characteristics of the disease. Average onset of organ systems appears to occur sequentially (**Figure 3**), with early-onset diabetes and enteropathy preceding hematologic manifestations and lung disease.

The enteropathy was frequently initially diagnosed as pseudo-celiac, with villous atrophy, but without the usual serologic signature. Only half of the patients tested had the high risk HLA (compared to more than 95% in classic celiac disease). Gluten free diet had no effect in most patients. STAT3 GOF patients presenting with early-onset IBD like phenotypes lacked the classical histologic findings of IBD and lymphocyte infiltration.

Early-onset endocrinopathies including neonatal diabetes and early onset thyroiditis were among the first symptoms occurring during infancy. Polyautoimmunity (encountered in 29/42 patients) included arthritis, autoimmune hepatitis, alopecia areata and autoimmune cytopenias notably later in life. Intra-uterine growth retardation was encountered in 1/3 of patients and post-growth failure in 3/4, with 5 patients < -4SD. Growth failure is a predominant feature of STAT3 GOF syndrome. This could be the consequence of digestive disease (malabsorption), diabetes, hypothyroidism, respiratory insufficiency, frequent infections or corticosteroid treatment. However, it could also be inherent to the *STAT3* GOF mutations. Indeed, *STAT5b*, is the major transcription factor downstream of GH signaling^{31,33}, and *STAT3* activation can inhibit *STAT5* phosphorylation¹⁶.

STAT3 GOF appears as a new etiology of ILD, mainly LIP as well as other forms of ILD such as COOP or DIP. Pathology showed fibrosis in 2 patients. LIP results from pulmonary interstitial accumulation of lymphocytes. Several

publications have suggested that STAT3 plays a role in regulating fibroblast function in fibrotic lung diseases and that aberrant STAT3 signaling plays a critical role in usual interstitial pneumonia and idiopathic pulmonary fibrosis pathogenesis³⁴.

Hematologic disease was frequent, onset after infancy and included lymphoproliferation, immunodeficiency with infection susceptibility (mainly recurrent LRTI and rare opportunistic infections), and autoimmune cytopenias. Despite the well-known association of somatic STAT3 GOF mutations with malignancy, cancer was only reported in 2 patients. Given the young average age of STAT3 GOF patients, long term followup will be critical to fully understand the risk of malignancy in STAT3 GOF syndrome.

The immunologic consequences of *STAT3* GOF mutations would be predicted to result in expansion of Th17 cells and inhibition of Treg cells, which plays a critical role in the development of autoimmunity^{5,10}. This is because, among other triggers, STAT3 is activated by IL-6 and plays an important role in differentiation of CD4 T-cells⁵. We hypothesize that, due to the severity of disease in most symptomatic STAT3 GOF patients, the reported T cell subsets were not performed in treatment naïve patients and that therapy with immunosuppression impacted Th17 development.

STAT3 interacts with other STATs mainly via suppressor of cytokine signalling 3 (SOCS3), which is a downstream target of STAT3 and negative regulator of STAT3 signaling³⁻⁵. Through this mechanism, STAT3 is believed to inhibit STAT1 and STAT5 phosphorylation. Disruption of STAT5 activation leads to inhibition of Treg development. In fact, most STAT3 GOF patients with reported T-cell subsets did have decreased Treg cells counts, but, to date, the majority of the few patients examined have been found to have normal levels of Th17 cells.

Non-targeted immunosuppressive treatments were disappointing in this cohort. Available biotherapies and bone marrow transplant constitute a real hope for these patients. Use of targeted therapy with tocilizumab and ruxolitinib or other JAK inhibitors may change the natural course of the disease. Bone marrow transplant could be a curative option when life-threatening complications occur, but available data are insufficient to determine safety or optimal conditions for this therapeutic option⁷.

This systematic review has some limitations. Four patients did not have any functional tests and many papers did not include specific details of clinical aspects, most notably the timing of onset of the different clinical features. A study reporting the complete natural history of the disease with timing of the onset of the different manifestations would be worth investigating. Nevertheless, the clinical summaries of STAT3 GOF made in this systematic review will be a useful tool to select among patients with early onset autoimmunity and lymphoproliferation to direct specific testing for STAT3.

STAT3 GOF syndrome is heterogeneous and patients do not universally present with hematologic autoimmunity, or obvious lymphoproliferation, immunodeficiency or short stature. They can also have a wide variety of solid organ manifestations such as pseudo-celiac disease, pseudo-juvenile arthritis, endocrinopathies, interstitial lung disease, and cutaneous disease. However, notably, very early-onset diabetes or atypical celiac disease are clues to this syndrome and can be for a long time the only manifestation.

