HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

J Allergy Clin Immunol. 2015 August ; 136(2): 402–412. doi:10.1016/j.jaci.2014.12.1945.

The extended clinical phenotype of 64 patients with DOCK8 deficiency

A full list of authors and affiliations appears at the end of the article.

Abstract

Background—Mutations in *DOCK8* cause a combined immunodeficiency (CID) also classified as autosomal-recessive hyper-IgE syndrome (HIES). Recognizing patients with CID / HIES is of clinical importance due to a difference in prognosis and management.

Objectives—Define the clinical features that distinguish DOCK8 deficiency from other forms of HIES and CIDs; study the mutational spectrum of DOCK8 deficiency; and report on the frequency of specific clinical findings.

Methods—Eighty-two patients from 60 families with CID and the phenotype of autosomal-recessive HIES with (64 patients) and without (18 patients) *DOCK8* mutations were studied. Support vector machines were used to compare clinical data from 35 patients with DOCK8 deficiency with 10 AR-HIES patients without a *DOCK8* mutation and 64 patients with *STAT3* mutations.

Results—DOCK8-deficient patients had a median IgE of 5,201 IU, high eosinophil levels of usually at least 800/μl (92% of patients), and low levels of IgM (62%). About 20% of patients were lymphopenic, mainly due to low $CD4^+$ and $CD8^+$ T cells. Fewer than half of the patients tested produced normal specific antibody responses to recall antigens. Bacterial (84%), viral (78%), and fungal (70%) infections were frequently observed. Skin abscesses (60%) and allergies (73%) were common clinical problems. In contrast to STAT3 deficiency, there were few pneumatoceles, bone fractures, and teething problems. Mortality was high (34%). A combination of five clinical features was helpful in distinguishing patients with *DOCK8* mutations from those with *STAT3* mutations.

Conclusions—DOCK8 deficiency is likely in patients with severe viral infections, allergies, and/or low IgM levels, who have a diagnosis of HIES plus hypereosinophilia and upper respiratory tract infections in the absence of parenchymal lung abnormalities, retained primary teeth, and minimal trauma fractures.

Keywords

Primary combined immunodeficiency; Hyper-IgE syndromes; HIES; Autosomal recessive hyper-IgE syndrome; DOCK8; STAT3; *Molluscum contagiosum*

[#]**Corresponding author:** Bodo Grimbacher, MD, Universitätsklinikum Freiburg, Center for Chronic Immunodeficiency, Engesser Straβe 4, 79108 Freiburg, Phone: +49-(0)761 270-77731, bodo.grimbacher@uniklinik-freiburg.de.

[*]S.K. and A.A.S. contributed equally to this work.

INTRODUCTION

DOCK8 deficiency is an autosomal recessive immunodeficiency syndrome characterized by a combined defect in humoral and cellular immunity.[1,2] This disease overlaps phenotypically to some extent with the autosomal dominant form of HIES caused by *STAT3* mutations.[3–6] Shared symptoms of DOCK8 and STAT3 deficiency include eczema, recurrent staphylococcal skin abscesses, frequent upper and lower respiratory tract infections, candidiasis, high serum levels of IgE, and hypereosinophilia. However, individuals with *STAT3* mutations may develop pneumatoceles, which are rarely seen in DOCK8-deficient patients. Mutations in *STAT3* are often associated with non-immune symptoms involving dentition, bone and connective tissue. In contrast, DOCK8-deficient patients present frequently with allergies, severe and refractory cutaneous viral infections, and sometimes with neurological symptoms. However, not all patients demonstrate the full spectrum of this syndrome, especially in early childhood; therefore it can sometimes be difficult to diagnose DOCK8 deficiency based on clinical presentation and laboratory results alone.

This study aims to obtain a more detailed picture of the clinical phenotype of DOCK8 deficiency based on 64 patients lacking intact DOCK8 (Figure E1), to establish diagnostic measures that help distinguish HIES patients with a *DOCK8* mutation from other patients with a combined immunodeficiency and from those with a *STAT3* mutation, thus helping to guide clinicians in their work-up of patients and to recognize this primary immune deficiency as early as possible to avoid diagnostic delay.

