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## **Key findings to expedite the diagnosis of hyper-IgE syndromes in infants and young children**

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**Running title:** diagnosing HIES early in life

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**Key findings to expedite the diagnosis of hyper-IgE syndromes in infants and young children**

*Pediatr Allergy Immunol*

**Abstract:**

**Background:** Hyper-IgE syndromes (HIES) are primary immunodeficiency disorders characterized by elevated serum-IgE, eczema and recurrent infections. Despite the availability of confirmatory molecular diagnosis of several distinct HIES entities the

differentiation of HIES particularly from severe forms of atopic dermatitis remains a challenge. The two most common forms of HIES are caused by mutations in the genes *STAT3* and *DOCK8*.

**Methods:** Here we assess the clinical and immunological phenotype of DOCK8- and STAT3-HIES patients including the cell activation, proliferation, and cytokine release after stimulation.

**Results:** Existing HIES scoring systems are helpful to identify HIES patients. However, those scores may fail in infants and young children due to the age-related lack of clinical symptoms. Furthermore, our long term observations showed a striking variation of laboratory results over time in the individual patient. Reduced memory B cell counts in concert with low specific antibody production are the most consistent findings likely contributing to the high susceptibility to bacterial and fungal infection. In DOCK8-HIES T cell lymphopenia and low IFN $\gamma$  secretion after stimulation were common, likely promoting viral infections. In contrast to STAT3-HIES, DOCK8-HIES patients showed more severe inflammation with regard to allergic manifestations, elevated activation markers (HLA-DR, CD69, CD86, CD154), and significantly increased inflammatory cytokines (IL1 $\beta$ , IL4, IL6, IFN $\gamma$ ).

**Conclusion:** To differentiate HIES from other diseases like atopic dermatitis early in life is essential for patients because treatment modalities differ. To expedite the diagnosis process we propose here a diagnostic workflow.

**Keywords:**

hyper-IgE syndrome, HIES, dedicator of cytokinesis 8, DOCK8, signal transducer and activator of transcription 3, STAT3, immune dysregulation, host defense

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## **Introduction:**

The presence of eczema, elevated serum IgE, and eosinophilia are hallmarks of atopic dermatitis, the IPEX syndrome, Loeys-Dietz syndrome and combined immunodeficiencies such as the hyper-IgE syndromes (HIES), Wiskott-Aldrich, Omenn, and Comèl-Netherton syndrome (1-3). Despite the availability of confirmatory molecular diagnosis of several distinct HIES entities (TYK2-, STAT3-, DOCK8-, PGM3-HIES) particularly the differentiation of HIES from severe forms of atopic dermatitis remains a challenge (1, 4, 5).

STAT3-HIES and DOCK8-HIES are the two most common molecularly defined forms of HIES (4-12). STAT3-HIES patients often present with pneumatocele formation after pneumonia and skeletal and connective tissue findings which are rarely present in DOCK8-HIES patients (5, 9, 13). DOCK8-HIES differs from STAT3-HIES by presenting, in addition to bacterial and fungal infections, with more severe viral infections particularly of the skin (14, 15). DOCK8 is involved in intracellular signaling networks, proliferation, differentiation, migration, synapsis formation, adhesion, and survival of cells affecting innate and adaptive immunity (16-21). The STAT3 protein is known for its central role in cell development and inflammation control. Heterozygous loss-of-function mutations in STAT3 cause a dominant negative effect on STAT3 function resulting in the clinical and immunologic phenotype of STAT3-HIES (11, 12, 22).

Here, we provide a long-term observation of DOCK8- and STAT3-HIES patients with regards to clinical and laboratory findings and assessed the activation and cytokine secretion of lymphocytes after *in vitro* stimulation in comparison to healthy individuals and STAT3-HIES patients.

**Methods:** see online repository

**Results:**

**Clinical and immunological phenotype of DOCK8- and STAT3-HIES exhibits impaired host defense and immune regulation**

All our HIES patients presented with chronic eczema and recurrent infections and NIH scores confirmed suspicion of HIES (Figure 1 and 2, Table E1 and E2) (23). The later proposed STAT3-HIES score focuses on the quantity of pneumonias and pathologic bone fractures as well as on the presence of a newborn rash, a cathedral palate and the characteristic face of STAT3-HIES (24). This STAT3-HIES score was false-positive in one of our DOCK8-HIES patients (presenting with more than three pneumonias, a newborn rash, and a cathedral palate). Five of our STAT3-HIES patients were not identified due to the lack of pathologic fractures, pneumonia or cathedral palate. The recently proposed DOCK8-HIES score evaluates absolute eosinophil counts and the quantity of upper respiratory tract infections and allocates negative points for findings seen in STAT3-HIES patients such as parenchymal lung abnormalities, retained primary teeth and pathological bone fractures (4). This DOCK8-HIES score proposed DOCK8 mutations in all DOCK8-HIES and in six STAT3-HIES patients which had very high eosinophil counts, many upper respiratory infections, and lacked characteristic STAT3-HIES findings.

*Staphylococcus aureus* was isolated from mucocutaneous sites of all patients including methicillin resistant *Staphylococcus aureus* in four DOCK8- and two STAT3-HIES patients. Further major bacterial pathogens in descending order were *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Enterococcus* species isolated from DOCK8-HIES patients and *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Streptococcus* species isolated from STAT3-HIES patients. Most of our HIES patients presented with chronic mucocutaneous candidiasis, while about half of our DOCK8-HIES patients had also generalized *Molluscum contagiosum* skin infection. In our DOCK8-HIES patients additional recurrent viral infections, particularly of the skin, included *Herpes simplex virus*, *Human herpesvirus 6*, *Varicella zoster virus*, and *Human papilloma virus* infections. Only patient STAT3 #2 had recurrent Herpes zoster and patient STAT3 #11 recurrent *Molluscum contagiosum* infection (Figure 1). More than half of the DOCK8-HIES patients had at least once a long-lasting and difficult to treat keratitis or conjunctivitis. Five DOCK8-HIES patients developed benign tumorous mucosa proliferations most likely triggered by *Herpes simplex virus*. Three tumors progressed from the internal eyelid (DOCK8 #1, #5, and #6) and two from oral mucosa (DOCK8 #7 and DOCK8 #11). One patient died six months after diagnosis of a squamous cell carcinoma in the genital region at 16 years of age (DOCK8 #8). Patient STAT3 #5 developed B- and T-cell lymphoma.

Recurrent upper respiratory tract infections were equally seen in both HIES entities while bronchial hyperreactivity was predominantly present in DOCK8-HIES patients. Defective healing after pulmonary infection resulted in bronchiectases in eight of our STAT3-HIES and two DOCK8-HIES patients and in pneumatocele formation in nine STAT3-HIES patients but none of our DOCK8-HIES patients.

All DOCK8-HIES patients presented with severe food allergies, whereas STAT3-HIES patients did not show any clinical signs of food allergy. Central nervous system symptoms were present in 27% of our DOCK8-HIES patients including hemiplegia, infection, or cerebral bleeding. Autoimmune hemolytic anemia occurred in patient DOCK8 #1. In contrast

to our STAT3-HIES cohort, half of our DOCK8-HIES patients presented with a failure to thrive resulting in a short stature below the 3<sup>rd</sup> growth curve percentile.

Absolute cell counts showed pronounced eosinophilia in all DOCK8-HIES patients (Figure E1), whereas two of our STAT3-HIES patients presented without eosinophilia (STAT3 #12, STAT3 #13). Median eosinophil counts per patient were significantly higher in DOCK8-HIES patients in comparison to STAT3-HIES patients (Mann-Whitney test  $p < 0.001$ ). While most DOCK8-HIES patients presented with lymphopenia, lymphocyte values were within the normal range or increased in STAT3-HIES patients except for patients STAT3 #5 and STAT3 #7.