Current diagnostic methods for STAT3 GOF syndrome include genetic testing paired with functional analysis of new mutations. This process is available only in expert centers, using mainly luciferase reporter assays. SOCS3 level of expression has also been used as a functional assay. Levels of GOF determined by luciferase reporter assay seem to be the best assessment in order to potentially delineate genotype-phenotype correlations in the future.

Conclusion

STAT3 GOF syndrome caused by heterozygous, germline *STAT3* GOF mutations is a new clinical entity to think about when confronted with early-onset poly-autoimmunity with lymphoproliferation, enteropathy, interstitial lung disease, and associated with severe growth failure. Heterogeneity of the phenotype makes a precise clinical definition difficult. Currently, genetic analysis is the only valid diagnostic method. At this time, defined therapeutic guidelines are still lacking but use of anti-IL-6 receptor and JAK inhibitor biologics are attractive possibilities while other future options, such as anti-STAT3 therapeutics, are being developed.

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Figure legends

Figure 1. PRISMA flow diagram.

Fig. 2 Schematic of the STAT3 protein showing the location of mutations reported to cause STAT3 GOF.

STAT3 is divided into 6 domains: the N-terminal domain, coiled-coil domain, DNA binding domain, linker domain, Src-homology 2 (SH2) domain and transactivation domain (TA). Mutations shown represent mainly missense mutations and the resulting amino acid changes, but there is also one deletion. Mutations replicated in several patients are indicated in red by the number of patients reported and using bold lines.

Figure 3. Age at onset.

Age at onset of the disease and at onset of different clinical manifestations.

Figure 4. Weight and height z-scores at birth and last visit.

Weight and height z-scores at birth and last visit.

Figure 5. Kaplan-Meier survival curves.

Kaplan-Meier survival curves of (A) overall survival, (B) diabetes mellitus, (C) enteropathy, (D) lymphadenopathy, (E) hepatosplenomegaly, (F) autoimmune cytopenias, (G) arthritis, and (H) interstitial lung disease (ILD). On the X axes, time is expressed in years. Y axes represent either the percentage of survival (in A) or the number of patients free of the disease (B-H). Solid lines represent survival curves and dotted lines the confidence intervals.

630 **Figure 6. Venn diagram of clinical manifestations.**

631 Venn diagram of endocrine, gut, hematologic, articular and pulmonary clinical
632 manifestations.

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Figure 1. PRISMA Flow diagram

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

Age (years)

Z-score

Table 1. Treatments reported in STAT3 GOF patients

Treatments	Total	Positive effect	Negative effect / no effect	Not determined
Non targeted therapies				
Systemic steroids	17 ^{1,6,7,10,14,16,17,21,22,24}	3 ^{14,16,17}	6 ^{1,16,22,24}	8 ^{6,7,10,21}
Inhaled steroids	2 ^{1,21}	-	2 ^{1,21}	-
Mycophenolate mofetil	5 ^{1,4,7,17,22}	2 ^{4,17}	1 ⁷	2 ^{1,22}
Tacrolimus	4 ^{1,7,10}	1 ¹	2 ^{7,10}	1 ¹
Azathioprine	4 ^{7,19,24}	-	4 ^{7,19,24}	-
Rituximab	3 ^{7,15,19}	1 ¹⁵	1 ⁷	1 ¹⁹
Sirolimus	3 ^{7,21}	-	3 ^{7,21}	-
Anti TNF-alpha	3 ^{7,24}	-	3 ^{7,24}	-
Infliximab	1 ²⁴	-	1 ²⁴	-
Adalimumab	1 ²⁴	-	1 ²⁴	-
Methotrexate	2 ^{7,24}	-	2 ^{7,24}	-
Cyclophosphamide	2 ⁷	-	2 ⁷	-
Cyclosporine	1 ²¹	-	1 ²¹	-
Abatacept	1 ²¹	-	1 ²¹	-
Alemtuzumab	1 ⁷	-	1 ⁷	-
Anti-IL1	1 ⁷	-	1 ⁷	-
HSCT	5 ^{4,7,16,21}	1 ⁴	4 ^{7,16,21}	-
Targeted therapies				
Tocilizumab	9 ^{4,7,14,20,24}	8 ^{4,7,14,20,24}	1 ⁷	-
Addition of jakinib	8 ^{4,7,20,24}	5 ^{7,20,24}	3 ^{4,7}	-

Table 2. Clinical features of single-gene defects with immune dysregulation^{3,4,7,26–32}

