Diagnostic approach to the hyper-IgE syndromes: Immunologic and clinical key findings to differentiate hyper-IgE syndromes from atopic dermatitis

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Background: Hyper-IgE syndromes (HIES) are primary immunodeficiency disorders characterized by *Staphylococcus aureus* abscesses, recurrent pneumonia, increased serum IgE levels, and eczema. The association of heterozygous signal transducer and activator of transcription 3 *(STAT3)* mutations with autosomal dominant (AD)–HIES allows the differentiation of AD-HIES from disorders associated with eczema and increased serum IgE levels, such as other primary immunodeficiencies and atopic dermatitis.

Objective: To facilitate early diagnosis of AD-HIES to initiate appropriate therapy.

Methods: The clinical phenotype (suggested by a National Institutes of Health [NIH] score of ≥40 points), *STAT3* genotype, and T_H17 cell counts were compared in a cohort of 78 patients suspected of having HIES.

Results: Heterozygous *STAT3* missense mutations and in-frame deletions were identified in 48 patients, all but 2 with an NIH score ≥40 points. Patients with *STAT3* mutations with HIES showed significantly lower T_H17 cell counts compared with patients with wild-type *STAT3* and control subjects. Only 1 patient with wild-type *STAT3* had both an NIH score ≥40 points and abnormal T_H17 cell counts (≤0.2% of $CD4^+$ cells), with this exception being identified with a homozygous dedicator of cytogenesis 8 protein *(DOCK8)* mutation. Pathologic shedding of primary teeth was present in 3 patients with wild-type *STAT3* and 33 patients with *STAT3* mutations. Internal abscesses and severe infections were exclusively seen in patients with *STAT3* mutations, who also had increased pneumatocele formation and skeletal or connective tissue manifestations compared with patients with wild-type *STAT3*.

Conclusion: We expanded the number of *STAT3* mutations and validated that the NIH score sensitively identifies patients with HIES. Based on our patient cohort, we propose key findings that, when combined with T_H17 cell numbers, predict patients with AD-HIES with *STAT3* mutations, supporting early diagnosis of AD-HIES. (J Allergy Clin Immunol 2010;126:611-7.)

Key words: *Atopic dermatitis, dedicator of cytogenesis 8 protein* (DOCK8)*, hyper-IgE syndrome, Job syndrome, National Institutes of Health score, signal transducer and activator of transcription 3 (*STAT3*), T_H17 cells, tyrosine kinase 2 (*TYK2*)*

The hyper-IgE syndromes (HIES) are rare primary immune deficiency diseases (PIDD) that are inherited in an autosomal dominant (AD) or autosomal recessive (AR) manner or, in some cases, occur sporadically. Patients with AD-HIES often present with *Staphylococcus aureus* abscesses, recurrent episodes of pneumonia with pneumatocele formation, increased serum IgE levels, and eczema.[1] The clinical entity was originally reported in 1966 as Job syndrome in 2 unrelated girls who had recurrent "cold" staphylococcal abscesses and chronic lung disease.[2] The term "hyper-IgE syndrome" was introduced in 1972 when high serum IgE levels and coarse facial features were reported.[3] Additional features of this syndrome include hyperextensible joints, abnormal shedding of primary teeth, scoliosis, bone fractures with minimal trauma, and AD inheritance.[1,4] A rare AR entity (AR-HIES) complicated by recurrent viral infections and involvement of the central nervous system but without connective tissue and bone abnormalities has been described several years later and

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Abbreviations used
AD: Autosomal dominant
AR: Autosomal recessive
DOCK8: Dedicator of cytogenesis 8 protein
HIES: Hyper-IgE syndrome
IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
NIH: National Institutes of Health
PBMC: Peripheral blood mononuclear cell
PIDD: Primary immune deficiency disease
SH2: Src homology domain 2
STAT3: Signal transducer and activator of transcription 3
TYK2: Tyrosine kinase 2

has now been associated with dedicator of cytogenesis 8 protein *(DOCK8)* mutations.[5-7] The identification of heterozygous signal transducer and activator of transcription 3 *(STAT3)* mutations as the cause of AD-HIES[8-12] was made possible by the discovery that a homozygous mutation in the tyrosine kinase 2 *(TYK2)* gene was associated with a variant form of AR-HIES,[13] suggesting that defects in JAK/STAT signaling might be involved in other forms of HIES. STAT3 belongs to the STAT family of transcriptional regulators, which are known to play key roles in cell development and differentiation and in cell death. The precise mechanisms by which heterozygous *STAT3* mutations with a dominant negative effect on STAT3 function cause the various findings observed in patients with AD-HIES are only partially understood.[8,14-16] The generation of a nonfunctional *STAT3* allele affects the development of IL-17 producing T_H17 effector T cells,[11,17-19] which are thought to play a prominent role in controlling common infectious agents observed in HIES, such as extracellular bacteria and fungi.[20]

