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STAT3 GAIN-OF-FUNCTION MUTATION IN AN ADULT PATIENT

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Abstract Germline gain-of-function (GOF) mutation of the signal transducer and activator of transcription 3 (*STAT3*) gene causes a disease clinically characterized by a significant lymphoproliferation, including lymphadenopathy and/or hepatosplenomegaly, as well as childhood onset autoimmunity. Here we present an adult patient who, during his early years of life, presented recurrent infections, autoimmune hemolytic anemia and benign lymphoproliferative disease, characterized by hepatosplenomegaly and lymphadenopathy, being diagnosed with common variable immunodeficiency (CVID) at 13 years of age. He was diagnosed with lymphocytic interstitial pneumonia at the age of 20. When he was 40 years old, after a diagnostic review, it was decided to perform genetic studies. A heterozygous mutation in *STAT3* NM_003150 c.2141C>T, p.P714L was detected by whole exome sequencing and validated by Sanger. Previously published functional studies performed in two siblings showed that this mutation resulted in gain-of-function. They were initially diagnosed with autoimmune lymphoproliferative syndrome, and later with *STAT3* GOF as a second genetic defect. Our patient developed severe pulmonary disease and died, without access to treatment targeted to his molecular defect due to the advanced nature of his pulmonary involvement and the fact that many of the therapies were still in development at that time. The diagnosis of *STAT3* GOF mutations should be suspected in patients with early-onset of lymphoproliferative disease, autoimmunity and hypogammaglobulinemia. This must be considered especially in the group of CVID patients with these characteristics, in order to allow the implementation of treatments targeting the molecular defect (JAK inhibitors and Il-6 receptor antagonists) that could modify the disease evolution.

Key words: common variable immunodeficiency, signal transducer and activator of transcription 3, gain of function, lung disease

Resumen ***Paciente adulto portador de una mutación con ganancia de función en el gen STAT3.*** Mutaciones en línea germinal con ganancia de función (GOF) del gen transductor de señales y activador de la transcripción 3 (*STAT3*) provocan una enfermedad caracterizada por importante linfoproliferación, incluyendo linfadenopatías y/o hepatoesplenomegalia, así como autoinmunidad de inicio en la infancia. Presentamos un paciente adulto que, durante sus primeros años de vida, presentó infecciones recurrentes, anemia hemolítica autoinmune y enfermedad linfoproliferativa benigna, caracterizada inicialmente por hepatoesplenomegalia y linfoadenopatías, diagnosticado de inmunodeficiencia común variable (IDCV) a los 13 años. A los 20 años, al ser estudiado por compromiso pulmonar, se diagnosticó neumonía intersticial linfocítica. A los 40 años, tras revisión diagnóstica se decidió realizar estudios genéticos. Por secuenciación del exoma completo se detectó una mutación heterocigota en *STAT3* NM_003150 c.2141C>T, p.P714L, que se validó por Sanger. Estudios funcionales previamente publicados realizados en dos hermanos con diagnóstico inicial de síndrome linfoproliferativo autoinmune, mostraron que esta mutación daba lugar a una ganancia de función. Nuestro paciente desarrolló enfermedad pulmonar grave y falleció a los 41 años, sin posibilidad de acceder a tratamiento dirigido a su defecto molecular por lo avanzado de su compromiso pulmonar y a que muchas de las terapias se encontraban en ese momento en desarrollo. El diagnóstico de mutaciones *STAT3* GOF debe sospecharse en pacientes con enfermedad linfoproliferativa temprana, autoinmunidad e hipogammaglobulinemia. Esto debe ser considerado especialmente en pacientes con IDCV con estas características, para permitir la implementación de tratamientos dirigidos al defecto molecular (inhibidores de JAK y antagonistas del receptor de Il-6) que podrían modificar la evolución de la enfermedad.

