

CORRESPONDENCE

Hyper IgE syndrome: anaphylaxis in a patient carrying the N567D *STAT3* mutation

DOI:10.1111/pai.12217

To the Editor,

Autosomal-dominant hyper IgE syndrome (AD-HIES) is a rare primary immunodeficiency caused by dominant-negative mutations in the *STAT3* gene and is characterized by eczema, recurrent staphylococcal infections of the skin and lungs, pneumatocele formation, mucocutaneous candidiasis, other non-immunologic features, and, obviously, high IgE titers. Despite this feature (and differently from autosomal recessive hyper IgE syndrome), the incidence of allergy in AD-HIES patients is low and anaphylaxis has never been reported in the literature (1).

Here, we report the case of a 30-year-old man, diagnosed with HIES at the age of 13 months, who presented to our department after a documented episode of anaphylaxis. One month before, he was seen at our emergency department suffering from dyspnoea, oral itching, and urticaria: he reported a sudden onset of symptoms after eating pasta with pesto. At physical examination, he presented wheezing and hives localized to the face, neck, and upper trunk; vital signs, in particular blood pressure, were normal. Intravenous antihistamine and corticosteroid were administered, with progressive clinical improvement. About two hours after resolution of symptoms, he exhibited a new reaction, similar to the first, again readily treated with the same medications. After a few hours of observation, he was discharged with the diagnosis of 'unspecified allergy' and it was suggested that he undergo specific allergy testing.

He was subsequently seen at our department where a complete workup was undertaken. His past medical history was typical of AD-HIES (1): newborn rash; eczema; sepsis caused by *Staphylococcus aureus* at the age of 1 month; frequent episodes of oral candidiasis; elevated levels of serum IgE (Table 1A); staphylococcal lung infection with formation of pneumatocele which required two lobectomies at the age of 13 months and 9 yr; recurrent skin infections, otitis, and suppurative lymphadenitis; bone fragility with frequent fractures; and typical *facies* (NIH score at consultation = 74). However, genetic testing had not yet been performed. At the time of the visit, he was on prophylaxis with trimethoprim/sulfamethoxazole and itraconazole with good control of infections. Skin prick tests, ImmunoCAP specific IgE, and ImmunoCAP ISAC were performed: They showed multiple sensitizations, in particular for seeds (peanut, hazelnut, and walnut) and peach (precisely for their non-specific lipid transfer proteins (nsLTPs) allergenic components) (Table 1B).

Given the strong suspicion of AD-HIES (1), sequencing of the *STAT3* gene was performed. *STAT3* (NM_139276.2) sequencing analysis revealed a heterozygous substitution in exon 19 [cDNA: NM_139276.2:c.1699A>G, DNA Level: Chr17 (GRCh37):g.40475327T>C, protein level: p.Asn567Asp or

Table 1 A: Patient's total IgE over time. B: Results of skin prick tests and specific IgE antibodies (ImmunoCAP specific IgE and ImmunoCAP ISAC)

		Total IgE (IU/ml)	
		Patient	Normal values
A			
Age			
13 months		199	<20
3 yr		1537	<80
5 yr		17,862	<100
9 yr		14,560	<100
18 yr		21,138	<120
30 yr		19,714	<120
		Skin prick tests (mean diameter of the weal)	
		Specific IgE (kU/l)	ImmunoCAP ISAC
B			
Positive control	4 mm		
Negative control	0 mm		
Peanut	2 mm	rAra h 9 = 38.5	nAra h 1–3 < 0.3 ISU
Pine nuts	2 mm	14.7	
Walnut	11 mm	95.9	
Hazelnut	8 mm	24.7	rCor a 8 = 18 ISU
Kiwi	5 mm	n.p.	nAct d 1 = 5.2 ISU
Peach	n.p.	n.p.	nPru p 3 = 38 ISU
Cat	3 mm	n.p.	rFel d 1 = 10 ISU

n.p., not performed; ISAC, Immuno Solid-phase Allergen Chip.

p.N567D] (Fig. 1). The mutation resulted in a substitution of asparagine to aspartic acid at amino acid position 567 (p.Asn567Asp) in one of the four helices connecting the DNA-binding domain and the SH2 domain (connector or linker domain). This mutation was not found in the single nucleotide polymorphism database (dbSNP135; www.ncbi.nlm.nih.gov/projects/SNP/). Further study of family members revealed that neither parent carried the mutation: both resulted homozygous for the wild-type allele, indicating that the *STAT3* mutation occurred *de novo*. The mutation was present in a moderately conserved nucleotide (phyloP: 2.63 [–14.2; 6.4]). Moreover, comparative genomics analysis, by aligning nucleotide sequences of 11 different species, from mammals to amphibian, showed that p.N567 is a highly conserved amino acid. Functional predictions of the p.N567D mutation were performed with the bioinformatic methods: Alamut (version 2.1), Mutation Taster (<http://www.mutationtaster.org/>), and Sorting Intolerant from Tolerant