In contrast to mainly normal T cell counts in STAT3-HIES, these counts were commonly low in DOCK8-HIES (Figure E2A). Th17 cells ( $CD3^+CD4^+IL17^+IFN\gamma^-$ ) were normal in all of our DOCK8-HIES patients except for patient DOCK8 #11 (0.16% of CD4<sup>+</sup> cells). In contrast STAT3-HIES patients presented with decreased or almost absent Th17 cells except for patients STAT3 #7 and STAT3 #9 with low normal values. DOCK8-HIES patient DOCK8 #1 had consistently decreased absolute NK cell counts in contrast to only single reduced values in STAT3-HIES patients (Figure E2B).

Total B cell counts ( $CD19^+$  cells) were normal in all our DOCK8-HIES patients except for single measurements, whereas patients STAT3 #5 and STAT3 #14 had low B cell counts (Figure E3A). All assessed DOCK8- and STAT3-HIES patients presented with a decreased B cell memory compartment ( $CD19^+CD27^+$  cells) including decreased absolute non-class-switched and class-switched memory B cell counts. The compartment of switched memory B cells was more severely affected than non-class-switched memory B cells in both patient groups demonstrated by an increased ratio of non-class-switched to class-switched memory B cells (DOCK8-HIES: median: 2.2; range: 0.9 to 11.5; STAT3-HIES: median: 1.3; range: 0.5 to 4.4).

IgE levels were highly elevated in all DOCK8- and STAT3-HIES patients except for a few normal measurements in patient DOCK8 #11 (Figure E3B). IgE levels were not significantly different between DOCK8- and STAT3-HIES patients. Hypogammaglobulinemia was not observed in all DOCK8- and STAT3-HIES patients. About half of the DOCK8-HIES patients had high or high normal IgA values. Most STAT3-HIES patients had normal IgA levels. Serum IgM levels were not decreased in STAT3-HIES patients, while two-thirds of DOCK8-HIES patients presented consistently with low serum IgM values. However, most DOCK8-HIES patients showed both, low and normal IgM levels at various age. Specific IgM production was impaired in general as demonstrated by reduced IgM isohaemagglutinin titers in all tested DOCK8-HIES patients with a maximum titer of 1:8 in three of the tested patients (normal range of 1:32 to 1:128).

The vaccination response against *Haemophilus influenzae* and *Streptococcus pneumoniae* was reduced in 89% of DOCK8- and 50% of STAT3-HIES patients. Specific antibody titers against tetanus toxoid were insufficient or equivocal in 89% of the DOCK8- and 75% of STAT3-HIES patients.

### **DOCK8-deficient in contrast to STAT3-mutated lymphocytes show diminished proliferation, increased cell activation, and altered cytokine secretion**

DOCK8-deficient lymphocytes showed significantly decreased proliferation after T cell- and B cell specific mitogen stimulation (Figure 3A). Mitogen stimulation of STAT3-mutated PBMCs was comparable to control cells except for SAC stimulation. Proliferation after stimulation with an antigen-mixture was significantly reduced in both patient groups. The stimulation responses, however, were variable as shown exemplary by ten measurements over time of patient DOCK8 #9 (Figure 3B). The responses to PHA (1 µg/ml), SAC, and the antigen-mixture were most consistent. Therefore, we used PHA as a T cell mitogen and SAC as a B cell mitogen for the subsequent functional experiments.

Despite the fact that unstimulated DOCK8-deficient lymphocytes already expressed the activation markers CD69, CD86, and CD154 in a significantly larger proportion compared to healthy controls, the percentage of positive cells significantly increased further after PHA and SAC stimulation (Figure 4A). In general, the percentage of activated DOCK8-deficient lymphocytes was significantly higher compared to controls after stimulation. STAT3-mutated lymphocytes were mostly comparable to control cells. Furthermore, the percentage of activated T lymphocytes (HLA-DR<sup>+</sup> CD3<sup>+</sup> of lymphocytes) was elevated in more than half of our DOCK8-HIES patients, in contrast to normal percentages in our STAT3-HIES patients (Figure 4B).

Consistent with the high activation levels of lymphocytes, inflammatory cytokines such as IFNgamma, IL4, IL1beta, and IL6 were secreted in significantly higher concentrations from unstimulated DOCK8-deficient PBMCs compared to healthy control cells (Figure 5). Cytokine secretion levels of IL12 comparable to controls hint to normal Th1 cell differentiation in DOCK8- and STAT3-HIES, while a significantly reduced PHA-induced IFNgamma secretion in DOCK8-deficient PBMCs indicated a distorted Th1 cell function. Th2 cell differentiation and function assessed by IL4 release was significantly increased in unstimulated DOCK8-deficient PBMCs compared to controls. IL1beta and IL6 – cytokines important for inflammation and Th17 differentiation – were both secreted in higher concentrations from DOCK8-deficient PBMCs without and with stimulation compared to healthy controls. PHA-stimulated DOCK8-deficient and STAT3-mutated cells, however, released significantly lower amounts of IL17 compared to control cells. The anti-inflammatory cytokine IL10 was secreted in comparable amounts by PBMCs from all three groups without and with stimulation.

## Discussion:

Because the eczema resembled severe atopic dermatitis several of our DOCK8-HIES patients were referred from their primary care doctor first to a dermatologist before being sent to a specialized center for primary immunodeficiencies. In our STAT3-HIES patients eczema was present but the primary suspicion of an immunodeficiency was mainly raised due to elevated serum IgE, severe and recurrent infections of the respiratory tract or due to recurrent abscesses. In everyday practice the important question remains when a suspicion of HIES should be raised and further work-up should be initiated particularly in infants. To facilitate early diagnosis, we assessed clinical and immunologic key findings of our HIES patients to develop a diagnostic workflow.

In addition to the hallmarks of HIES: eczema, elevated serum IgE, and recurrent bacterial and fungal infections, findings directing to DOCK8-HIES and also observed in our cohort were recurrent viral infections, bronchial hyper-reactivity, food allergies, and consanguinity (8, 14, 15, 25-27). One of our DOCK8-HIES patients presented with bronchiectasis, which has been reported with varying occurrence, and none had pneumatocoles, which are frequently seen in STAT3-HIES patients (5, 13-15, 25-27). Skeletal abnormalities such as bone fractures without adequate trauma and scoliosis were not present in our DOCK8-HIES patients but characteristic for STAT3-HIES (4, 5, 10, 13, 15, 25-27). In agreement with the literature, we saw central nervous system symptoms, autoimmune hemolytic anemia, and a failure to thrive resulting in a short stature in our DOCK8-HIES patients (14, 15, 25-27). Malignancy was present in one DOCK8-HIES and one STAT3-HIES patient (5, 14, 15, 26-28). Furthermore, others and we reported virus-triggered benign tumorous proliferation of mucosal epithelium in DOCK8-HIES patients (29, 30).

The higher susceptibility to viral infections in DOCK8-HIES in contrast to STAT3-HIES may be explained by reduced CD8<sup>+</sup> T cells, NK cell cytotoxicity and our here reported reduced induction of IFN $\gamma$  upon PHA stimulation in DOCK8-deficient PBMCs (19, 25). The

reduced IFN $\gamma$  secretion after stimulation is likely due to an intrinsic defect, since CDC42, which is activated by DOCK8 (17), is required for IFN $\gamma$  secretion by CD4<sup>+</sup> cells at the immunological synapse (31).