Other single-gene defects resulting in PIDD with associated eczema, increased serum IgE levels, and recurrent infections include Omenn syndrome (caused by hypomorphic mutations in genes such as *RAG1, RAG2, ARTEMIS, ADA,* and *RMRP*); Wiskott-Aldrich syndrome (caused by mutations in the *WAS* gene); immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX; caused by mutations in the gene *FOXP3*); and Comèl-Netherton syndrome (caused by mutations in *SPINK5*).[21] Severe atopic dermatitis, however, is the most common disease presenting with eczema and increased serum IgE levels. Atopic dermatitis is a clinical diagnosis based on chronic pruritic eczema with a distinct appearance and distribution often associated with asthma, allergic rhinitis, food allergy, white dermographism, and high IgE levels.[22] True abscesses are rare in patients with atopic dermatitis. Because of impaired innate immunity and decreased barrier function of the skin, patients with atopic dermatitis are at risk for disseminated viral infections and bacterial colonization, resulting in staphylococcal skin infections.[23]

Before the association with heterozygous *STAT3* mutations was discovered, the diagnosis of AD-HIES depended on clinical findings and was often delayed until complications developed, such as pneumatocele formation after pneumonia. The National Institutes of Health (NIH) scoring system based on the presence and severity of 21 clinical and laboratory findings[24] has been widely used to identify patients with HIES. The discovery that heterozygous *STAT3* mutations cause AD-HIES confirmed that patients with an NIH score of 40 points or greater have a high probability for a *STAT3* mutation.[9,11]

To explore the possibility that patients with only a few clinical findings of HIES carry *STAT3* mutations, we assembled a cohort of patients (n = 78) with a broad range of HIES-associated findings to perform *STAT3* mutation analysis and T_H17 cell enumeration with the aim of identifying the most straightforward and sensitive criteria defining AD-HIES. The ultimate goal of our study was to differentiate atopic dermatitis patients (patients with wild-type *STAT3*) from patients with *STAT3* mutation in order to initiate optimal therapy at an early age before serious complications occur.

METHODS

Subjects

We enrolled 78 patients (50 male and 28 female patients; age range, 8 months to 57 years; median age, 20 years) from 75 unrelated families with diverse ethnic backgrounds. Participants were selected from a group of patients referred to our clinics for possible HIES based on increased serum IgE levels, eczema, staphylococcal infections, no other well-defined PIDD, and NIH scores of greater than 20 points, except for 3 patients with an NIH score as low as 14 points. The original source of referrals included primary care physicians, general hospitals, and centers specialized in PIDD. The NIH score was determined based on medical history, physical examination, and laboratory results.[24] Thirty-eight of the 78 patients had been reported previously.[2,5,10,11] The study was approved by local institutional review boards. Written informed consent was obtained.

Mutation analysis

The *STAT3* gene was amplified from gDNA by using specific oligonucleotide primers and PCR, as previously described.[11] Briefly, genomic DNA was prepared from venous blood by using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, Calif). The amplified gene fragments were sequenced with the ABI Big Dye Terminator mix (Applied Biosystems, Foster City, Calif) and analyzed with a 3730xl DNA Analyzer (Applied Biosystems). Mutations are reported by using the nomenclature of den Dunnen and Antonarakis.[25]

PolyPhen (Polymorphism Phenotyping), a bioinformatic method to predict the possible effect of an amino acid substitution on the structure and function of a human protein by using physical and comparative considerations, was used to estimate the effect of newly identified *STAT3* missense mutations. PolyPhen scores the resulting protein damage as "putative benign" (0.00-1.50), "possibly damaging" (1.51 to 2.00), and "probably damaging" (>2.00). After standard SDS-PAGE and Western blotting, total STAT3 was visualized in selected patients by using a murine anti-STAT3 mAb (Cell Signaling, Danvers, Mass) and secondary horseradish peroxidase–conjugated polyclonal anti-mouse antibody (Biosource-Invitrogen, Carlsbad, Calif). Blots were developed with Supersignal West Pico chemiluminescent substrate (Pierce, Rockford, Ill).