METHODS

Patients and controls

We enrolled a cohort of 82 patients from 60 families in a world-wide collaboration. All patients fulfilled the following inclusion criteria for this study: signed informed consent, a strong clinical suspicion of AR-HIES according to the referring immunologist, and an available sample of genomic DNA or RNA. Of the 82 patients, 40 were males and 42 females. The age of the patients at the time of clinical evaluation ranged between 6 months and 45 years. The ethnic origin, HIES score, and clinical information of each DOCK8-deficient patient are shown in Table E1. The laboratory measurements of each DOCK8-deficient patients are shown in Table E2.

All patients and controls or their parental or legal guardians provided written consent for the conducted studies, following local ethics committee requirements. The study was approved under the ethics committee at University College London (protocols #04/Q0501/119_AM03 for affected individuals and #07/H0720/182 for family members).

Genotyping and genetic linkage analysis

For many of the patients described here, microsatellite or SNP marker genotyping was performed as described in the Online Repository at www.jacionline.org or as previously reported.[1]

PCR and Sequence analysis

Genomic DNA and RNA of controls and patients were isolated from either whole blood or peripheral blood mononuclear cells (PBMCs). RNA was isolated using RNeasy Kit (Qiagen) according to manufacturer's instructions. RNA was reverse transcribed using Omniscript reverse transcriptase (Qiagen). Coding genomic sequences and cDNA of *DOCK8* were amplified and purified using the QIAquick PCR purification kit (Qiagen). Primer sequences are available upon request. Purified PCR products were sequenced with the ABI PRISM BigDye Terminator cycle ready reaction kit V3.1 (Applied Biosystems, Foster City, CA) using the PCR primers as sequencing primers. The sequencing was performed on a 3130xl Applied Biosystems Genetic Analyzer, and the data were analyzed with DNA Sequencing Analysis software version 5.2 (Applied Biosystems) and Sequencher™ version 4.8 (Gene Codes Corporation, Ann Arbor, USA).

Statistical analysis

We investigated the significance of each of 20 features on the NIH Score sheet using logistic regression. We also used the machine learning technique of Support Vector Machines (SVM) to reduce the number of features and produce a linear classifier that best distinguished this cohort of DOCK8 patients from a previously published cohort of STAT3 deficient patients; see the Methods section of this article's Online Repository at www.jacionline.org.

Several additional methods used in this study are described in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Identification of DOCK8 deficiency

Of the 82 individuals studied in 60 families, we diagnosed 64 individuals from 50 families with DOCK8 deficiency (Figure E1). For 60 individuals from 46 unrelated families, a homozygous or compound heterozygous mutation was identified in *DOCK8* (Figure 1 and Table E3), a total of 40 distinct mutations. For four patients from four families (ARH018, ARH019, ARH006, and ARH007), the *DOCK8* mutation could not be identified by sequencing due to the unavailability of cDNA or of additional genomic DNA. We summarize the evidence for DOCK8 deficiency in each of these four families in the results section of this article's Online Repository at www.jacionline.org.

Mutations in *DOCK8*

Of the mutations identified in this cohort, 14 distinct mutations in 21 individuals from 14 families were previously reported.[1] Any families appearing in both reference 1 and here have the same ARH identifiers, except that ARH017.1 was previously labeled ARH017 and ARH020.3 was previously labeled ARH020; both changes are necessitated by the ascertainment of second affected siblings in the same families. Twenty-five novel mutations are reported in this paper, including two previously reported patients[1] whose *DOCK8* mutation detection was completed as part of this study.