Remarkably, not only virus-triggered tumors developed from mucocutaneous sites also most other clinical symptoms of DOCK8- and STAT3-HIES focus on the skin or mucosa including the lung and in DOCK8-HIES the gastrointestinal tract. Severe chronic eczema, persistent sinusitis, otitis media and externa in both patient groups and in DOCK8-HIES additionally severe keratitis and conjunctivitis result in a constant state of infection (4-6, 10, 12-15, 22, 25-27, 32). Particularly the lack of DOCK8 protein seems to cause a vicious circle where chronic inflammation and mucocutaneous barrier defect initiate and trigger infection, inflammation, and allergic symptoms and vice versa. Elevated serum-IgE, eosinophilia, and Th2 cell shift are common features of allergic diseases and DOCK8-HIES (1, 33, 34). IL4, CD86, and CD154 are known to mediate IgE synthesis (35, 36) and were all increased in our DOCK8-HIES patients, likely contributing together with the observed eosinophilia to high IgE levels. Despite chronic infections in STAT3-HIES, cytokine secretion and cell activation of our STAT3-HIES patients did not differ from normal controls. This might explain the impaired inflammation response demonstrated by the formation of cold abscesses and the lack of allergic findings in STAT3-HIES patients (5, 6, 12, 13, 22, 32).

STAT3-HIES and DOCK8-HIES patients both present with recurrent bacterial and fungal infections. Low Th17 cell counts in STAT3-HIES patients are suggested to explain these infections (22, 37-39). There are reports on diminished Th17 cell differentiation in DOCK8-HIES patients (14, 40). However, we have reported normal Th17 cell counts in DOCK8-HIES patients previously (1). We assume that rather than reduced quantitative Th17 cell counts impaired IL17 secretion upon PHA stimulation in DOCK8-HIES leads to insufficient IL17 immunity to clear bacterial and fungal infections. This hypothesis of a cell intrinsic defect is supported by the finding that IL1 $\beta$ , IL6, and IL12 to initiate Th17 and Th1 cell differentiation are normally secreted.

Recurrent infections in HIES are also explained by the lack of specific antibodies observed in STAT3- and DOCK8-HIES patients. Total B cell counts were normal in DOCK8- and STAT3-HIES patients, whereas SAC-induced proliferation was significantly reduced, which may be the consequence of an impaired TLR9/MyD88 signaling (41). Despite reduced proliferation, activation of DOCK8-deficient and STAT3-mutated lymphocytes is not impaired and sufficiently induces the T-B co-signaling ligands CD154 and CD86 (18). The expression of these important T-B ligands together with the normal differentiation of Th1 and Th2 cells indicates an intact T-B cell interaction in HIES (21). Germinal center reactions, however, seem to be impaired in DOCK8- and STAT3-HIES (21, 42) suggesting defective affinity maturation of specific antibodies. Thus, despite normal serum IgG levels DOCK8- and STAT3-HIES patients likely benefit from immunoglobulin substitution therapy by overcoming low affinity antibody production. The infused high affinity antibodies may fight infections more effectively and thus reduce the rate of infection in HIES patients.

Our data underline the complexity and severity of HIES and hence the need for an early diagnosis.

The existing HIES scoring systems were helpful to identify HIES patients in our cohort and are recommended to be performed on patients with suspicion of HIES. However, these HIES scoring systems do not provide absolute diagnostic certainty and particularly infants and small children, where the evaluated symptoms may be missing due to young age, may not be recognized. To overcome the challenge of diagnosing infants with STAT3-HIES we suggested in the past to initiate a diagnostic work-up with the presence of one of seven key findings including abscesses of internal organs, other severe infections, pneumatoceles, nail or mucocutaneous candidiasis, bone fractures without adequate trauma, scoliosis, and a positive family history of HIES (13). To expedite early diagnosis of HIES particularly in infants and small children we suggest here a diagnostic work-flow (Figure 6).

We recommend to refer patients to a specialized center for further analysis if – together with the initial observation of eczema, recurrent infections or elevated IgE – at least one of the following findings is present: severe, recurrent or difficult to treat infections, healing defect after infection, failure to thrive, severe food allergies, CNS findings, skeletal abnormalities, positive family history, consanguinity, unexplained death of a close relative, alterations in differential blood count or serum immunoglobulin levels or if HIES is indicated by one of the existing HIES scoring systems (Figure 6). The specialized center will exclude differential diagnoses by carefully evaluating immunologic parameters, perform molecular diagnostics and initiate appropriate treatment.

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## Figure legends:

### Figure 1: Mucocutaneous inflammation and infections in DOCK8-HIES.

Severe eczema, molluscum contagiosum, fungal skin infections, and benign tumorous mucosa proliferations progressing from the inner eyelid and from oral mucosa are clinical signs of DOCK8-HIES.

### Figure 2: Comparison of HIES scoring systems.

The NIH score (23) is predictive for HIES with  $\geq 40$  points and indicates the suspicion of HIES with 20 to 39 points, the STAT3-HIES score (24) and DOCK8-HIES score (4) suggest STAT3-HIES and DOCK8-HIES, respectively, with scores  $\geq 30$  points.

### Figure 3: Lymphocyte proliferation of DOCK8-HIES and STAT3-HIES patients.

(A) Diminished lymphocyte proliferation is shown by the most recent proliferation assessment of DOCK8-HIES and STAT3-HIES patients after stimulation with Phytohaemagglutinin (PHA), anti-CD3 (OKT3), Concanavalin A (ConA), Pokeweed mitogen (PWM), *Staphylococcus aureus* Cowan I (SAC) and an antigen mixture (antigen). (B) Proliferation assessment over-time of patient DOCK8 #9. All ten evaluations of patient DOCK8 #9 before bone marrow transplantation are shown in comparison to ten control measurements.

Black dots: healthy controls, white dots: DOCK8-HIES patient(s), grey dots: STAT3-HIES patients. \* indicates significant differences ( $P < 0.05$ ), and \*\* and \*\*\* indicate highly significant differences ( $P < 0.01$  and  $P < 0.001$ ) as calculated by Mann–Whitney test.

#### **Figure 4: Lymphocyte activation of DOCK8-HIES and STAT3-HIES patients.**

(A) Percentages of lymphocytes from five DOCK8-HIES patients and eight STAT3-HIES patients expressing the activation markers CD69, CD86 and CD154 as measured by cell surface stain and flow cytometry compared to ten healthy controls. All groups showed significant increases after stimulation compared to unstimulated cells. US: unstimulated, PHA: stimulated with 1 µg/ml PHA, SAC: stimulated with 0.001% SAC. Black dots: healthy controls, white dots: DOCK8-HIES patients, grey dots: STAT3-HIES patients. \* indicates significant differences ( $P < 0.05$ ), and \*\* and \*\*\* indicate highly significant differences ( $P < 0.01$  and  $P < 0.001$ , respectively) as calculated by Mann–Whitney test. (B) Percentage of HLA-DR<sup>+</sup>CD3<sup>+</sup> cells of total lymphocytes from DOCK8-HIES and STAT3-HIES patients compared to age-related normal range (filled area).

#### **Figure 5: Cytokine release from PBMCs of DOCK8-HIES and STAT3-HIES patients.**

Cytokine concentrations in supernatants from unstimulated and with 1 µg/ml PHA or 0.001% SAC stimulated (PHA; SAC) PBMCs from five DOCK8-HIES patients compared to ten healthy controls and eight STAT3-HIES patients. Black dots: healthy controls, white dots: DOCK8-HIES patients, grey dots: STAT3-HIES patients. \* indicates significant differences ( $P < 0.05$ ), and \*\* and \*\*\* indicate highly significant differences ( $P < 0.01$  and  $P < 0.001$ , respectively) as calculated by Mann–Whitney test.

#### **Figure 6: Diagnostic work-flow of patients with suspicion of HIES.**

If - together with the initial observation of eczema, recurrent infections or elevated IgE - at least one of the findings listed under “Basic work-up by primary care physician” is present, it is recommended to refer this patient to a specialized center for further work-up and final diagnosis.