T_H17 cell assessment

PBMCs were isolated with Ficoll-Paque PLUS (Biochrom AG, Berlin, Germany). T_H17 cells were identified by means of intracellular staining of $CD4^+$ T cells for the production of IL-17, as previously described.[11] Briefly, 2.5×10^6 PBMCs from patients and control subjects were stimulated overnight with 10 ng/ml phorbol 12-myristate 13-acetate and 1 μg/mL ionomycin (Sigma-Aldrich, St Louis, Mo) in the presence of GolgiPlug (BD Biosciences, San Jose, Calif). After cell-surface staining with phycoerythrin-conjugated anti-CD4 (eBioscience, San Diego, Calif), cells were fixed, permeabilized (Cytofix/Cytoperm, BD Biosciences), and stained with Alexa Fluor 647–conjugated anti–IL-17-A (eBioscience). As a control for cellular activation and intracellular staining, $CD4^+$ T cells were evaluated for IFN-γ production (fluorescein isothiocyanate–conjugated anti–IFN-γ; eBioscience). Flow cytometric studies were performed on a Calibur instrument (BD Biosciences) and analyzed with Cell Quest Pro Version 4.0.2 (BD Biosciences). T_H17 cells are

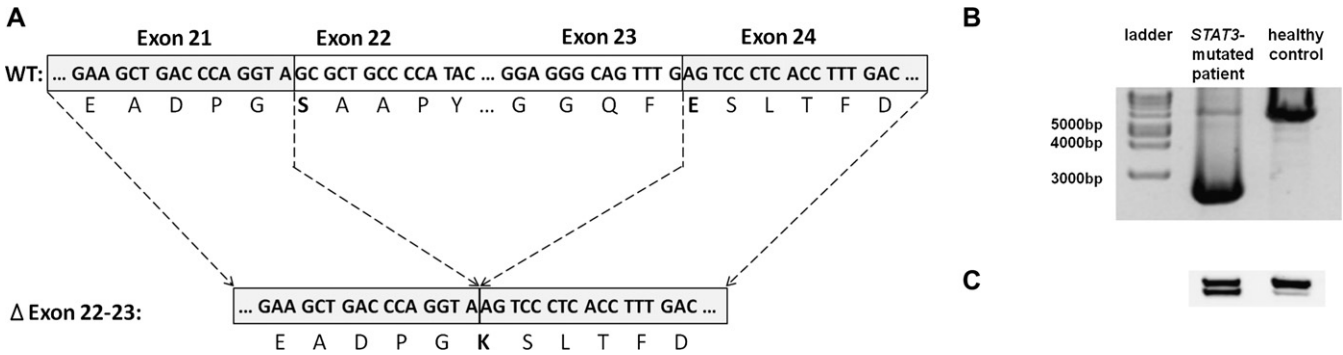

FIG 1. *STAT3* genomic deletion comprising exons 22 and 23. **A**, Large deletion (c.2101+2332_o*STAT3*:c.2257+772del3933bp) of exons 22 and 23 results in a 53-amino-acid shortened protein with a single amino acid change at the junction between exons 21 and 24. *WT*, Wild-type. **B**, Confirmed deletion with a 2,500-bp fragment instead of a 6,600-bp fragment by means of gDNA amplification with primers in exons 21 and 24. **C**, STAT3 Western blotting with 2 differently sized protein products in the patients compared with normal control T-cell extracts.

reported as a percentage of total $CD4^+$ T cells. The normal T_H17 cell range was defined by evaluating 28 healthy adult control subjects.

Lymphocyte proliferation test

Lymphocyte proliferation in response to mitogen and antigen was performed by using standard protocols.[26]

Statistics

The percentage of T_H17 cells from patients with wild-type *STAT3*, patients with *STAT3* mutations, and healthy control subjects were analyzed with the Student *t* test by using SPSS software (SPSS, Inc, Chicago, Ill). A *P* value of less than .01 was considered significant. Sensitivity and specificity of symptoms were assessed in contingency tables in various combinations to determine the prediction value of being a valid symptom or finding to successfully define patients with *STAT3* mutations.

RESULTS

Mutation analysis

All 78 patients enrolled were evaluated for mutations in *STAT3*. Forty-eight patients from 45 unrelated families were found to have heterozygous *STAT3* mutations. The spectrum includes 7 novel and 17 previously reported mutations.[8-12] Six of the 7 novel mutations were missense mutations located in the DNA-binding domain (995 A>T, H332L; 1406 A>G, Q469R), Src homology domain 2 (SH2; 1850 G>A, G617E; 1979 T>C, M660T), and the transactivation domain (2129 T>C, F710C; 2132 T>C, I711T). The missense mutations M660T and F710C were "possibly damaging," with a PolyPhen score of 1.62 and 1.63, respectively, whereas H332L, Q469R, G617E, and I711T, with scores of 2.18, 2.38, 2.18, and 2.03, were "probably damaging." None of these mutations were found in 200 control chromosomes, and all emerged *de novo* because none of the parents carried any of these mutations. In addition, sequencing across intron regions led to the detection of a novel large deletion in the transactivation domain (c.2101+2332_o*STAT3*: c.2257+772del3933bp) that removed exons 22 and 23 and resulted in the in-frame deletion of 53 amino acids (Fig 1). The exact religation point was intron 21–gcgatgtcagcgttt deletion aatcagttaaggtgg–intron 23. This mutation was identified in a previously reported patient (ID no. 35) with an NIH score of 72 points and low T_H17 cells[11] in whom we initially failed to detect a *STAT3* mutation.