Thirty-three of 46 families (72%) had insertions or deletions (indels): one homozygous 2-bp insertion, one homozygous 2-bp deletion, six homozygous single exon deletions, 24 homozygous multi-exon deletions, spanning at least two exons to as much as nearly the whole gene including neighboring gene(s), and one compound heterozygous multi-exon deletion with an overlap of 27 deleted exons (Figure 1 and Table E3). Eleven families had homozygous point mutations, which were either nonsense (6/11) or splice site mutations (5/11). In family ARH028, no specific point or splice site mutation was identified, but 56 intronic nucleotides plus an additional G were retained between exons 29 and 30 in the mRNA and caused a frame shift leading to a premature stop codon. In family ARH020, we found an absence of *DOCK8*-specific mRNA expression. Of the 40 distinct genetic alterations found, one abrogates gene transcription, and 37 result in an mRNA that, if translated at all, would lead to a severely truncated DOCK8 protein. Only two mutations lead to an mRNA with an in-frame deletion of a single exon: Ex27del and the splice donor site mutation leading to skipping of exon 25. These in-frame deletions are located between the two DHR domains of DOCK8 (Figure 1).

Affected individuals identified as unlikely to have DOCK8 deficiency

We excluded 14 patients from eight consanguineous families from further *DOCK8* mutation detection after homozygosity mapping with microsatellite or SNP markers showed that they were heterozygous in a genetic interval including *DOCK8* (see Methods and Results in this article's Online Repository at www.jacionline.org.). Some families also had other candidate loci excluded (See Results in the Online Repository).We did not investigate the possibility of compound heterozygous mutations in these patients due to parental consanguinity. Of these 14 patients, homozygous mutations in *PGM3* were subsequently found in nine patients from three families[7]; two other research groups have also reported patients with overlapping phenotypes and biallelic mutations in *PGM3*.[8–9] Moreover, based on sequencing of *DOCK8*, we concluded that four affected individuals from two families did not have DOCK8 deficiency. One individual was sequenced from each of these two families. Neither person had exonic mutations or mutations in flanking splice sites. For both individuals, *DOCK8* mRNA was expressed normally.

The clinical phenotype of DOCK8 deficiency

In our cohort of 64 DOCK8-deficient patients, 30 patients were male and 34 were female. Of the 50 families with DOCK8 deficiency, 40 were consanguineous, and ten were not known to be consanguineous. Among the 10 families without DOCK8 deficiency, 6/10 are also consanguineous (Table E1), so our results are primarily, but not exclusively about consanguineous families. The mean age of patients in our cohort was 10 years (range: 6 months to 45 years) at the time of last evaluation. Thirty-nine patients were in their first decade of life (61%), 21 in the second (33 %), two in their third and two in their fifth decade of life (Figure 2A and Table E1). The two eldest patients are brothers (family ARH010) who have a *DOCK8* splice site mutation, allowing for some residual protein expression.

Clinical data was not complete for all of the patients due to the loss of patients during follow-up and lack of proper documentation. For example, mortality data was only available for 58 of the 64 patients. The mortality rate in our cohort was 34% (20 of 58 patients), with

death occurring at a mean age of 9 years 3 months (range: 1.5–19 years); 14 patients died in the first, and six in the second decade of life (Figure 2A). Causes of death included encephalitis (3 patients), viral and fungal infections (3 patients), sepsis (2 patients), cerebral non-Hodgkin and Burkitt lymphoma (1 patient each), wasting and metabolic derangement (1 patient), respiratory failure (1 patient), rupture of an aortic aneurysm (1 patient), and JC-virus-negative PML (1 patient) (Table E1). Survival by the age of 10 years was 67% (95% confidence interval: 54–83%), but by the age of 18 years it dropped to 48% (95% confidence interval: 31–73%) (Figure 2B).

Fifty-seven of 64 patients were evaluated with the NIH HIES scoring system[10] and 46/57 of the score sheets were complete; 31/46 (67%) scored at least 40 points (highest 67 points), indicating that the diagnosis of HIES is probable, and 14 (30%) scored between 20 and 40 points, suggesting HIES is possible (Figure 2C). Only one DOCK8-deficient individual had a low score of 13; he was a healthy six-month-old brother of a patient and was diagnosed with DOCK8 deficiency by sequencing only due to his sibling's diagnosis.