**Figure E1: Eosinophil and lymphocyte cell counts of DOCK8-HIES and STAT3-HIES patients.**

Absolute cell counts of eosinophils and lymphocytes from DOCK8-HIES and STAT3-HIES patients compared to age-related normal ranges (filled area).

**Figure E2: T and NK cell counts of DOCK8-HIES and STAT3-HIES patients.**

(A) Absolute cell counts of total T cells ( $CD3^+$ ), T helper cells ( $CD3^+CD4^+$ ) and cytotoxic T cells ( $CD3^+CD8^+$ ), percentage of IL17-producing T cells ( $CD3^+CD4^+IL17^+IFN\gamma^-$ ) of  $CD3^+CD4^+$  cells (normal range  $>0.2\%$ ; dotted line), and (B) absolute cell counts of total NK cells ( $CD16^+CD56^+$ ) of DOCK8-HIES and STAT3-HIES patients compared to age-related normal ranges (filled area).

**Figure E3: B cell counts and serum immunoglobulin levels of DOCK8-HIES and STAT3-HIES.**

(A) Absolute cell counts of total ( $CD19^+$ ) and memory B cells ( $CD19^+CD27^+$ ) and (B) concentrations of immunoglobulin classes in serum of DOCK8-HIES and STAT3-HIES patients; normal ranges: filled area.

