A novel mutation in the linker domain of the signal transducer and activator of transcription 3 gene, p.Lys531Glu, in hyper-IgE syndrome

To the Editor:

Hyper-IgE syndrome (HIES) is a rare primary immunodeficiency disorder characterized by eczema, recurrent abscesses, pneumonia with pneumatocele, and an increased serum IgE level. Most cases are sporadic, but both autosomal dominant and autosomal recessive forms have been described.[1]

Recently, mutations in the signal transducer and activator of transcription factor 3 gene *(STAT3)* have been determined to be the cause of autosomal dominant HIES.[2,3] Most *STAT3* mutations were located in the DNA-binding domain or Src homology 2 domain, whereas missense mutations were also found in the transactivation domain downstream of the Src homology 2 domain.[2-4] Here we report the first *STAT3* mutation in the linker domain of the protein in a boy with HIES.

A 5-year-old Korean boy was admitted because of fever, cough, and otorrhea for a week. His parents were not consanguineous. He had no remarkable perinatal problems and had been vaccinated as scheduled. He had a history of atopic dermatitis since 6 months of age. Ingestion of eggs and milk had been restricted until 43 months of age, when he passed the open challenge test. Of note, past medical history also revealed recurrent infections, including multiple episodes of oral candidiasis. He was treated with antibiotics for an abscess on the scalp caused by group A β-hemolytic *Streptococcus* species at 4 months of age. He had a history of 3 hospitalizations for pneumonia without pneumatocele. Suppurative cervical lymphadenitis caused by *Staphylococcus aureus*, which occurred at 18 months of age, was successfully treated with incision, drainage, and antibiotics, but it recurred several times afterward. At 4 years of age, tympanostomy tubes were inserted in the eardrums bilaterally for recurrent otitis media caused by *Streptococcus pneumoniae*. Family history revealed no members with relevant allergic or immunologic diseases.

On physical examination, his height was 104.6 cm (10th percentile), and his weight was 17 kg (25th percentile). He had coarse facial features with a low hairline and hypertelorism and genu valgum. A whitish plaque was found on the soft palate. The left external auditory canal was wet, and the tympanostomy tube was not *in situ* in his left ear. A 1.0 × 1.0 cm, hard, tender lymph node was palpable in the left supraclavicular area. Fine crackles were heard on both lung fields, and his skin was slightly dry. Complete blood cell counts demonstrated a normal total eosinophil count (<400 cells/μL), although his eosinophil count had been up to 7000 cells/μL at 6 months of age. Plain chest radiographic analysis demonstrated bilateral peribronchial infiltrates, and *Haemophilus influenzae* was isolated from sputum culture. The serum IgE level was increased at 3980 IU/mL. Results of other immunologic studies, such as serum immunoglobulin level measurement and the nitroblue tetrazolium test, were normal. Lymphocyte subset analyses revealed normal levels of both $CD4^+$ and $CD8^+$ T cells (1950/μL and 1500/μL, respectively). His National Institutes of Health score was 45 points ("probably affected"; Table I).[5]

Written informed consent was obtained from the parents for molecular genetic studies for *STAT3* mutations. Direct sequencing analysis of the *STAT3* gene was performed with genomic DNA isolated from peripheral blood leukocytes. The Wizard Genomic DNA Purification Kit was used according to the manufacturer's instructions (Promega, Madison, Wis). As a result, we identified an amino acid–changing variation in the linker domain of the *STAT3* gene; it was an A-to-G transition at

TABLE I. National Institutes of Health score for the patient

	Point										
Clinical findings	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Highest serum IgE level (IU/mL)											O
Skin abscesses			O								
Pneumonia (episodes over lifetime)									O		
Parenchymal lung anomalies	O										
Retained primary teeth	O										
Scoliosis, maximum curvature	O										
Fractures with minor trauma	O										
Highest eosinophil count (cells/μL)							O				
Characteristic face				O							
Midline anomaly	O										
Newborn rash					O						
Eczema (worst stage)					O						
Upper respiratory tract infections per year					O						
Candidiasis		O									
Other serious infections	O										
Fatal infections	O										
Hyperextensibility	O										
Lymphoma	O										
Increased nasal width	O										
High palate	O										
Young age correction				O							