All but two patients had eczema (59/61 pts) and 16 patients (35%) presented with a newborn rash (Table 1). Skin abscesses were common (34/57 pts; 60%). Three patients had abscesses in organs such as liver, kidney, lung and brain. In one patient, *S. aureus* was isolated from a renal abscess, and in another patient, a brain abscess was positive for *Aspergillus*.

Mucocutaneous infections with *Candida* spp. (37/58 pts; 64%) and viruses (41/60 pts; 68%) were common. Severe and refractory skin infections with *Herpes simplex* (22/58 pts; 38%) and *Varicella zoster* virus (11/58 pts; 19%), *Molluscum contagiosum* virus (21/56 pts; 38%), or Human papilloma virus (16/55 pts; 29%) were frequent findings (Table 1). Non-cutaneous viral infections included the fatal JC virus-associated progressive multifocal leukoencephalopathy (PML) in two patients; pneumonia, meningitis, encephalitis, retinitis, keratitis and/or conjunctivitis caused by Herpes family viruses in nine patients; rotavirus enteritis in one individual and viral hepatitis (caused by HAV, HBV and HCV, respectively) in three patients (Table 2). Two patients had systemic *Candida* infections, of which one had pneumonia and one sepsis. Lung colonization, sinusitis, or chronic infection with the fungus *Aspergillus* occurred in three patients, and one other was diagnosed with allergic bronchopulmonary aspergillosis (ABPA). Other fungal infections were rare: among them, one patient presented with *Tinea cruris* and two with *Cryptococcus neoformans* (one CNS infection, one in skin abscess). Three Turkish patients had infections with the parasite *E. histolytica* and in one patient the protozoan parasite *Cryptosporidium* was found. Eighty-four % of patients (43/51 pts) had infections with bacteria, mainly with Gram-positive cocci (41/51 pts; 80%), especially *S. aureus*. Again, infections were predominantly confined to the skin as abscesses; however some were more severe infections including bacterial sepsis, meningitis, and pneumonia (Table 2).

Upper and/or lower respiratory tract infections occurred in all but one patient (59/60 pts) (Table 1). Ninety % of patients (54/60 pts) had at least one episode of pneumonia, with 35% (21/60 pts) having had more than five. Infections could result in abnormalities of the lung; 20 individuals developed bronchiectasis and two had pneumatoceles (Table E1 and Figure

E2). Seventeen out of 56 patients (30%) presented with asthma, which was sometimes linked to allergies.

Allergies are another feature of DOCK8 deficiency with 73% of patients being affected (41/56 pts), mostly by food allergies (36 pts) (Table 1). Eighteen patients reacted to environmental and inhalation allergens, three to latex and four to drugs. Poor growth and failure to thrive were present in 59% of individuals (32/54 pts) (Table E1).

Neurological symptoms and signs as sequelae of infectious disease, inflammation or malignancy frequently occurred in our DOCK8-deficient cohort. Some of these were fatal, in particular encephalitis (3 pts), CNS lymphoma (2 pts), JC virus-associated PML (2 pts), and non-JC viral encephalopathy (1 pt) (Table 3). In total, 20 patients had CNS involvement, including CNS vasculitis (3 pts), a vascular aneurysm (1 pt), meningitis (4 pts), brain abscesses (4 pts), or a brain infarct/stroke (3 pts). Apart from the two patients with CNS lymphoma (Burkitt and non-Hodgkin lymphoma), one other individual had a retropharyngeal Burkitt lymphoma and two had squamous cell carcinoma, summing to 8% of DOCK8-deficient patients with malignancies. Two patients had autoimmune hemolytic anemia.

Symptoms that cannot be attributed directly to immunodeficiency were present in our cohort of DOCK8-deficient patients (Table 3). Rare or unusual features observed in the cohort are listed in Table E4.