**Table E1: Clinical findings in DOCK8-HIES patients.**

**Table E2: Clinical findings in STAT3-HIES patients.**

Figure 1



**Figure 2**

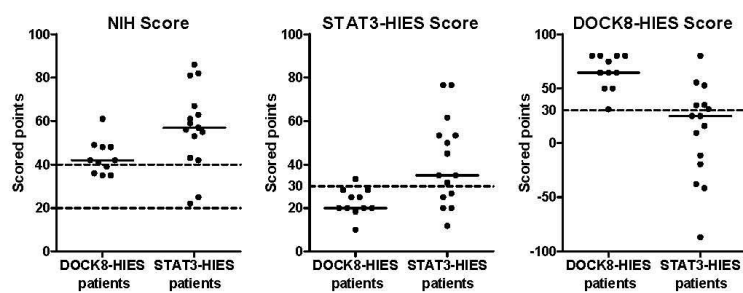


Figure 3

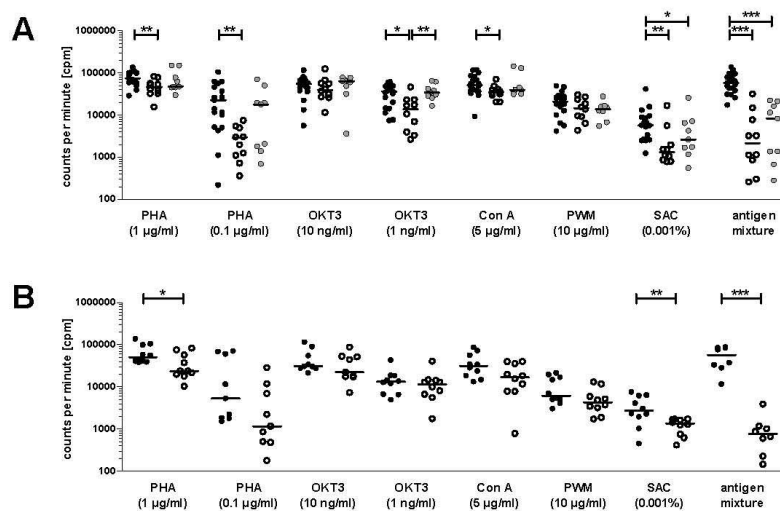


**Figure 4**

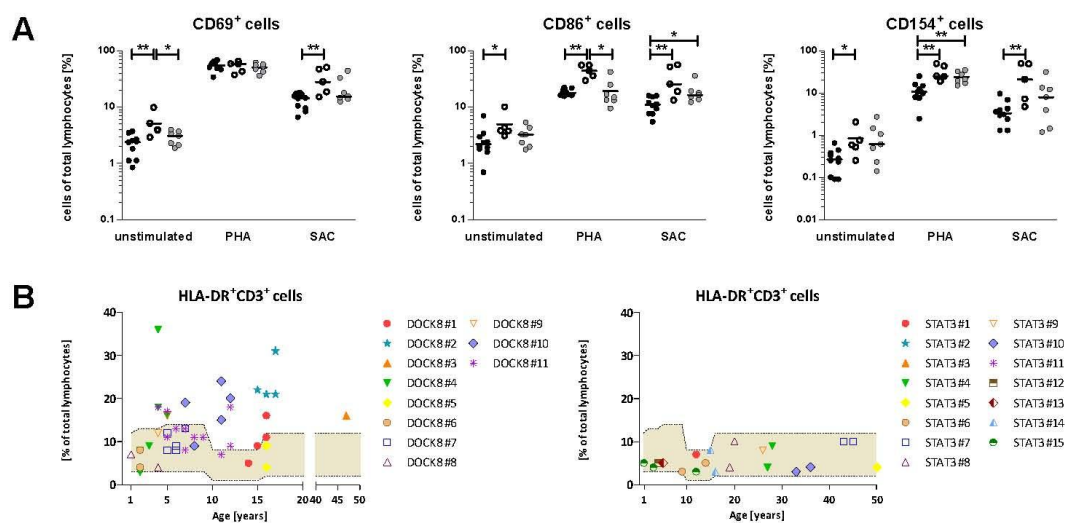


Figure 5

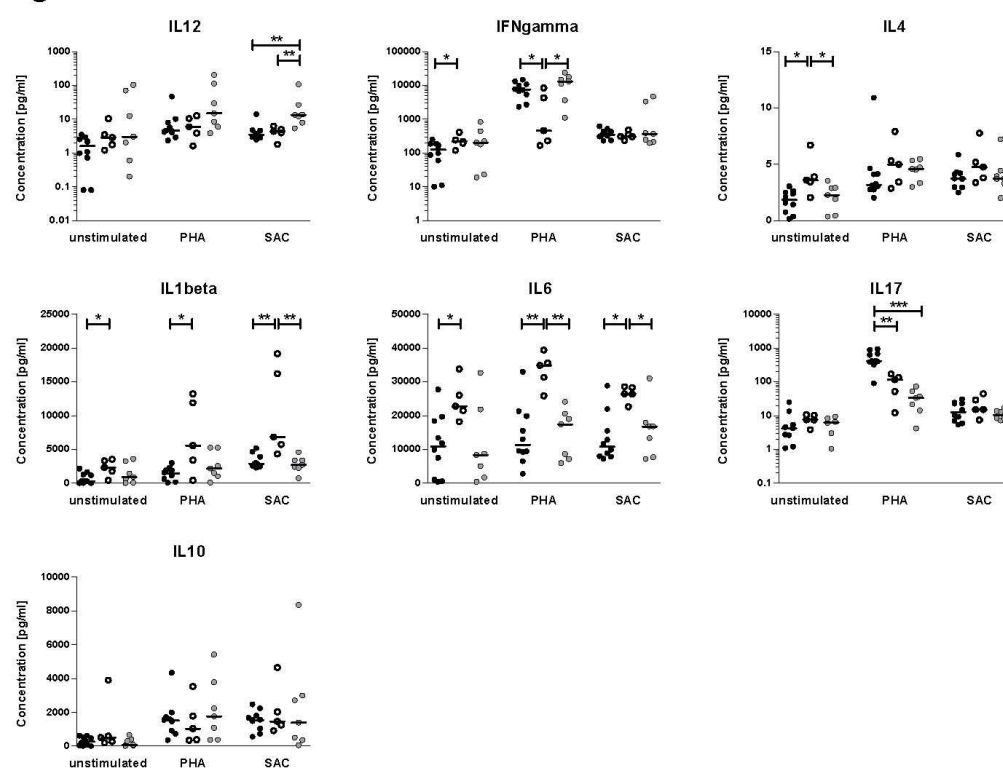


Figure 6

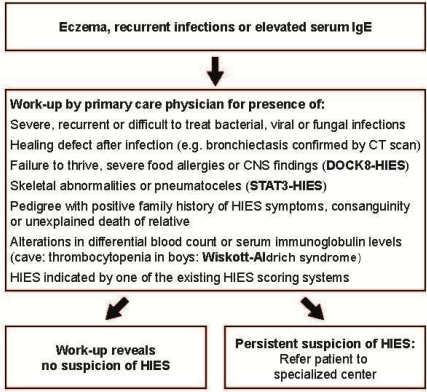
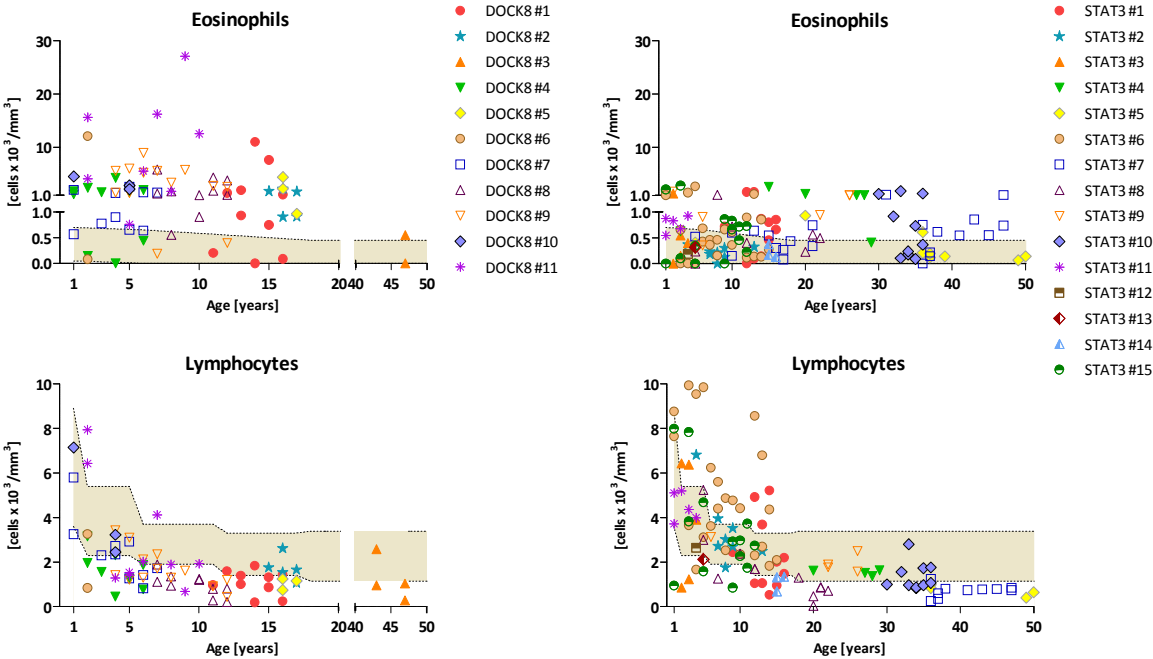
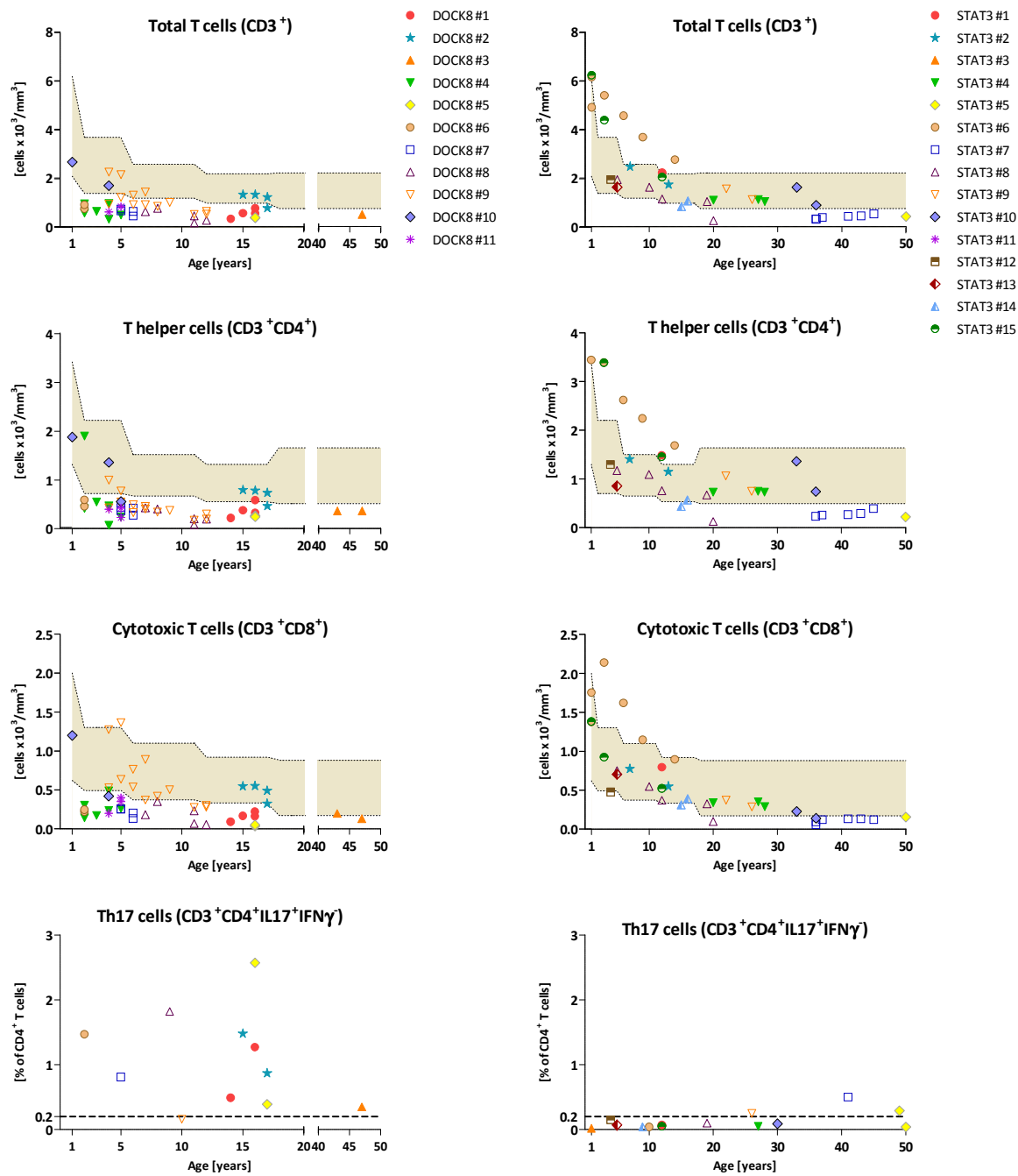