TABLE I. Clinical presentation of patients with and without *STAT3* mutations

Findings	Patients with *STAT3* mutation (n = 48)	Patients without *STAT3* mutation (n = 30)
Increases serum IgE levels*	96%	97%
Blood eosinophilia	93%	77%
Eczema	98%	90%
Newborn rash	74%	40%
Skin abscesses	85%	37%
Internal abscesses	46%	0%
Pneumonia	94%	53%
Pneumatoceles	48%	3%
Susceptibility to infections	89%	70%
Severe infections#	35%	0%
Oral candidiasis	56%	24%
Nail/mucocutaneous candidiasis	54%	13%
Pathologic second dentition	79%	19%
Pathologic bone fractures	60%	3%
Scoliosis	42%	3%
Hyperextensible joints	60%	23%
Characteristic face	90%	17%
Broad nasal bridge	79%	22%
High palate	59%	22%
Atopic disease besides eczema	57%	88%
NIH score ≥40 points	96%	10%

*Serum IgE levels increased 10 times above the mean of age-matched control subjects.
#Sepsis, meningitis, osteomyelitis.

Clinical findings associated with *STAT3* mutations

Eczema, increased serum IgE levels, and blood eosinophilia were the most common findings in all patients independent of STAT3 status (Table I). Pneumonia, abscess formation, and oral candidiasis were reported in both patients with *STAT3* mutations and patients with wild-type *STAT3*, but the combination of all 3 infectious symptoms was present in only 3 of 30 patients with wild-type *STAT3*, including a patient with a homozygous *DOCK8* mutation, compared with 23 of 48 patients with *STAT3*

TABLE II. Sensitivity and specificity of clinical findings in patients with HIES and *STAT3* mutations

Findings	Sensitivity (%)	Specificity (%)
Increased serum IgE levels (≥10 times normal)	95.8	3.3
Blood eosinophilia	93.5	23.3
Eczema	97.9	10
Newborn rash	73.9	60
Skin abscesses	85.4	63.3
Internal abscesses	45.8	100
Pneumonia	93.8	46.7
Pneumatoceles	47.9	96.7
Increased susceptibility to infections	89.4	30
Severe infections	34.8	100
Oral candidiasis	56.3	74.1
Nail/mucocutaneous candidiasis	54.2	86.7
Pathologic second dentition	78.6	75
Fractures without adequate trauma	60.4	96.7
Scoliosis	41.7	96.7
Hyperextensible joints	59.6	76.7
Characteristic facies*	95.8	60
Positive family history of HIES	6	100
Additional atopic findings	57.1	11.5

*Characteristic facial features, wide nose, and high palate.

mutations. Persistent pneumatocele formation was exclusively associated with *STAT3* mutations. Empyema (n = 8), internal abscesses (eg, liver [n = 6], lymph node [n = 5], lung [n = 4], dental [n = 2], testis [n = 2], maxilla [n = 1], *perirenal* [n = 1], and peritonsillar [n = 1]), and severe infections, such as bacterial meningitis (n = 1), osteomyelitis (n = 3), and sepsis (n = 12), were only present in patients with *STAT3* mutations. The frequency of scoliosis, prolonged retention of primary teeth, pathologic bone fractures, characteristic facies, and hyperextensible joints was also increased in patients with *STAT3* mutations when compared with patients with wild-type *STAT3*. Unexpectedly, pathologic second dentition described as pathognomonic for AD-HIES[4] was observed in 3 of 16 patients with wild-type *STAT3* 10 years of age or older. In 33 (79%) of 42 evaluable patients with *STAT3* mutations (≥9 years of age), pathologic second dentition included retained primary teeth, formation of double rows of teeth during second dentition, or the need to have teeth pulled because of failure of root resorption.