DOCK8-deficient patients had a median IgE of approximately 5,201 IU Nearly all patients (54/59 pts; 92%) presented with hypereosinophilia that was characterized by elevated levels of > 800 cells/μl (range: 245–37,880 cells/μl) (Figure 3B). Total numbers of lymphocytes were normal in 45/58 patients (78%), despite an elevated white blood cell count (WBC) in 17/53 patients (32%). Nineteen percent of patients (11/58) were lymphopenic which mainly affected absolute T cell counts (Table 4 and Figure 3A). Within the T-cell compartment, low absolute levels were detected in CD4+ and CD8+ T cells (16/56 patients (29%) , and 16/55 patients (29%), respectively, of which 9 patients had low levels of both T-cell subtypes), but only CD8+ T cells showed elevated levels in 7/55 patients (13%). One patient had highly elevated NK cell counts (Figure 3B), which was not due to a general increase of leukocytes.

Apart from the symptom-free DOCK8-deficient 6-month-old infant, all patients with reported serum immunoglobulins had elevated serum IgE with levels ranging from 400 to 90,910 IU/ml (average 12,893 IU/ml; median 5,201 IU/ml) (Table 4 and Figure 3B). Twenty-four of 62 patients (39%) had levels of more than 10,000 IU/ml. In the majority of patients, serum IgM levels were low (36/58; 62%) (Figure 3C). Low or absent specific antibody responses to recall antigens such as Pneumococcus, diphtheria, tetanus, and Candida were documented in 16 of 31 patients (52%), and low isohemagglutinin titers in 10 of 31 patients (32%) (Table E1).

In four patients from one family investigated, cytotoxic T cell (CTL) cytotoxicity and degranulation were normal (Figure E3), as was NK cell degranulation (Figure E3). In one patient of this family, NK cell cytotoxicity was assessed and proved to be normal (data not shown). For 15 patients, information could be gathered on memory B- and/or T-cell

numbers. There was a reduction in memory B cells and switched memory B cells, down to near absence (Table E2). T cell memory was more variable with either normal or decreased levels of CD45RO+ memory T cells (Table E2). In one patient, CD8+ naïve T cell numbers were higher than the corresponding numbers of memory cells (Table E2).

Statistical Analysis

We performed logistic regression (See Table E5 in the Online Repository) and SVM analysis to select five features and create a linear classifier that attempts to distinguish DOCK8- deficient patients from STAT3- deficient patients (see Supplementary Online Methods and Results) The five features chosen were lung abnormalities, eosinophilia, upper respiratory infections, retained primary teeth, and fractures with minimal trauma; the new SVM scoring system is shown in Table E6 in the Online Repository.

The leave-one-out error rate (see Supplementary Online Methods) for the chosen set was 11.1% with sensitivity for predicting a *DOCK8* mutation of 91.4% and specificity of 87.5%. By a Wilcoxon rank-sum test, the generated linear classifier is significantly predictive of a *DOCK8* mutation (two-sided P-value 3.6×10^{-13}). It should be emphasized, however, that leave-one-out testing is a technique used to analyze the robustness of a classifier on the training set, and the effectiveness of the classifier has not been evaluated on a prospective cohort of patients.

DISCUSSION

Here, we report 25 new mutations causing human DOCK8 deficiency and symptoms that were previously unrecognized to occur in DOCK8 deficiency. Early diagnosis of DOCK8 deficiency is important to facilitate an adequate treatment such as HSCT.[11–15]

DOCK8 deficiency has a high mortality at a young age with more frequent severe infections and malignancy, so HSCT should be considered. In contrast, conflicting results have been reported for HSCT as an effective treatment for AD-HIES due to *STAT3* mutations, the most common cause of HIES.[4,16] One AD-HIES patient had a relapse of HIES symptoms four years after transplantation;[17] however, long-term follow up of this patient revealed no further infectious damage (unpublished data). Two other transplanted STAT3-deficient patients were considered cured ten and 14 years later, respectively.[18] Due to its risks, HSCT would be considered only for STAT3 deficiency with severe complications, such as lymphoproliferative disease; while in DOCK8 deficiency, HSCT will probably be considered in the majority of patients. Because HSCT is best done as early as possible, early identification of HIES patients presenting with characteristics of a DOCK8 deficiency followed by a firm molecular diagnosis is essential to manage these patients appropriately.