Palabras clave: inmunodeficiencia común variable, transductor de señales y activador de la transcripción 3, ganancia de función, enfermedad pulmonar

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Signal transducer and activator of transcription 3 (STAT3) is a protein encoded by the *STAT3* gene that controls cell proliferation, growth and apoptosis through the regulation of gene expression in response to extracellular cytokines, hormones and growth factors[1]. The germline gain of function of the *STAT3* gene causes an autosomal dominant disease, clinically characterized by a significant lymphoproliferation, including lymphadenopathy and/or hepatosplenomegaly, as well as childhood onset autoimmunity[1]. Autoimmune cytopenias are common, including autoimmune haemolytic anaemia, neutropenia and/or idiopathic thrombocytopenia. Patients may also show eczema, hypogammaglobulinemia, severe and recurrent infections, and growth failure[1-3].

Common variable immunodeficiency (CVID) is the most frequent symptomatic antibody deficiency, characterized by an impaired antibody production. Over 90% of patients suffer from extracellular capsulated bacterial infections, mainly of the respiratory tract; 25-30% develop autoimmune diseases, and 8-22% benign lymphoproliferative or granulomatous disease. These patients are also more susceptible to oncological diseases, mainly lymphomas and gastric adenocarcinoma. The most common autoimmune diseases are idiopathic thrombocytopenia and autoimmune haemolytic anaemia. Lungs, lymph nodes and spleen are the most frequently affected organs due to benign lymphoproliferative disease[4].

We present an adult patient who, during his first years of life, presented severe benign lymphoproliferative disease, recurrent infections and autoimmune haemolytic anaemia and was initially diagnosed as CVID. During his adulthood and after a diagnostic review, a gain-of-function (GOF) mutation in *STAT3* was found. *STAT3* GOF was first described about 35 years after our patient disease onset. When molecular diagnosis could be made, he already had significant deterioration of lung function, limiting the therapeutic possibilities.

Clinical case

The patient was a 40-year-old male; son of non-consanguineous and healthy parents. He developed lymphadenopathies at 18 months of age. A lymph node biopsy was performed, reporting non-specific lymphoid hyperplasia with a preserved follicular structure. At 21 months, five and seven years of age, he presented lymphadenopathy again with the same results in lymph node biopsies. When he was five he developed hepatoesplenomegaly. He had several episodes of pneumonia during childhood, with good response to antibiotic treatment. At the age of eight, autoimmune haemolytic anaemia was diagnosed and treated with corticosteroids evolving with exacerbation and remission periods. When he was 13 years old, he was referred to a pediatric immunologic center where low serum IgG, IgM and IgA levels were found, with an impaired post-vaccination antibodies response, low hemagglutinin titers but normal number of B lymphocytes. CVID was diagnosed and he began treatment with intravenous immunoglobulin replacement.

At the age of 20, he had a new episode of pneumonia, where a thorax computed axial tomography showed mediastinal adenopathies, pulmonary infiltrates adopting ground glass configuration, and accentuation of the reticular interstitium with interlobular thickening diffusely compromising both lung fields (Fig. 1). A lymph node and a pulmonary biopsy were performed, reporting nonspecific lymphoid hyperplasia and lymphocytic interstitial pneumonia, respectively, and treatment with corticoids was performed with good response. At the age of 22, he started follow-up in our hospital unit. Enterovirus meningoencephalitis was diagnosed and was treated with acyclovir until HSV was ruled out and the interval between doses of immunoglobulin was reduced, receiving one dose upon hospital admission. Infection resolved with residual seizures. He had lymphocytic interstitial pneumonia relapse when turning 24 years old and was treated with steroids at high doses. A lymph node biopsy performed 10 years afterwards reported lymph node hyperplasia. Several lymphoid phenotypes showed lymphopenia, inversion of CD4/CD8 ratio, normal B cell count, $CD21^{low}$ expansion, decreased switched memory B cells, normal double negative ($CD3^+$ $TCR\alpha\beta$ + $CD4^-$ $CD8^-$) and NK cells. An autoimmune lymphoproliferative syndrome (ALPS) was excluded based on a normal double negative cell count and normal levels of vitamin B12 and soluble FasL expression.