Based on an NIH score of 40 points or greater, 49 of the 78 patients qualified as having clinically defined HIES (NIH score range, 40-89). All but 3 carried a heterozygous *STAT3* mutation. One of the 3 patients with wild-type *STAT3* (NIH score, 52 points) has a homozygous *DOCK8* mutation.[6,7] The other 2 patients with wild-type *STAT3*, both with an NIH score of 43 points, had severe eczema, pneumonia, and mucocutaneous *Candida albicans* and recurrent staphylococcal skin infections. The 9-year-old boy had characteristic facial features (classified as mild in terms of NIH score), and the 2-year-old girl reported an atraumatic femur fracture. Only 2 of 29 patients with an NIH score ranging from 20 to 39 points carried heterozygous *STAT3* mutations. One is a previously reported now 3-year-old boy (NIH score, 34 points),[10] whose paternal grandmother and father, both of whom were included in this study, have a *STAT3* hotspot mutation (NIH scores of 83 and 89 points, respectively). This boy presented with eczema as a newborn and with staphylococcal pneumonia with empyema at 3 months of age. The other, a 19-year-old man (NIH score, 37) with a high serum IgE level (>5,000 IU/mL), eczema, skin abscesses, scoliosis, and a characteristic facies, never had pneumonia, pathologic fractures, or retained primary teeth; he carries one of the novel mutations in *SH2* domain (G617E).

To define reliable criteria for selecting candidate patients to undergo *STAT3* sequencing, particularly among pediatric patients, we compared clinical, genetic, and immunologic findings from our entire cohort of patients with and without *STAT3* mutations. Clinical hallmarks consistently present in both groups of patients were eczema, increased serum IgE levels, and blood eosinophilia (>5% of circulating leukocytes), reflecting high sensitivity but low specificity for HIES with *STAT3* mutations (Table II). Whereas these findings distinguished patients with HIES and patients with atopic dermatitis from healthy subjects, they did not differentiate them from each other. Based on the specificity distribution of the findings listed in Table II, we identified 7 key findings for HIES with *STAT3* mutations that achieved specificities of greater than 85%, including abscesses of internal organs, other severe infections, pneumatoceles, nail/mucocutaneous candidiasis, bone fractures without adequate trauma, scoliosis, and a positive family history of HIES. All patients with *STAT3* mutations from our cohort had at least one of those key findings. Three or more key findings were present in 32 (67%) patients with *STAT3* mutations, including up to 5 findings in 5 patients. In contrast, only 6 (20%) of the 30 patients without *STAT3* mutations had 1 or at most 2 of those findings.

T_H17 cell numbers in patients with HIES

The threshold value of normal T_H17 cell numbers expressed as a percentage of $CD4^+$ T cells was determined in healthy adult control subjects (n = 28) and set as greater than 0.2%. T_H17 cells were measured in 53 patients (28 patients with *STAT3* mutations and 25 patients with wild-type *STAT3*). In patients with wild-type *STAT3*, T_H17 cells (average, 0.53% of $CD4^+$ T cells; range, 0.1% to 1.9%) were comparable with those seen in healthy control subjects (average, 0.62% of $CD4^+$ T cells; range, 0.2% to 2.0%). In contrast, T_H17 cells were either absent or markedly reduced in patients with *STAT3* mutations (average, 0.08% of $CD4^+$ T cells; range, 0% to 0.3%). The difference in the number of T_H17 cells between patients with *STAT3* mutations and patients with wild-type *STAT3* or healthy control subjects was highly significant ($P < .001$), and the overlap of T_H17 cell values of patients with *STAT3* mutations and patients with wild-type *STAT3* was minimal (Fig 2).

Correlation of *STAT3* genotype, NIH scores, and T_H17 cell percentages

NIH scores, T_H17 cell percentages, and *STAT3* genotype were available for correlation in 53 patients and are shown in Fig 3. In 83% the NIH scores, as well as the T_H17 cell counts, matched the *STAT3* sequencing results. In 8 patients (5 patients with *STAT3* mutations and 3 patients with wild-type *STAT3*) either the T_H17 cell number or the NIH score fell outside the defined quadrant. Among the patients with wild-type *STAT3*, only the patient with *DOCK8* mutation had an increased NIH score, as well as a low T_H17 cell count, which is similar to the majority of patients with *STAT3* mutations. The T_H17 cell percentages intersected between 0.2% and 0.3% in patients with and without *STAT3* mutations, with the exception of a 2-year-old patient with wild-type *STAT3* who had low (0.11%) T_H17 cell counts (Fig 3). Nine