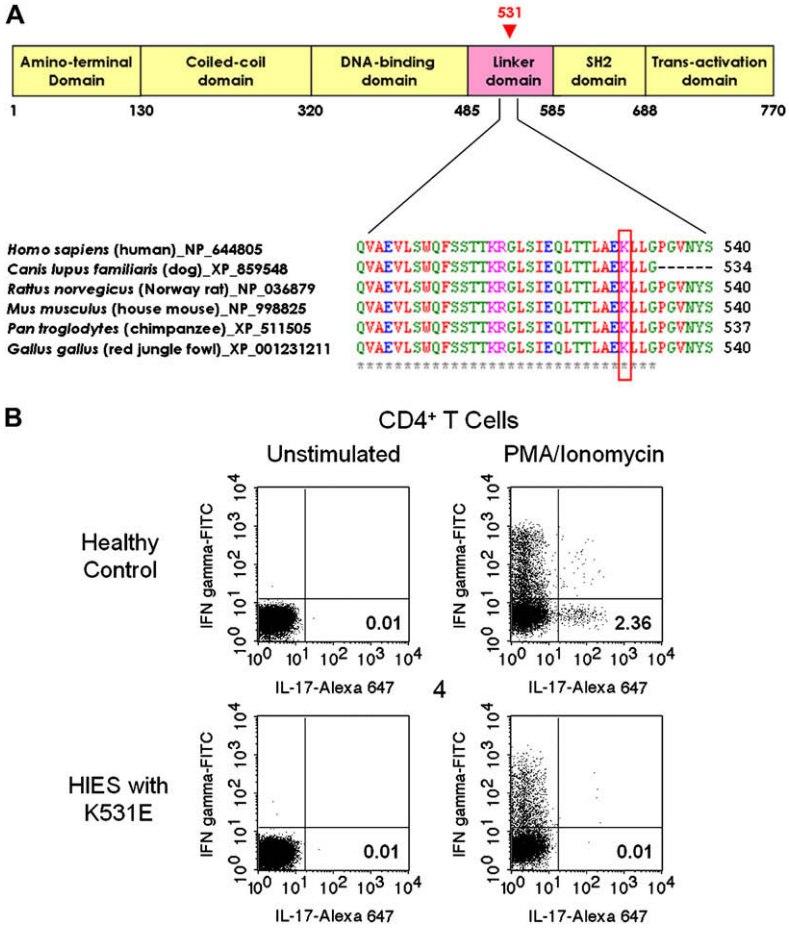

FIG 1. A, The affected residue, K531, was located in the linker domain of *STAT3 (red arrowhead)*. Sequence alignment of the STAT3 protein with CLUSTALW showed that the K531 residue was perfectly conserved across different species *(red box)*. The numbers indicate amino acid positions. **B**, Flow cytometric analyses by means of intracellular staining for IL-17 production in phorbol 12-myristate 13-acetate/ionomycin–activated $CD4^+$ T cells. Note the markedly low level of IL-17–secreting $CD4^+$ T cells (T_H17 cells) in the patient compared with the corresponding level in a healthy control subject (0.01% vs 2.36%).

the nucleotide 1591 position (c.1591A>G) leading to substitution of lysine at the amino acid position 531 to glutamic acid (p.K531E). K531E was not found in the single nucleotide polymorphism database (dbSNP; www.ncbi.nlm.nih.gov/projects/SNP/) nor has it been reported as a mutation in patients with HIES. Family study revealed that neither of the patient's parents had the mutation, and his 3-year-old younger sister is also homozygous for the wild-type allele. Comparative genomics analyses performed by aligning nucleotide sequences of different species showed that the K531 residue in the linker domain is perfectly conserved (Fig 1, *A*). Functional prediction of the variation with the PolyPhen (Polymorphism Phenotyping) program (http://genetics.bwh.harvard.edu/pph/) and the Sorting Intolerant From Tolerant program (http://blocks.fhcrc.org/sift/SIFT.html) predicted "probably damaging (PSIC score difference, 2.064)" and "deleterious (SIFT score 0)" values, respectively. We also observed that the K531E variation did not occur in 200 control chromosomes from 100 healthy individuals (frequency, 0%). Lastly, we performed flow cytometric analyses to demonstrate the functional deficit of the STAT3 protein, as recently described.[4] As a result, we observed that the patient had impaired generation of IL-17–secreting $CD4^+$ T cells (T_H17 cells) on stimulation compared with control levels (Fig 1, *B*). Collectively, all these findings indicate that this *de novo* heterozygous missense mutation, K531E, in the linker domain of *STAT3* was the disease-causing mutation in the patient.

It was reported that *STAT3* mutations were not always found in patients with HIES.[3,4] In addition, homozygous mutations in the tyrosine kinase 2 gene are the genetic defect in a subgroup of patients with HIES.[6] The K531E mutation described in this report is the first mutation in the linker domain of *STAT3*, further expanding the genetic heterogeneity of HIES. According to recent studies,[4,7,8] patients with HIES with *STAT3* mutations showed absent or markedly decreased levels of T_H17 cells in their peripheral blood. By comparison, patients with HIES or HIES-like symptoms (without recurrent candidiasis/staphylococcal abscess or lung infection) without *STAT3* mutations were shown to have higher levels of T_H17 cells than those with mutations, albeit still significantly lower than levels seen in control individuals. The functional prediction by means of bioinformatics analyses and population screening supported that K531E is a mutation affecting a highly conserved amino acid residue with a potential to adversely affect the linker domain. Indeed, we could demonstrate that the T_H17 response was markedly decreased in the patient

compared with that seen in healthy control subjects. The level of decrease was comparable with that seen in previously reported patients with *STAT3* mutations in other domains.