Figure E1

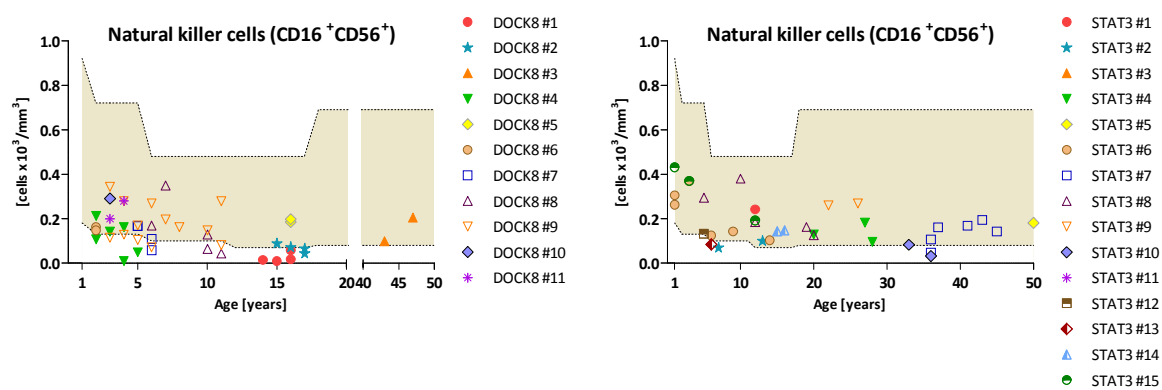


# Figure E2

**A**

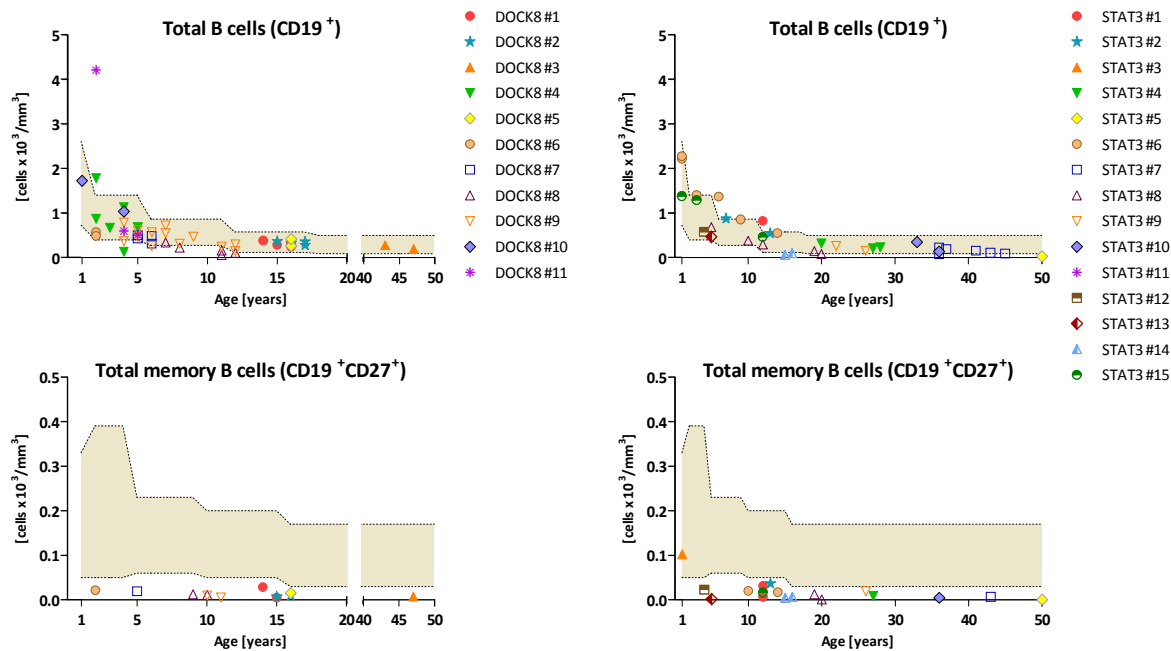


**B**



# Figure E3

**A**



**B**

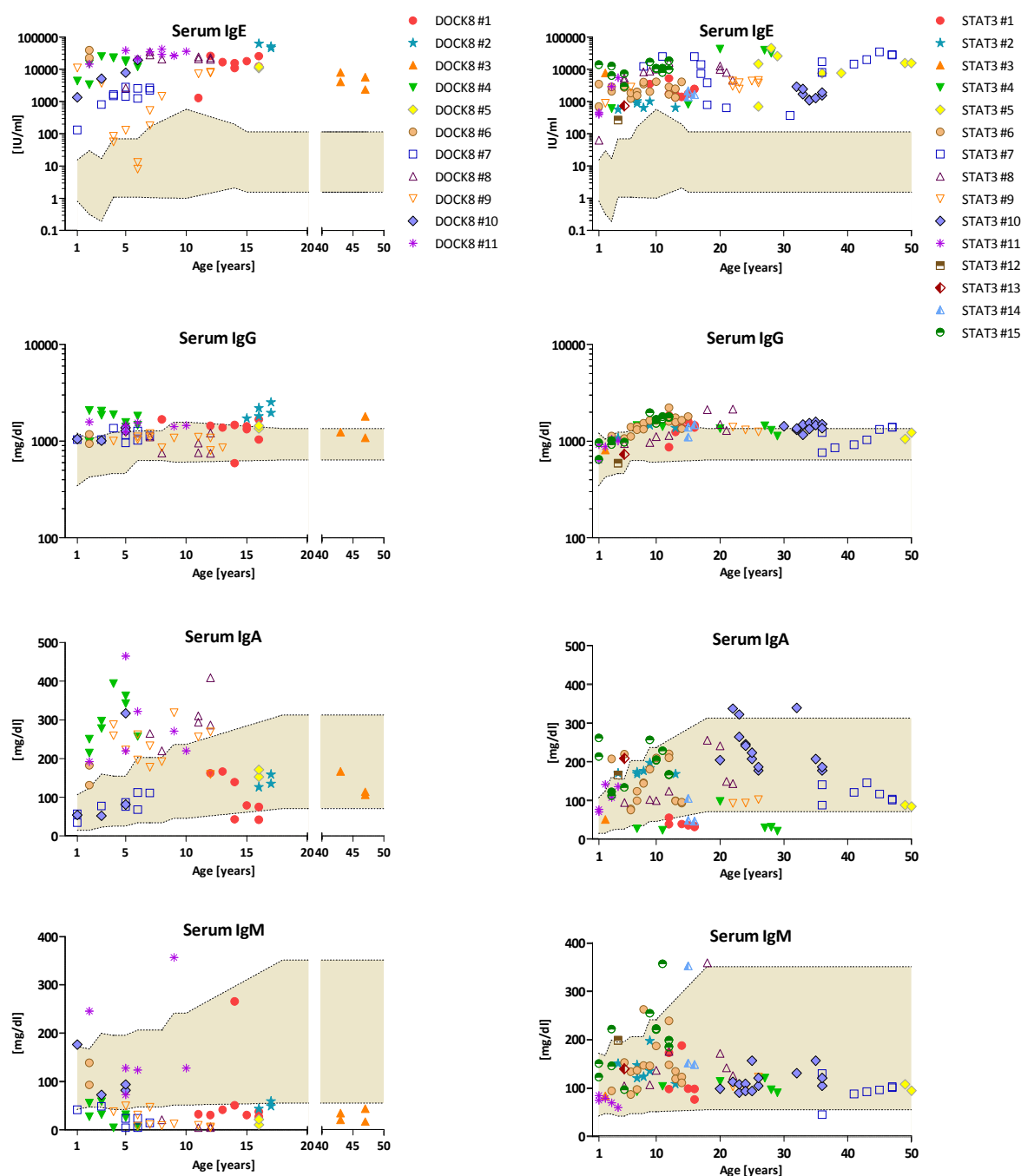


Table E1

| ID                    | Age at last evaluation | Gender | Eczema     | Recurrent mucocutaneous infections |           |            | Recurrent sinopulmonary infections |                |            | Recurrent keratitis/conjunctivitis | Chronic lung disease |               |                  | Food allergies | CNS findings | Failure to thrive | NIH score <sup>1</sup> | STAT3-HIES score <sup>2</sup> | DOCK8-HIES score <sup>3</sup> | DOCK8 mutation                                                 |
|-----------------------|------------------------|--------|------------|------------------------------------|-----------|------------|------------------------------------|----------------|------------|------------------------------------|----------------------|---------------|------------------|----------------|--------------|-------------------|------------------------|-------------------------------|-------------------------------|----------------------------------------------------------------|
|                       |                        |        |            | bacterial                          | fungal    | viral      | Sinusitis/Otitis                   | Otitis externa | Pneumonia  |                                    | Bronchiectasis       | Pneumatoceles | Hyper-reactivity |                |              |                   |                        |                               |                               |                                                                |
| DOCK8 #1              | 16 y                   | female | yes        | yes                                | yes       | yes        | yes                                | no             | yes        | yes                                | yes                  | no            | yes              | yes            | yes          | yes               | 48                     | 20                            | 50                            | homozygous splice donor site mutation c.3120+1G>T              |
| DOCK8 #2              | 17 y                   | female | yes        | yes                                | yes       | yes        | yes                                | no             | yes        | no                                 | no                   | no            | yes              | yes            | no           | yes               | 41                     | 20                            | 65                            | homozygous splice donor site mutation c.3120+1G>T              |
| DOCK8 #3              | 47 y                   | male   | yes        | yes                                | yes       | yes        | yes                                | yes            | yes        | yes                                | yes                  | no            | yes              | yes            | no           | no                | 38                     | 10                            | 50                            | homozygous splice donor site mutation c.3120+1G>T              |
| DOCK8 #4              | 6 y                    | female | yes        | yes                                | yes       | yes        | yes                                | no             | yes        | no                                 | no                   | no            | yes              | yes            | no           | yes               | 42                     | 20                            | 80                            | homozygous nonsense mutation c.5132C>A                         |
| DOCK8 #5              | 18 y                   | male   | yes        | yes                                | yes       | yes        | no                                 | no             | yes        | yes                                | no                   | no            | yes              | yes            | no           | yes               | 61                     | 28                            | 75                            | homozygous deletion of exons 11 to 48                          |
| DOCK8 #6              | 3 y                    | female | yes        | yes                                | yes       | yes        | yes                                | yes            | yes        | yes                                | no                   | no            | no               | yes            | no           | yes               | 36                     | 25                            | 65                            | homozygous deletion in exon 8 c.850_851delCT                   |
| DOCK8 #7              | 7 y                    | male   | yes        | yes                                | yes       | yes        | yes                                | yes            | yes        | no                                 | no                   | no            | no               | yes            | no           | no                | 35                     | 25                            | 65                            | homozygous deletion in exon 8 c.850_851delCT                   |
| DOCK8 #8              | 12 y                   | male   | yes        | yes                                | yes       | yes        | yes                                | yes            | yes        | yes                                | no                   | no            | yes              | yes            | no           | yes               | 49                     | 18                            | 31                            | homozygous deletion upstream of exon 1 including exon 2        |
| DOCK8 #9              | 13 y                   | female | yes        | yes                                | no        | yes        | yes                                | yes            | yes        | yes                                | no                   | no            | yes              | yes            | no           | no                | 39                     | 20                            | 80                            | homozygous deletion upstream of exon 1 including exon 26       |
| DOCK8 #10             | † 16 y                 | female | yes        | yes                                | yes       | yes        | yes                                | yes            | yes        | no                                 | no                   | no            | yes              | yes            | yes          | no                | 42                     | 28                            | 80                            | homozygous deletion upstream of exon 1 including exon 19       |
| DOCK8 #11             | 17 y                   | female | yes        | yes                                | yes       | yes        | yes                                | no             | yes        | no                                 | no                   | no            | no               | yes            | yes          | no                | 48                     | 33                            | 80                            | chrom9 del 30 910 - 388 506 including deletion of exon 1 to 26 |
| Positive for finding: |                        |        | 100% 11/11 | 100% 11/11                         | 91% 10/11 | 100% 11/11 | 91% 10/11                          | 55% 6/11       | 100% 11/11 | 55% 6/11                           | 18% 2/11             | 0% 0/15       | 73% 8/11         | 100% 11/11     | 27% 3/11     | 55% 6/11          |                        |                               |                               |                                                                |

† age at death

n.d. not determined

<sup>1</sup>Grimbacher B et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. Am J Hum Genet. 1999;65:735-44

<sup>2</sup>Woellner C et al. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. J Allergy Clin Immunol. 2010;125:424-32 e8

<sup>3</sup>Engelhardt KR et al. The extended clinical phenotype of 64 patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol. 2015

Table E2

| ID                    | Age at last evaluation | Gender | Eczema | Recurrent mucocutaneous infections |        |       | Recurrent sinopulmonary infections |                |           | Recurrent keratitis/ conjunctivitis | Chronic lung disease |               |                  | Food allergies | CNS findings | Failure to thrive | NIH score <sup>1</sup> | STAT3-HIES score <sup>2</sup> | DOCK8-HIES score <sup>3</sup> | STAT3 mutation                                 |