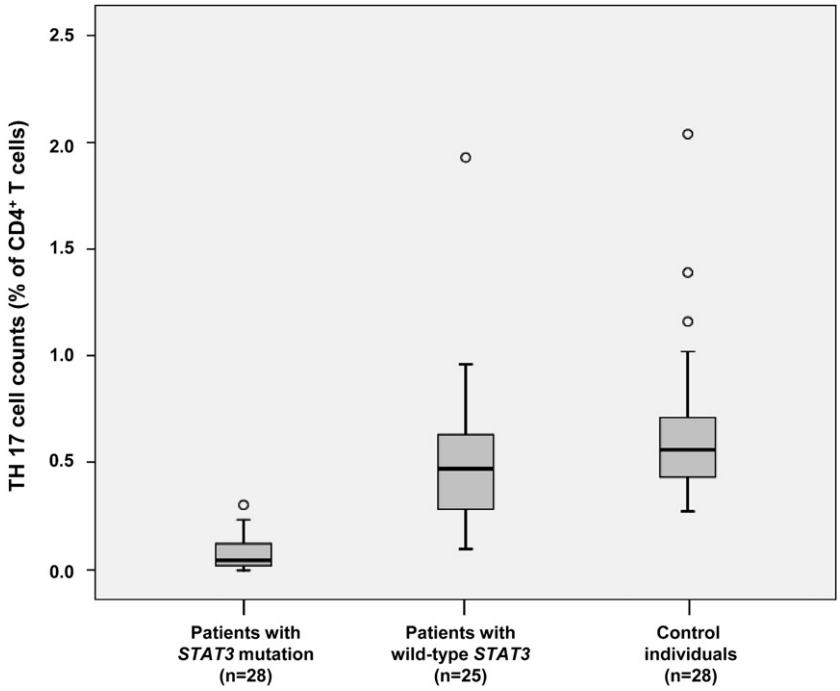

FIG 2. T_H17 cell counts in patients with HIES with *STAT3* mutations, patients with HIES with wild-type *STAT3*, and control subjects. Box plots of peripheral blood T_H17 cells (shown as percentage of $CD4^+$ T cells) are shown. There is a significant difference ($P < .001$) between T_H17 cell percentages in patients with *STAT3* mutations versus patients with wild-type *STAT3* and patients with *STAT3* mutations versus healthy control subjects.

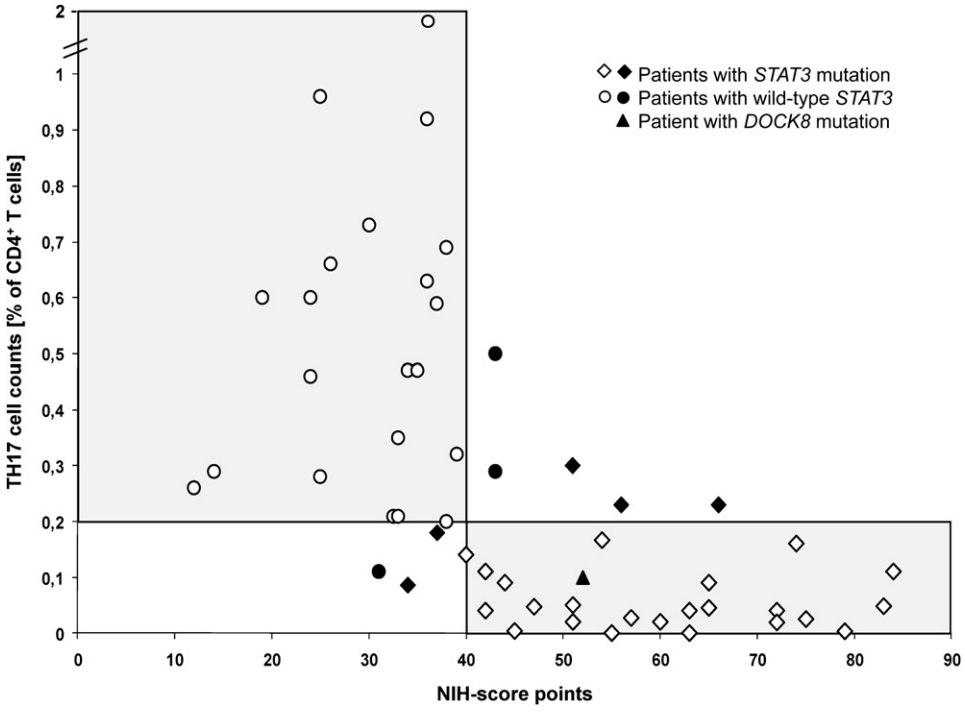

FIG 3. Correlation of NIH scores, T_H17 cell counts, and *STAT3* mutation status in 51 patients. Most patients with *STAT3* mutations have an NIH score of 40 points or greater and T_H17 cells at less than 0.2% of $CD4^+$ T cells, whereas the majority of patients with wild-type *STAT3* have an NIH score of less than 40 points and T_H17 cells of 0.2% or greater of $CD4^+$ T cells. Patients with NIH scores, T_H17 cell counts, or both criteria being outside the established region are marked with *solid symbols*.