In summary, we identified the first mutation in the linker domain of *STAT3* in a patient with HIES. Further cases are needed to elucidate any genotype-phenotype correlations in mutations occurring in the linker domain in comparison with those in other *STAT3* domains.

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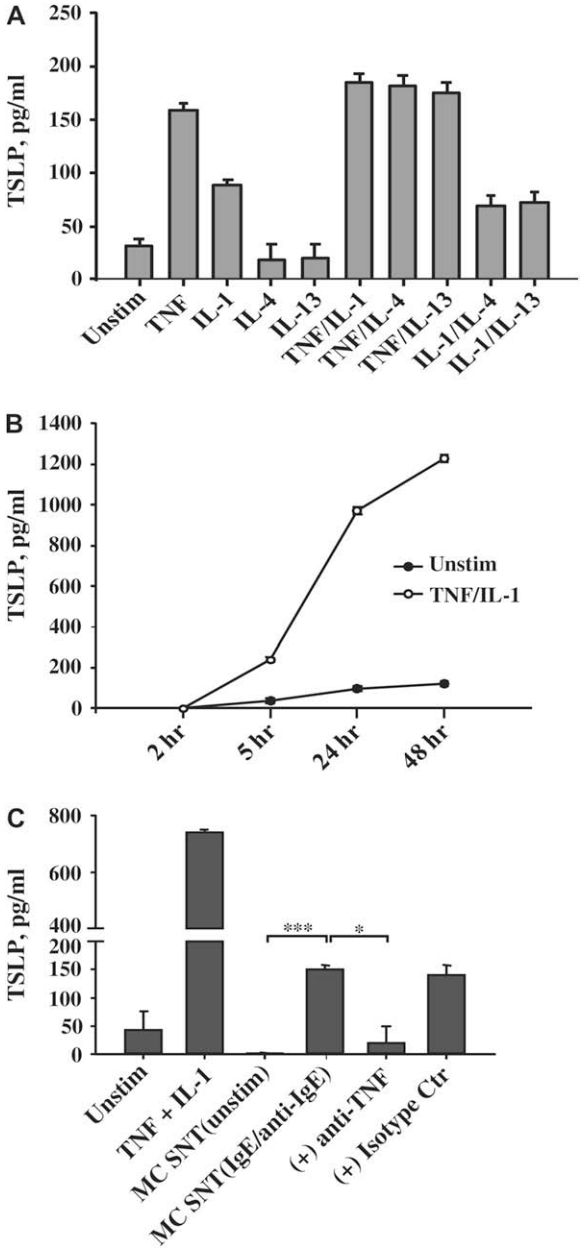

FIG 1. **A**, TSLP production by human BSM cells stimulated with TNF (25 ng/mL), IL-1α (10 ng/mL), IL-4, and IL-13 (50 ng/mL each). Data are representative of 4 experiments. **B**, Time course of TSLP production by BSM cells stimulated with IL-1/TNF. Data are representative of 2 experiments. **C**, Supernatants of IgE/anti-IgE–activated primary MCs (50% vol/vol), prepared as in Allakhverdi et al,[5] with or without antibody to TNF or isotype control (10 μg/mL each). Data are representative of 4 experiments. *$P < .05$, ***$P < .001$. *Unstim*, Unstimulated; *SNT*, supernatant.

Thymic stromal lymphopoietin as a mediator of crosstalk between bronchial smooth muscles and mast cells

To the Editor:

Accumulation of chronically activated mast cells (MCs) within the bronchial smooth muscle (BSM) bundles is a key immunopathologic feature of asthma, regardless of the severity of the disease.[1] This MC microlocalization is thought to contribute to the development of BSM hypertrophy, hyperplasia, and hyperreactivity. MCs might mediate these effects by releasing autacoids; granule-associated molecules, such as tryptase; and the cytokines IL-13 and TNF.[2] Indeed, expression of IL-13 by the MCs localized within the BSM bundles is observed in patients with mild and severe asthma.[3] We here provide evidence that the recently described epithelial cell–derived cytokine thymic stromal lymphopoietin (TSLP) might also be implicated in this process. TSLP is known to initiate typical IgE- and T_H2 cell–dependent allergic diseases through its effects on dendritic cells, whereas its ability to potently stimulate MCs might contribute to the T cell– and IgE-independent allergic inflammation.[4,5]

In agreement with a previous study,[6] here we show that primary human BSM cells from nonasthmatic donors (commercially