|-----------------------|------------------------|--------|--------|------------------------------------|--------|-------|------------------------------------|----------------|-----------|-------------------------------------|----------------------|---------------|------------------|----------------|--------------|-------------------|------------------------|-------------------------------|-------------------------------|------------------------------------------------|
|                       |                        |        |        | bacterial                          | fungal | viral | Sinusitis/ Otitis                  | Otitis externa | Pneumonia |                                     | Bronchiectasis       | Pneumatoceles | Hyper-reactivity |                |              |                   |                        |                               |                               |                                                |
| STAT3 #1              | 16 y                   | male   | yes    | yes                                | yes    | no    | yes                                | no             | yes       | no                                  | yes                  | yes           | no               | no             | no           | no                | 56                     | 27                            | 35                            | heterozygous STAT3 mutation c.1145G>A; p.R382Q |
| STAT3 #2              | 13 y                   | male   | yes    | yes                                | yes    | yes   | yes                                | yes            | yes       | no                                  | no                   | no            | no               | no             | no           | no                | 55                     | 45                            | 53                            | heterozygous STAT3 mutation c.995A>T; p.H332L  |
| STAT3 #3              | 5 y                    | male   | yes    | yes                                | no     | no    | yes                                | no             | yes       | no                                  | no                   | yes           | yes              | no             | no           | no                | 43                     | 20                            | 9                             | heterozygous STAT3 mutation c.1027G>C; p.V343L |
| STAT3 #4              | 29 y                   | male   | yes    | yes                                | yes    | no    | yes                                | yes            | yes       | no                                  | yes                  | yes           | no               | no             | no           | no                | 61                     | 35                            | 25                            | heterozygous STAT3 mutation c.1152T>A; p.F348L |
| STAT3 #5              | 50 y                   | male   | yes    | yes                                | yes    | no    | yes                                | no             | yes       | no                                  | no                   | no            | no               | no             | no           | no                | 81                     | 53                            | -42                           | heterozygous STAT3 mutation IVS 12-2A>G        |
| STAT3 #6              | 14 y                   | male   | yes    | yes                                | yes    | no    | yes                                | yes            | yes       | no                                  | yes                  | yes           | yes              | no             | no           | no                | 82                     | 77                            | -20                           | heterozygous STAT3 mutation c.1144C>T; p.R382W |
| STAT3 #7              | 47 y                   | male   | yes    | yes                                | yes    | no    | yes                                | no             | yes       | no                                  | no                   | no            | no               | no             | no           | no                | 53                     | 32                            | 56                            | heterozygous STAT3 mutation c.1144C>T; p.R382W |
| STAT3 #8              | 22 y                   | female | yes    | yes                                | yes    | no    | yes                                | yes            | yes       | no                                  | yes                  | yes           | yes              | no             | no           | no                | 86                     | 77                            | -38                           | heterozygous STAT3 mutation c.1145G>A; p.R382Q |
| STAT3 #9              | 26 y                   | male   | yes    | yes                                | yes    | no    | yes                                | yes            | no        | no                                  | yes                  | yes           | no               | no             | no           | no                | 57                     | 20                            | -12                           | heterozygous STAT3 mutation IVS 12+1g>t        |
| STAT3 #10             | 36 y                   | female | yes    | yes                                | yes    | no    | yes                                | no             | yes       | no                                  | yes                  | yes           | no               | no             | no           | no                | 67                     | 50                            | 25                            | heterozygous STAT3 mutation c.1144C>T; p.R382W |
| STAT3 #11             | 4 y                    | male   | yes    | yes                                | yes    | yes   | yes                                | no             | no        | no                                  | no                   | no            | no               | no             | no           | no                | 42                     | 12                            | 80                            | heterozygous STAT3 mutation c.1144C>T; p.R382W |
| STAT3 #12             | 4 y                    | male   | yes    | no                                 | no     | no    | yes                                | yes            | yes       | no                                  | no                   | no            | no               | no             | no           | no                | 22                     | 35                            | 16                            | heterozygous STAT3 mutation c.1145G>A; p.R382Q |
| STAT3 #13             | 5 y                    | male   | yes    | yes                                | no     | no    | yes                                | yes            | no        | no                                  | no                   | no            | no               | no             | no           | no                | 25                     | 25                            | 31                            | heterozygous STAT3 mutation c.1145G>A; p.R382Q |
| STAT3 #14             | 16 y                   | female | yes    | yes                                | no     | no    | yes                                | yes            | yes       | no                                  | yes                  | yes           | no               | no             | no           | no                | 63                     | 62                            | -87                           | heterozygous STAT3 mutation c.1145G>A; p.R382Q |
| STAT3 #15             | 12 y                   | female | yes    | yes                                | yes    | no    | yes                                | no             | yes       | no                                  | yes                  | yes           | no               | no             | no           | no                | 62                     | 53                            | 35                            | heterozygous STAT3 mutation c.1909G>A; p.V637M |
| Positive for finding: |                        |        | 100%   | 93%                                | 73%    | 13%   | 100%                               | 53%            | 80%       | 0%                                  | 53%                  | 60%           | 20%              | 0%             | 0%           | 0%                |                        |                               |                               |                                                |
|                       |                        |        | 15/15  | 14/15                              | 11/15  | 2/15  | 15/15                              | 8/15           | 12/15     | 0/15                                | 8/15                 | 9/15          | 3/15             | 0/15           | 0/15         | 0/15              |                        |                               |                               |                                                |