patients with wild-type *STAT3* with scores in the intersection area (≥34 points) had T_H17 cell percentages of greater than 0.3%, with 7 being most likely severe forms of atopic dermatitis rather than HIES. Key findings associated with HIES due to *STAT3* mutations were present in 2 of those 7 patients: one with a scoliosis and one with a questionable pneumatocele. Three patients with wild-type *STAT3* with NIH scores between 33 and 38 points and T_H17 cell counts at or slightly greater than 0.2% had severe atopic dermatitis without additional key findings, as did the young patient with only 0.11% T_H17 cells and an NIH score of 31 points. Three patients with wild-type *STAT3*, including the patient with a *DOCK8* mutation, had NIH scores of 39, 43, and 52 points and T_H17 cell counts at 0.32%, 0.29%, and 0.1% combined with recurrent viral infections, suggesting a T-cell deficiency. None of the other patients with wild-type *STAT3* presented with a significant T-cell deficiency clinically or in regard to *in vitro* testing of lymphocyte proliferation to mitogen.

DISCUSSION

Some of the most common clinical findings observed in patients with HIES, such as eczema, increased serum IgE levels, and recurrent skin infections, overlap with those associated with other PIDD as well as with severe forms of atopic dermatitis. Because of the discovery that AD-HIES is caused by *STAT3* mutations, we correlated the *STAT3* genotype with the clinical and laboratory findings of patients suspected of having HIES to determine the most effective screening criteria to predict which patients with HIES most likely have *STAT3* mutations, with the aim of improving early and accurate diagnosis of AD-HIES.

The *STAT3* gene, located on chromosome 17, encodes a protein consisting of 6 major domains: the N-terminal, coiled-coil, DNA-binding, linker, SH2, and transactivation domains.[14] Sequence analysis of *STAT3* carried out in a large cohort selected for the presence of severe eczema, staphylococcal infections, and increased serum IgE levels identified heterozygous mutations in more than half of the 78 patients enrolled. This outcome might be biased because our cohort was preselected by being referred to tertiary centers specialized in PIDD.

Initial reports described functionally relevant mutations in the DNA-binding domain, SH2, and the transactivation domain.[8-11] All mutations identified to date,[8-12,27,28] including those described here, are heterozygous missense mutations or in-frame deletions that allow expression of a mutant protein. Although we did not directly prove a dominant negative effect on STAT3 function by the novel mutations described, our findings support the hypothesis that haploinsufficiency caused by loss of 1 *STAT3* allele is not sufficient to cause the diverse clinical phenotypes of AD-HIES but rather that coexpression of a mutant protein with dominant negative function is required.[15]

No apparent genotype-phenotype correlation was identified within our cohort of patients with *STAT3* mutations. Highly increased serum IgE levels, eczema, and increased eosinophil counts were present in the majority of our 78 patients independent of STAT3 status (Table I). The major difference between the patients with and without *STAT3* mutations was the incidence of severe infections and internal abscess formation characteristic of patients with *STAT3* mutations, highlighting the critical role that this transcription factor plays in inflammation and the coordination of appropriate immune responses. The clinical phenotypes of our patients based on the NIH scoring system strongly correlated with the presence or absence of *STAT3* mutations, confirming the specificity of the NIH scoring system for AD-HIES and for AR-HIES caused by *DOCK8* mutations. Nevertheless, the incidental occurrence of some HIES-specific findings in patients with wild-type *STAT3* underlines the fact that each symptom by itself is nonspecific. In particular, subjective findings associated with AD-HIES, such as characteristic facial features, were also observed in patients with wild-type *STAT3*. Comparing the clinical findings included in the NIH scoring system, we identified 7 key findings to be highly specific for AD-HIES. Those were abscesses of internal organs, severe infections, pneumatoceles, nail and mucocutaneous candidiasis, bone fractures, scoliosis, and a positive family history of HIES. On the other hand, allergic asthma, allergic rhinitis, and severe food allergies were more frequently associated with patients with wild-type *STAT3* compared with patients having *STAT3* mutations. A large proportion of the 29 patients with wild-type *STAT3* met the criteria of severe atopic dermatitis with its multifactorial cause; in most of these, single-gene defects are not expected to be identified. None of our patients with wild-type *STAT3* met the criteria of Omenn syndrome, Wiskott-Aldrich syndrome, IPEX, or Comèl-Netherton syndrome, which have in common early-onset eczema and increased serum IgE levels.[21] However, those patients with wild-type *STAT3* who present with sufficient additional findings uncommon in atopic dermatitis, such as the key findings described above, atypical mycobacteriosis, recurrent viral infections, or cerebral hemorrhages, might eventually be found to have a single-gene defect. Indeed, one of our patients with wild-type *STAT3*, a previously reported patient with AR-HIES,[5] was found to have a homozygous *DOCK8* mutation.[6,7] Patients with mutations in *TYK2* and *DOCK8* present with infections that are characteristic for cellular immunodeficiency, including severe cutaneous viral infections, recurrent sinopulmonary diseases, and increased serum IgE levels.[6,13] Two additional patients with wild-type *STAT3* in our cohort were found to have a significant T-cell deficiency and are currently being evaluated for *DOCK8* mutations, whereas none of the patients with wild-type *STAT3* have a history of *Salmonella* or atypical *Mycobacterium* species infections, findings described in the only patient with a *TYK2* mutation reported to date.[13]