Considering the association of respiratory infections, autoimmunity and benign lymphoproliferation of childhood onset, genetic studies were decided. In our country it is still difficult to access sequencing techniques, which, in many cases, delays and makes diagnosis difficult. Patients without health insurance cannot access these type of studies. After signing informed consent, the patient was enrolled in a National Institutes of Health (NIH, Bethesda, MD) diagnostic protocol (05-I-0213 Screening and Baseline Assessment of Patients with Abnormalities of Immune Function, registered at ClinicalTrials.gov) and whole exome sequencing (WES) was performed according to the manufacturer's protocol (Illumina Exome with Enrichment Flex/HiSeq 2500). A heterozygous mutation, *STAT3* NM_003150 c.2141C>T, p.P714L was detected by WES and confirmed by Sanger sequencing (Life Technologies, BigDye Terminator v1.1 Cycle Sequencing Kit/3500 Genetic Analyzer) (Fig. 2). Previously published functional studies showed that this mutation resulted in GOF[5].

During the last years the patient developed multiple respiratory infections, progressive decline in lung function without

Fig. 1.– Thorax computed axial tomography. Pulmonary infiltrates adopting ground glass configuration, accentuation of the reticular interstitium with interlobular thickening diffusely compromising both lung fields

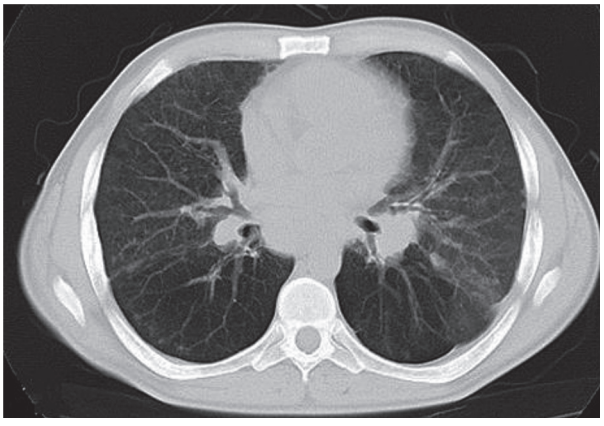
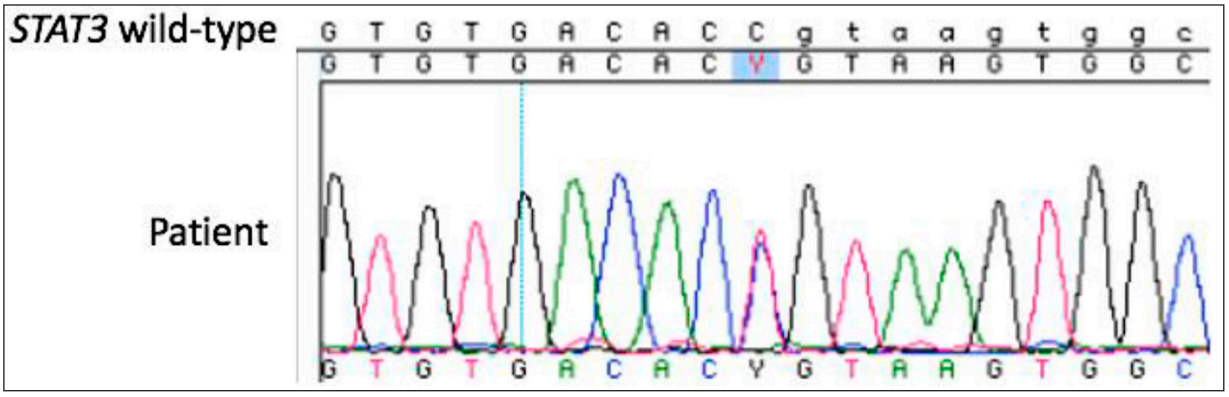


Fig. 2.– Sanger sequencing confirmation of WES-detected heterozygous variant *STAT3* NM_003150 c.2141C>T, p.P714L. The patient's Sanger sequencing trace data was aligned to the *STAT3* NM_003150 reference (wild-type) sequence. The IUPAC-IUB code 'Y' in the patient's data denotes the presence of both cytosine (C) and a thymine (T) peaks at that position



significant changes in thorax computed tomography scans, and right ventricular systolic function impaired. He died at the age of 41 due to an infectious complication that worsened his lung disease. He was waiting for a lung transplant.