	STAT3 GOF	STAT3 DN (AD-HIES)	STAT1 GOF	STAT5b deficiency	APDS	ALPS	IPEX	LATAIE	CHAI
Gene	STAT3	STAT3	STAT1	STAT5b	PIK3CD	TNFRSF6	FOXP3	LRBA	CTLA-4
Transmission	AD	AD	AD	AR	AD	70% AD (some somatic mutations)	X-linked	AR	AD
Onset of Symptoms	Childhood	Early infancy	Childhood	Childhood	1-3 years	1-3 years	Early infancy	Childhood	Childhood
Lymphoproliferation	SM, HM	-	SM, HM	-	LAD, SM, ENT lymphoid hyperplasia	80% chronic lymphoproliferation (>6 months): LAD, SM, HM	-	LAD, SM, HM	LAD, SM
Autoimmune disorder (immune dysregulation)	AIHA, ITP Early-onset pseudo-celiac enteropathy, Interstitial lung disease, Early-onset diabetes and thyroiditis, Arthritis, Alopecia	-	Thyroiditis, Alopecia, Psoriasis, Scleroderma, Lupus, Pseudo-celiac enteropathy, Colitis, Autoimmune hepatitis, Autoimmune cytopenia	Interstitial lung disease, ITP, Thyroiditis, Arthritis	30% Cytopenias, Insulin-dependent diabetes, Arthritis	70% Cytopenias: AIHA, ITP or neutropenia, Rare other autoimmune manifestations: nephritis, hepatitis, biliary cirrhosis, colitis, arthritis, vasculitis	Early-onset enteritis with villous atrophy & lymphocytic infiltrates, Endocrinopathy: diabetes (sometimes neonatal) & thyroiditis, Hemolytic anemia	Enteropathy, AIHA, ITP, Interstitial lung disease, Diabetes, Hepatitis, Psoriasis	>50% Enteropathy >50% Interstitial lung disease, AIHA, ITP
Other	Herpesvirus infections, Fungal infections, Bacterial infections,	Dermatitis, Neonatal rash, Skin abscesses,	Fungal infections (especially chronic mucocutaneous	Growth failure, Dysmorphic features, Eczema,	Upper & lower respiratory infections,	Rare recurrent infections	Early-onset failure to thrive, Neonatal	Upper & lower respiratory infections, Bronchiectasis,	Upper & lower respiratory infections

	Respiratory infections, Short stature	Pneumonias, Bronchiectasis, Pneumatocele, Fungal infections, Bacterial infections, Herpes virus infections, Dysmorphic features, Vascular abnormalities	candidiasis), Severe viral infections, Bacterial infections, Mycobacterial infections, Bronchiectasis, Asthma, Eczema, Dysphagia, Failure to thrive	Severe herpesvirus infections, Recurrent infections	Pneumonia, Bronchiectasis, Chronic rhinosinusitis, Severe or recurrent herpesvirus infections		erythema, Eczema, Psoriasiform dermatitis, Alopecia	Viral infections, Fungal infections, Bacterial infections	
Increased risk of malignancy	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes
Laboratory parameters	Hypogammaglobulinemia, Reduced switched memory B-cells & Tregs	Elevated IgE, Eosinophilia, Decreased Th17 cells, B and T memory lymphopenia	Reduced switched memory B cells, Decreased Th17 cells, Low IgG2 and IgG4,	Treg deficiency and dysfunction, Reduced T cells and NK cells, Hypergammaglobulinemia	Decreased IgA/IgG +/- increased IgM, Naive CD4 T-cell & Memory B-cell lymphopenia with elevated transitional B- cells	Direct Coombs positive, Hypergammaglobulinemia, Elevated DNT cells, Elevated plasma sFASL, Elevated plasma IL-10, Elevated plasma vitamin B12	Elevated IgE, Eosinophilia, Anti-enterocyte antibodies	50% Hypogammaglobulinemia, Reduced Tregs, Reduced switched memory B-cells	Hypogammaglobulinemia

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Footnotes: ALPS: Autoimmune Lymphoproliferative Syndrome; LAD: lymphadenopathy; SM: splenomegaly; HM: hepatomegaly; AIHA: autoimmune hemolytic anemia; ITP: Immune thrombocytopenia; DNT: double-negative T-cells (CD3+TCR $\alpha\beta$ +CD4⁻CD8⁻); LATAIE: LRBA deficiency with autoantibodies, Treg defects, autoimmune infiltration, and enteropathy; CHAI: CTLA-4 haploinsufficiency with autoimmune infiltration ; AD-HIES : autosomal-dominant hyper-immunoglobulin E syndrome, GOF : gain-of-function ; DN : dominant negative, AD : autosomal dominant, AR : autosomal recessive.