To aid in the clinical management of DOCK8-deficient patients, we compiled all symptoms of the patients in our cohort. This adds information to findings compiled by other groups following DOCK8-deficient patients.[19,20] Some of these rare symptoms (gastrointestinal tract problems, sclerosing cholangitis and CNS lymphoma) have also been reported in singleton patients by Sanal et al.,[20] suggesting that they might be associated with the lack of DOCK8. However, as most of the patients are born to consanguineous parents (40/50

families), additional homozygous defects may be present. We also have to caution that clinical findings very specific to STAT3 deficiency, such as pneumatoceles may also occur in DOCK8-deficient patients (Figure E2). Our study did include some non-consanguineous patients (10/50 families with DOCK8 deficiency and 4/10 families without), but the frequencies of various symptoms of DOCK8 deficiency could be significantly different in a sample with a lower rate of consanguineous parents.

In this present study, we describe the largest cohort of patients reported to date with *DOCK8* mutations: We identified *DOCK8* mutations in 60 patients from 46 unrelated families. Among those, there are 40 distinct mutations, with one compound heterozygous individual carrying two overlapping multi-exon deletions. Twenty-five of these mutations have not been previously reported. While the majority of mutations in our cohort are insertions and deletions (henceforth “indels”), there are nonsense and splice junction point mutations. We did not find any missense mutations. To date, only two missense mutations in DOCK8 have been described: p.C1447R and p. V797M.[20] The *DOCK8* mutation spectrum is quite different from that of *STAT3*, the latter being characterized by dominant-negative point mutations in the two important functional domains of STAT3.[4–6] The differences in the mutation spectra of the two diseases have important implications for the diagnosis in today’s era of personalized genomic medicine and high-throughput DNA sequencing. One implication is that some DOCK8 mutations whose presence is often initially identified by FACS or Western blot, can be characterized best at the nucleotide level by sequencing cDNA. Therefore, clinicians suspecting a diagnosis of DOCK8 deficiency should collect samples from which mRNA can be generated or which can be used for protein detection via flow cytometry[21] or immunoblotting.

At the Center for Chronic Immunodeficiency, Freiburg, Germany, DOCK8 deficiency is typically diagnosed by protein analysis via FACS or Western blot and genetically confirmed by targeted gene panel resequencing (including 16 genes involved in similar phenotypes), followed by CNV detection, PCR, or Sanger sequencing. As *DOCK8* is a large gene, it is important to reduce costs where possible. First, we show that there is a non-negligible proportion of patients (18/82 patients; 22%) diagnosed with AR-HIES who do not have DOCK8 deficiency. Thus, if a clinician receives from a molecular diagnostic laboratory a report indicating that *DOCK8* sequence is wild type (wt), this is a plausible result. However, a possible somatic reversion of the germline mutation may be present. [22] Eventually, genes mutated in the DOCK8-sufficient patients, such as *PGM3*,[7–9] will be identified, and the diagnostic sequencing strategy can be expanded to include more genes (see the Online Repository for exclusion of other candidate genes in some of our families that do not have mutations in either *DOCK8* or *PGM3*). In addition, a recent report[22] has demonstrated that in some patients DOCK8 gene expression can be reestablished in one or more subsets of cells through somatic reversion. When screening patients for DOCK8 mutations, somatic reversions might mask the identification of DOCK8 mutations in those patients, especially because the cells with reversions to wild type sequence may be selected for among cell populations that expand, such as T cells. We could not phenotypically distinguish DOCK8 deficiency in 35 families from other causes of AR-HIES in 10 families, among which three have PGM3 deficiency and seven are not yet explained genetically. It would be clinically

useful to distinguish DOCK8 deficiency from PGM3 deficiency, STK4 deficiency, and TYK2 deficiency. However, such a distinction cannot be made statistically because the clinical presentations of these three other immunodeficiencies are too heterogeneous given the small number of patients described to date. Moreover, our cohort did not include STK4-deficient or TYK2-deficient patients (see Online Repository regarding exclusion of these loci). In the case of PGM3, the clinical heterogeneity is at least partly due to the known mutations being hypomorphic mutations of varying severity and affecting different domains of the protein.[7–9] The reasons for the heterogeneity of STK4 and TYK2 deficiencies remain elusive. Another differential diagnosis to DOCK8 deficiency is chronic granulomatous disease (CGD), which, however, can be readily diagnosed by a test termed DHR.[23]