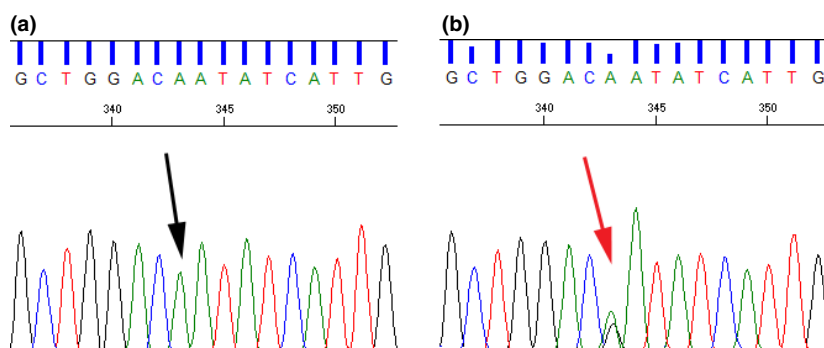


Figure 1 *STAT3* mutational analysis: (a) Chromatogram showing the wild-type *STAT3* sequence. The black arrow points to the wild-type *STAT3* allele present in both patient's parents. (b) Chromatogram showing the mutated *STAT3* sequence in our patient. The red arrow points to the N567D heterozygous mutation.

(SIFT, <http://sift.jcvi.org/>) programs. Alamut reported the substitution as 'class-3-unknown pathogenicity', Mutation taster as 'disease causing' (p value: 0.9987; the p value is the probability of the prediction), while SIFT classified it as 'tolerated' (score 0.12). Since the mutation has not been reported on dbSNP135, in the 1000 Genome Project (<http://www.1000genomes.org/data>) and in the Exome Sequencing Project (<https://esp.gs.washington.edu/drupal/>) databases, nor in our in-house database, containing data of 89 whole exomes, it has been suggested that the identified mutation could be causative of the patient's phenotype. This initial hypothesis about the causative role of the identified N567D mutation was supported by Chandresris et al. (2) who reported a I568F *STAT3* mutation in a French AD-HIES patient.

To confirm the pathologic relevance of this newly identified mutation, immunologic tests were performed, as previously described (3–5). Accordingly with diagnostic guidelines proposed for hyper-IgE syndrome (3), the induction of Th17 cells after stimulation with the superantigen SEB was strongly impaired in the patient (0.05%) compared with age-matched healthy controls (0.52–0.76%). Despite a normal frequency of

total CD4+ cells, we documented an increase in naïve CD4+ T cells (71%) compared with normal donors (median 42%, range 35%–53%), as previously described in *STAT3*mut individuals (4); moreover, stimulation with IL-12 resulted in a reduction of IL-21 expression in naïve CD4+ T cells (3.5%), compared with that observed in normal donors (median 32%, range (25%–39%)) (4). The addition of IL-10 to LPS-treated DC, contrary to what occurs in normal controls, did not affect their maturation, as indicated by the expression of maturation antigens CD1, CD80, and CD86 (5).

Using Deep View/Swiss Pdb Viewer (6), possible interactions of Asn 567 were investigated in the three-dimensional structure of mouse *STAT3* (7). Asn567 is contained in one of the four helices connecting the DNA-binding domain and the dimerization domain. The amide nitrogen is H-bonded to the oxygen atom of Lys 615, close to the highly conserved aminoacids 609–613 that constitute the phosphotyrosine-binding region. Since the human *STAT3* gene shows very high homology with the mouse protein, it is likely that the substitution of an amide nitrogen with a partially negative oxygen not only leads to the absence of the H-bond, but causes