Our results from studying a large cohort of patients confirmed that a reduced percentage of T_H17 cells in the peripheral blood correlates strongly with the presence of *STAT3* mutations. Because STAT3 is required for the development of T_H17 cells, several groups reported significantly decreased T_H17 cell counts in patients with *STAT3* mutations compared with those seen in healthy control subjects.[11,18,19,29] In contrast, most patients with atopic dermatitis, including those reported here, tend to have increased T_H17 cell counts in their peripheral blood.[30] Therefore T_H17 cells, in addition to the lack of allergic symptoms except eczema in AD-HIES patients, seem to be an excellent and rapid parameter to differentiate HIES from atopic dermatitis. However, T_H17 cell assessment is limited to specialized laboratories, and it might in some situations be easier to request *STAT3* sequencing in clinically suspicious patients. All patients with *STAT3* mutations had less than 0.3% T_H17 cells. In contrast, only one male infant (<2 years of age) with an NIH score of 31 points and wild-type *STAT3* had T_H17 cells of less than 0.2% of $CD4^+$ T cells. This decrease in T_H17 cells might have been age related. Although there are no validated age-matched normal values for T_H17 cell numbers, we have observed that T_H17 cell counts increase within the first 2 years of age (unpublished observations),

and data provided by Schaub et al[31] (see this article's Online Repository and Fig E1 at www.jacionline.org) confirm that T_H17 cells and IL-17 production increase with age. Low T_H17 cell counts are not unique to patients with HIES. Other diseases associated with low T_H17 cell counts in the peripheral blood include chronic mucocutaneous candidiasis, reflecting a possible disease overlap.[32] However, the clinical difference between chronic mucocutaneous candidiasis, which recently has been associated with variations in *DECTIN1* and *CARD9*,[33,34] and HIES is substantial and should be readily apparent based on other clinical features. The combined assessment of clinical, immunologic, and genetic data strongly suggests that high NIH scores for patients with AD-HIES are associated with low T_H17 cells and *vice versa* (Fig 3). Because early aggressive treatment might decrease the positive predictive value of any scoring system and its effects on T_H17 cell values are yet to be determined, it might be prudent to carefully evaluate patients for the presence of the 7 key findings we selected from the complex NIH score, allowing early diagnosis followed by effective medical treatment.

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Clinical implications: Differentiation of HIES from severe atopic dermatitis is a diagnostic challenge with important therapeutic implications. Clinical key findings combined with T_H17 cell counts predict patients with HIES with *STAT3* mutations.

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Online supplementary data: Interleukin 17 levels increase with age in childhood

Early in life, namely in cord blood of healthy donors, IL-17$^+$ cells are present, although in low percentages. Fig E1 shows that IL-17$^+$ cells increase with age.

IL-17 protein secretion (measured by means of LUMINEX) was inducible after PHA stimulation in healthy adults (n = 18; median, 710.01 pg/mL; interquartile range, 487.2-862.2 pg/mL) compared with undetectable values in unstimulated cells, as previously reported.[E1] In comparison, IL-17 secretion was 0.33 pg/mL after PHA stimulation of control cord blood mononuclear cells (n = 25) and therefore significantly lower than in adult healthy control subjects.

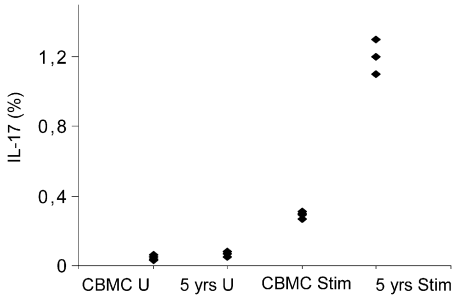

FIG E1. Percentage of IL-17$^+$ cells in cord blood mononuclear *cells* (*CBMC;* n = 5) versus cells of 3 children (4-6 years of age) unstimulated *(U)* and stimulated *(Stim)* with phorbol 12-myristate 13-acetate/ionomycin.

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