To aid in faster diagnosis, we investigated whether it was possible to distinguish AD-HIES from DOCK8 deficiency, even though the clinical manifestations of both disease are variable. In this article's Online Repository at www.jacionline.org we provide a modified, weighted HIES score based on a subset of DOCK8-relevant features that could assist physicians to predict which of DOCK8 deficiency or STAT3 deficiency is more likely in a specific patient. Most cases of STAT3 deficiency and DOCK8 deficiency can be correctly distinguished by a linear classifier using five items from the 20-item HIES clinical scoring sheet, which are parenchymal lung abnormalities, eosinophilia, sinusitis/otitis, retained primary teeth, and fracture with minor trauma. Our "DOCK8 score" could help to justify the expenditure for cDNA collection and targeted sequencing of *DOCK8* in samples of those patients with a high score

The DOCK8 score is statistically significant in distinguishing patients with a *DOCK8* mutation from those with a *STAT3* mutation (two-sided P-value 3.6×10^{-13}). It performs substantially better in leave-one-out testing than the NIH score or the STAT3 score (see this article's Online Repository at www.jacionline.org). The NIH score, while certainly indicative of the presence of disease, performed poorly at distinguishing DOCK8 from STAT3 patients (see Supplementary Online Results). However, the usefulness of the DOCK8 score has not been confirmed on a prospective cohort of patients with immunodeficiencies that present with high IgE and a strong clinical suspicion of HIES. Thus, the authors call for a validation on an independent cohort.

Since the NIH score and HIES clinical sheet were developed using a cohort of STAT3-deficient patients,[10] it is interesting to note that two of the features in the DOCK8 score, eosinophilia and upper respiratory infections, have positive coefficients indicating that they are more prevalent in DOCK8 deficiency. Other hallmarks of DOCK8 deficiency, such as viral infections and T cell lymphopenia unfortunately could not be used in the machine learning analysis because their presence/absence was not systematically recorded for STAT3-deficient patients.

New treatments for DOCK8 deficiency may eventually be found by investigating the cellular mechanisms of this peculiar disease. Some progress towards understanding the mechanisms of DOCK8 deficiency has been made by functional studies of Dock8-deficient mice. DOCK8 is a Cdc42-specific guanine nucleotide exchange factor (GEF) at the plasma membrane needed for spatial activation of Cdc42 at the leading edge of dendritic cells (DCs)

during interstitial migration. Absence of DOCK8 results in failure of DC migration to lymph nodes and in defective CD4+ T cell priming.[24] In that regard, the decreased presence of T cell recombination circles (TRECs) observed in the peripheral blood of DOCK8-deficient subjects may reflect impaired migration of mature thymocytes to the periphery.[25] In this context, it will be interesting to see whether infants with biallelic *DOCK8* mutations will be detected in the TREC-based SCID newborn screening program. In B cells, DOCK8 functions as an adaptor protein downstream of TLR9 and upstream of STAT3,[26] possibly explaining the interesting clinical overlap between these two forms of HIES. Moreover, Dock8-deficient mice do not form germinal centers and have a deficit of marginal zone B cells.[27] DOCK8 deficiency impacts long-term memory of B cells as well as of virus-specific CD8+ T cells,[26, 28–30] which might explain the susceptibility to bacterial and viral infections. In line with the mouse data, we also found a reduction in memory B cells and switched memory B cells in our patients.