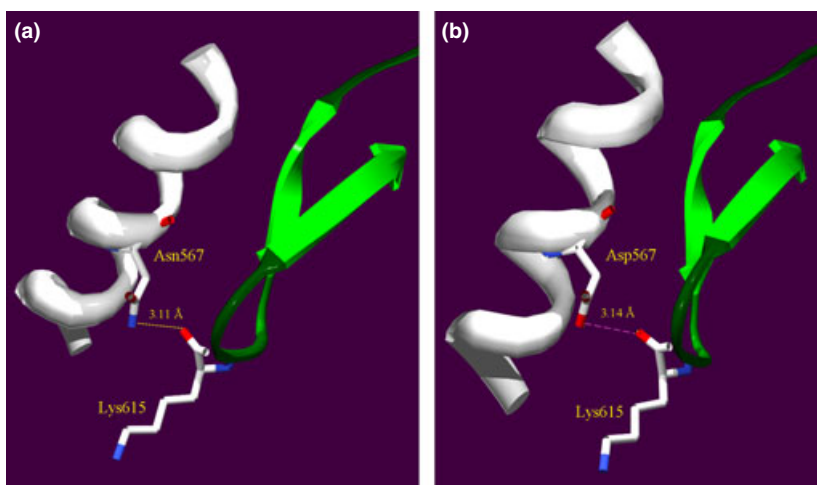


Figure 2 Three-dimensional structure of mouse *STAT3* (Deep View/Swiss Pdb Viwer: <http://www.expasy.org/spdbv>). Carbon Atoms in white, Oxygen Atoms in red, Nitrogen Atoms in blue; H-bond in yellow dashes. (a) Wild-type protein: on the left, helix $\alpha 8$ containing Asn 567; on the right, the loop connecting β -strand C to β -strand B, which contains the phosphotyrosine-binding site (not shown). (b) Simulation of mutated protein: In the best-scoring rotamer, the orientation of the carboxylate of the substituted Asp still shows a strong repulsion (violet dashed line) with Oxygen of Lys 615.

a repulsion of the backbone oxygen of AA615 (Fig. 2), thus resulting in a movement of the loop connecting the two β strands of the dimerization domain. One of these strands forms the phosphotyrosine-binding site, and it is therefore possible that this mutation affects the binding properties of the protein and eventually the stability of the dimer.

Although patients with AD-HIES may have elevated IgE to specific allergens due to their increased total IgE, the incidence of allergic diseases is low (2). In particular, to the best of our knowledge, this is the first description of anaphylaxis in a patient with HIES in the literature. Pathogenesis in AD-HIES has not yet been clearly defined, in particular concerning the paradox of increased IgE production in relation to few allergic reactions. Experimental data suggest that perturbation of the complex cytokine network (mainly IL-4, IL-21, IL-10, and IFN γ) that finely regulates IgE production results, at the naïve B cell level, in IL-4/STAT6-mediated IgE overproduction (8). On the other hand, it seems that STAT proteins are involved in mast cell growth/differentiation/survival, cytokine production, and release of mediators (9). It is therefore possible that the high number of non-specific IgE constitutively associated with Fc ϵ RI on the surface of mast cells interferes with the aggregation of receptors needed to initiate the signaling cascade and finally (together with an altered signal transduction) mast cell degranulation.

Anaphylaxis was not recognized in the emergency department, despite a clinical presentation clearly fulfilling the diagnostic criteria (10), and was not treated according to current guidelines. Skin prick tests and laboratory tests, together with the characteristic history, strongly indicate that walnuts, one of the main ingredients of Genoese pesto (all others tested resulted negative), were causative of the biphasic anaphylaxis. Therefore, the patient was supplied with adrenaline and told to avoid this food.

Clinical features and description of the newly identified mutation rendered this case of particular interest; data comparing the binding properties of the mutated and wild-type proteins should help clarify the pathogenetic mechanism of this condition.

Pietro Merli^{1,2}; Francesca Novara³; Daniela Montagna^{1,2}; Serena Benzo^{1,2}; Matteo Tanzi²; Ilaria Turin²; Mara De Amici²; Angelo Merli⁴; Orsetta Zuffardi³ & Gian Luigi Marseglia^{1,2}

¹Department of Clinical-surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy; ²Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; ³Department of Molecular Medicine, University of Pavia, Pavia, Italy; ⁴Department of Biosciences, University of Parma, Parma, Italy
E-mail: pietromerli@gmail.com

References

1. Zhang Q, Su HC. Hyperimmunoglobulin E syndromes in pediatrics. *Curr Opin Pediatr* 2011; **23**: 653–8.
2. Chandesris MO, Melki I, Natividad A, et al. Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. *Medicine (Baltimore)* 2012; **91**: e1–19.
3. Woellner C, Gertz EM, Schaffer AA, et al. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. *J Allergy Clin Immunol* 2010; **125**: 424–32 e428.
4. Ma CS, Avery DT, Chan A, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. *Blood* 2012; **119**: 3997–4008.
5. Giacomelli M, Tamassia N, Moratto D, et al. SH2-domain mutations in STAT3 in hyper-IgE syndrome patients result in impairment of IL-10 function. *Eur J Immunol* 2011; **41**: 3075–84.
6. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 1997; **18**: 2714–23.
7. Becker S, Groner B, Muller CW. Three-dimensional structure of the Stat3beta homodimer bound to DNA. *Nature* 1998; **394**: 145–51.
8. Avery DT, Ma CS, Bryant VL, et al. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. *Blood* 2008; **112**: 1784–93.
9. Morales JK, Falanga YT, Depczynski A, Fernando J, Ryan JJ. Mast cell homeostasis and the JAK-STAT pathway. *Genes Immun* 2010; **11**: 599–608.
10. Simons FE, Arduoso LR, Bilo MB, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J* 2011; **4**: 13–37.