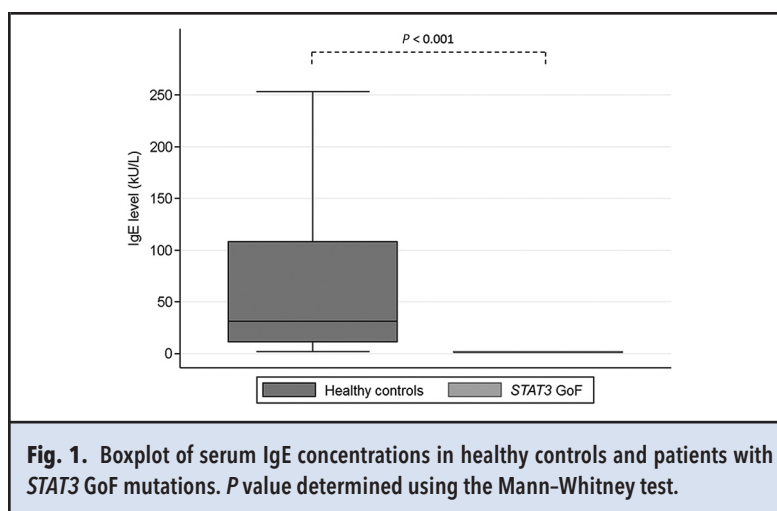


Low IgE Is a Useful Tool to Identify *STAT3* Gain-of-Function Mutations

To the Editor:

Germline gain-of-function mutations (GoF)¹ in signal transducer and activator of transcription 3 (*STAT3*)² were recently identified as the cause of early-onset autoimmune disease (MIM: 615952). This rare monogenic autoimmune disorder commonly encompasses autoimmune hematological disorders (identified in 70% of patients), recurrent infections (60%), and autoimmune enteropathies (50%)^(1–3). The phenotype observed contrasts to that of patients with loss-of-function (LoF) *STAT3* mutations causing hyper IgE syndrome (Job syndrome), typically characterized by increased immunoglobulin E (IgE), recurrent infections, and eczema⁽⁴⁾. Identifying patients with *STAT3* GoF mutations informs families and clinicians of prognosis and facilitates personalized treatment.

There is substantial clinical variability between patients with GoF *STAT3* mutations and also overlap in phenotype with other monogenic autoimmune disorders such as immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome (MIM: 304790) resulting from hemizygous loss-of-function forkhead box P3 (*FOXP3*) mutations, and also early-onset polygenic autoimmune disease. Distinguishing the specific genetic etiology using clinical characteristics alone is not possible and genetic testing, often performed sequentially until a pathogenic mutation is found, is essential for accurate diagnosis.



Genetic testing for *STAT3* is expensive and available only in a limited number of specialist centers. An inexpensive and widely available test to identify patients most likely to harbor GoF *STAT3* mutations is desirable as it will prevent inappropriate genetic testing. We assessed whether IgE would be a useful tool to aid in the identification of patients suitable for *STAT3* sequencing.

Total serum IgE concentration was measured by using the ImmunoCAP 1000 instrument (Phadia) for both healthy reference samples ($n = 1510$) and *STAT3* patient samples ($n = 6$). Calibration was performed with the 2nd WHO International Reference Preparation of Human Serum Immunoglobulin E (IgE) (NIBSC code 75/502) ⁽⁵⁾. Statistical analysis was performed in Stata®; specificity, sensitivity and binomial confidence intervals were calculated. The age of the patients with *STAT3* GoF mutations ranged from 1 to 9 years, and the median time from the first clinical symptom to IgE testing was 4 years.

Immunological assessment of individuals with *STAT3* GoF mutations identified total serum IgE below the lower reference limit of age-matched controls in all 6 cases, (<2 kU/L, range 0.7–2 kU/L; see Fig. 1)⁽¹⁾. Using 2 kU/L as a cutoff for

the assay for identification against all age groups gave a sensitivity and specificity of 100% (95% CI, 54.–100) and 97.2% (95% CI, 96.2–97.9), respectively.

We have demonstrated that serum IgE, an inexpensive and widely available diagnostic test, is likely to be very useful to facilitate identifying patients suitable for *STAT3* sequencing, and a 2-kU/L cutoff gives high sensitivity and specificity. The role we propose for IgE measurement is in patients with multisystem early-childhood autoimmune disease in whom *STAT3* GoF mutations are being considered. This is likely to be particularly helpful in distinguishing from multiple autoimmunity resulting from hemizygous *FOXP3* mutations as more than 90% of patients with IPEX syndrome have increased serum IgE. In this scenario, raised IgE would be helpful to rule out *STAT3* GoF mutations, reducing total genetic testing expenditure. In addition, despite recent advances in sequencing technologies, identifying pathogenic mutations from benign variants remains a challenge. Measuring IgE could be a tool to aid molecular geneticists in classifying *STAT3* variants found by DNA sequencing; serum IgE concentration below 2 kU/L supports pathogenic GoF.

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¹ Nonstandard abbreviations: GoF, gain-of-function mutations; LoF, loss-of-function; IgE, immunoglobulin E; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome.

² Human genes: *STAT3*, signal transducer and activator of transcription 3; *FOXP3*, forkhead box P3.

We have assessed IgE in a small number of patients with GoF *STAT3* mutations ($n = 6$) and assessment in further patients is required to increase confidence in the diagnostic utility, but it is notable that all 6 patients had IgE concentrations below the 1st centile of the reference population. Two previous case series of patients with *STAT3* GoF mutations measured IgE in a total of 9 additional individuals (4 of those previously reported are included in this study (1, 2)). One described 2 additional individuals with serum IgE below 2 kU/L(2), supporting our findings. The other reported more variability in IgE than we have observed (0.1–58.5 mg/dL; $n = 7$)(3), but we were unable to ascertain reference interval, testing platform or comparable units to our assay for these individuals and therefore, unable to make a direct comparison to our findings(3). A potential limitation of the diagnostic accuracy of IgE to identify patients with *STAT3* GoF mutations is that an allergic response or parasitic infection may increase serum IgE. Given that allergies are relatively common (for example, allergic rhinitis is present in >20% of the European population) this is an important consideration as this may decrease the performance of the test in these patients. In conclusion, total serum IgE concentration is an inexpensive and robust tool for determining which patients with multiple early-onset autoimmunity are likely to have a *STAT3* GoF mutation. In combination with the iden-

tification of clinical features of early-onset autoimmune disease, IgE concentrations could be assayed locally to facilitate appropriate, cost-efficient, and rapid genetic testing. Further work is warranted to determine IgE in other subtypes of monogenic autoimmunity.

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