Discussion

STAT3 GOF germline mutations were first described in five individuals with childhood-onset autoimmunity by Flanagan et al. in 2014[1]. Shortly thereafter Haapaniemi et al. reported three patients with *de novo* mutations, clinically characterized by multiorgan autoimmunity, lymphoproliferation, and late-onset mycobacterial disease[2]. Milner et al reported nine heterozygous germline mutations in thirteen individuals from ten families with lymphoproliferation autoimmunity and early-onset organ autoimmunity [3]. A total of 43 cases were later reported by different authors[5, 6].

STAT3 GOF disease has an autosomal dominant inheritance pattern. Although most identified mutations were *de novo*, six members of five families carrying a *STAT3* mutation were asymptomatic or had a less severe phenotype, indicating that there were carriers who showed an autosomal dominant inheritance with incomplete penetrance[3]. Since the parents of our patient did not show symptoms of the disease, but they were not studied, this defect could correspond to a *de novo* mutation but it cannot be ruled out that it is an autosomal dominant mutation with incomplete penetrance.

In most patients, genomic sequencing was necessary because the diagnostic approach is challenging, since clinical manifestations are variable and the similarity with other lymphoproliferative syndromes makes its presumption difficult. Initially, when *STAT3* GOF disease had not been yet described, a diagnosis of common variable immunodeficiency was made based on his clinical history of infections, autoimmune cytopenias, hypogammaglobulinemia and decrease switched memory B cells.

Lymphocyte phenotype as a diagnostic tool in *STAT3* GOF disease is controversial since the findings are variable, as are the levels of immunoglobulins[4-6]. Lymphoproliferation was accompanied by an increase in $CD3^+$ $CD8^-$ $CD4^-$ double negative T-cell count and defective mediated Fas apoptosis in some series[3,7]. An overlap in the phenotype with ALPS suggests that germline GOF mutations in *STAT3* produce a strong susceptibility to immune dysregulation. According to Nabhani et al, Fas expression was decreased and Fas-mediated apoptosis was deficient in patients with *STAT3* GOF mutations and ALPS like phenotype[7]. Thus, these diseases may have a common pathophysiologic mechanism at some point, involving the failure of self-tolerance. Consistent with this, our patient has an ALPS like phenotype. It is interesting to note that our patient's mutation had previously been described in two siblings diagnosed with ALPS, in whom clinical evolution led to the suspicion that there was another underlying mutation[5].

Therapeutic decisions are difficult to make due of a lack of prospective studies and the scarce number of reports. Immunoglobulin replacement therapy is indicated in patients with hypogammaglobulinemia and recurrent infections. It has been reported that the use of immunosuppressants like corticosteroids, mTOR inhibitors, mycophenolate mofetil and rituximab is the mainstay of the treatment for autoimmune manifestations[6-11]. However, the use of immunosuppressants could have significant side effects and often doesn´t result in clinical resolution of lymphoproliferative and autoimmune manifestations. Eight patients were successfully treated with tocilizumab, a monoclonal antibody against the Il-6 receptor, and specific inhibition of JAK molecules, including tofacitinib and ruxolitinib[3,8-11]. Until now, hematopoietic cell transplantation was performed in five patients, four of whom died[3, 10]. This would suggest that other therapeutic approaches, such as specific small-molecule inhibitors, are perhaps more appropriate therapies.

STAT3 gain of function disease was described about 35 years after our patient disease onset. When molecular diagnosis could be made, he already had significant deterioration of lung function. New promising treatments such as JAK inhibitors weren´t available in our country at that time.

The diagnosis of *STAT3* GOF mutations should be suspected in patients with lymphoproliferative disease, autoimmunity and hypogammaglobulinemia. It is important to consider this diagnosis in CVID patients that share these characteristics, regardless of their age. In order to determine the therapeutic efficacy of JAK inhibitors, Il-6 receptor antagonists and hematopoietic cell transplantation, long-term studies are needed. Nowadays, early diagnosis may allow the implementation of targeted precision therapies that could modify the disease evolution.

Conflict of interest: None to declare

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