<sup>1</sup>Grimbacher B et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. Am J Hum Genet. 1999;65:735-44

<sup>2</sup>Woellner C et al. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. J Allergy Clin Immunol. 2010;125:424-32 e8

<sup>3</sup>Engelhardt KR et al. The extended clinical phenotype of 64 patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol. 2015

## **Methods:**

### **Patients**

Eleven molecularly defined DOCK8-HIES patients (4 males and 7 females, age range at most recent evaluation 3 to 47 years of age; median 16 years of age) from eight unrelated families were enrolled resulting in a total observation time of 79 patient years (range of follow-up 2 to 16 years; median of follow-up 6 years). Ethnic background was Turkish in six families, and one family each of German and Chechen descent. All but one family (DOCK8 #8) reported consanguinity. DOCK8-HIES was genetically defined in all patients (Table E1). Two patients were included in the original AR-HIES report (DOCK8 #10, DOCK8 #11) (1). Fifteen molecularly defined STAT3-HIES patients (11 males and 4 females, age range at most recent evaluation 4 to 50 of age; median 16 years of age) from 13 unrelated families of German descent (range of follow-up 1 to 46 years; median of follow-up 10 years) (Table E2). Primer sequences are available upon request. Mutations were reported using the nomenclature of den Dunnen and Antonarakis (2). All patients except patients STAT3 #3 and STAT3 #11 were reported previously (1, 3-14). The study was approved by the local Institutional Review Board. Written informed consent was obtained.

### **Clinical and immunologic work-up**

All subjects had a complete history, review of medical records, physical exam, and laboratory investigations, and were assessed with the three to-date proposed HIES scores (15-17). Differential blood count, serum immunoglobulin level, isohaemagglutinin IgM titers and specific antibody titers against *Haemophilus influenzae*, *Streptococcus pneumoniae*, and tetanus toxoid were assessed. Cell subsets were defined as total T cells (CD3<sup>+</sup>), T helper cells (CD3<sup>+</sup>CD4<sup>+</sup>), cytotoxic T cells (CD3<sup>+</sup>CD8<sup>+</sup>), Th17 cells (CD3<sup>+</sup>CD4<sup>+</sup>IL17<sup>+</sup>IFN $\gamma$ ), total B cells (CD19<sup>+</sup>), memory B cells (CD19<sup>+</sup>CD27<sup>+</sup>), NK cells (CD16<sup>+</sup>CD56<sup>+</sup>) and activated T cells (HLA-DR<sup>+</sup>CD3<sup>+</sup>) and analyzed by flow cytometry (BD FACSCalibur, BD Biosciences, San Jose, CA, USA) as previously described (9, 13, 18). Non-class switched memory B cells and class switched memory B cells were identified as CD19<sup>+</sup>CD27<sup>+</sup>IgM<sup>+</sup>IgD<sup>+</sup> and CD19<sup>+</sup>CD27<sup>+</sup>IgM<sup>+</sup>IgD<sup>-</sup>. Ratios of non-class switched memory B cells to class switched

memory B cells were considered normal when  $\leq 0.5$  (own unpublished observation). Age-matched reference values were taken from previous reports (13, 19-26).

### **Lymphocyte activation tests**

Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood using Biocoll Separating Solution (BIOCHROM AG, Berlin, Germany). Lymphocyte proliferation in response to mitogen and antigen was assessed by [ $^3$ H]thymidine uptake adapted from a standard protocol (27) and the following mitogen concentrations: Phytohaemagglutinin (PHA) (Oxoid Limited, Basingstoke, UK) 1  $\mu$ g/ml and 0.1  $\mu$ g/ml, OKT3 (Immunotech, Marseille, France) 10 ng/ml and 1 ng/ml, Concanavalin A (ConA) (Sigma-Aldrich, St. Louis, MO, USA) 5  $\mu$ g/ml, Pokeweed mitogen (PWM) (Sigma-Aldrich) 10  $\mu$ g/ml, *Staphylococcus aureus* Cowan I (SAC) (Pansorbin<sup>®</sup>; Merck Chemicals GmbH, Schwalbach, Germany) 0.001% and an antigen mixture of Tetanus Toxoid Antigen and Diphtheria Toxoid Antigen (both Binding Site, Birmingham, UK).  $1 \times 10^5$  PBMCs were stimulated with mitogen for three days or with antigen for seven days.

Expression of the surface markers CD69, CD86, and CD154 was assessed in unstimulated and in 48 hours PHA 1  $\mu$ g/ml or SAC 0,001% stimulated  $0.5 \times 10^6$  PBMCs by flow cytometry on a FACS Calibur (BD Biosciences); all antibodies (CD154-PE, CD69-APC, CD86-PE-Cy5) from BD Biosciences. Supernatants of unstimulated and stimulated PBMCs ( $1 \times 10^6$  cells/ml) were assessed with the Bioplex Pro Human Cytokine Group I 7-plex Kit including IL1beta, IL4, IL6, IL10, IL12, IL17 and IFNgamma on a Bioplex 200 system as to the manufacturer's instruction (Bio-Rad Laboratories GmbH, Munich, Germany).

### **Statistics:**

Non-parametric statistical procedures were applied. Two-group comparisons were assessed by Mann-Whitney test. P values of below 0.05 were considered significant. Calculations and graphics were generated with IBM SPSS Statistics Version 21 (International Business Machines Corp., Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA).

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