Since B cell function is compromised in DOCK8 deficiency, Jabara et al. gave evidence for a mechanism of defective TLR9 signaling, interestingly involving DOCK8 and STAT3.[26] Such studies of B cell dysfunction have direct clinical relevance in the clinical management of DOCK8-deficient patients because they raise the question if Ig substitution is necessary and if vaccination is effective in these patients. The published reports on vaccination are contradictory and further investigations are needed. Al-Herz et al.[31] reported that antibody responses to vaccines were normal in DOCK8 deficiency, while Jabara et al.[26] reported that antibody responses to tetanus and other vaccines were attenuated in DOCK8-deficient patients. The “specific antibody responses” row of Table E1 adds some retrospective case report information to aid in studying the response to vaccinations.

In sum, we collected extensive clinical data on 82 patients of whom 64 have DOCK8 deficiency, 9 have PGM3 deficiency, and 9 are genetically unexplained. We also compared DOCK8 deficiency to STAT3 deficiency using statistical analysis. Our quantification of how common are the well-known symptoms of DOCK8 deficiency and our compilation of dozens of rare symptoms of DOCK8 deficiency should aid clinicians in recognizing and managing this life-threatening immunodeficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Karin R. Engelhardt, PhD[1,2,3], E. Michael Gertz, PhD[4], Sevgi Keles, MD[5,6,7,*], Alejandro A. Schäffer, PhD[4,*], Elena C. Sigmund, BSc[2], Cristina Glocker, PhD[2], Shiva Saghafi, MSc[8], Zahra Pourpak, MD, PhD[8], Ruben Ceja, MSc[5,7], Atfa Sassi, PhD[9], Laura E. Graham, BSc, MBChB[1], Michel J. Massaad, PhD[7], Fethi Mellouli, MD[10], Imen Ben-Mustapha, MD[9], Monia Khemiri, MD[11], Sara Sebnem Kilic, MD[12], Amos Etzioni, MD[13], Alexandra F. Freeman, MD[14], Jens Thiel, MD[2], Ilka Schulze, MD[2], Waleed Al-Herz, MD[15], Ayse Metin, MD, PhD[16], Özden Sanal, MD[17], Ilhan Tezcan, MD[17], Mehdi Yeganeh, MD[18], Tim Niehues, MD[19], Gregor Dueckers,

MD[19], Sebastian Weinspach, MD[20], Turkan Patiroglu, MD[21], Ekrem Unal, MD[22], Majed Dasouki, MD[23], Mustafa Yilmaz, MD[24], Ferah Genel, MD[25], Caner Aytekin, MD[26], Necil Kutukculer, MD[27], Ayper Somer, MD[28], Mehmet Kilic, MD[29], Ismail Reisli, MD[6], Yildiz Camcioglu, MD[30], Andrew R. Gennery, MD[3], Andrew J. Cant, MD[3], Alison Jones, MD[31], H. Bobby Gaspar, MD[31], Peter D. Arkwright, MD, DPhil[32], Maria C. Pietrogrande, MD[33], Zeina Baz, MD[34], Salem Al-Tamemi, MD[35], Vassilios Lougaris, MD[36], Gerard Lefranc, PhD[37], Andre Megarbane, MD, PhD[38], Jeannette Boutros, MD[39], Nermeen Galal, MD[39], Mohamed Bejaoui, MD[10], Mohamed-Ridha Barbouche, MD, PhD[9], Raif S. Geha, MD[7], Talal A. Chatila, MD, MSc[5,7], and Bodo Grimbacher, MD[1,2,#]

Affiliations

[1]Dept. of Immunology and Molecular Pathology, Royal Free Hospital and University College London, London, UK

[2]Center of Chronic Immunodeficiency (CCI), University Medical Center Freiburg and University of Freiburg, Germany

[3]Institute of Cellular Medicine, University of Newcastle upon Tyne, UK

[4]National Center for Biotechnology Information, National Institutes of Health, Department of Health and Human Services, Bethesda, USA

[5]Division of Immunology, Allergy and Rheumatology, Department of Pediatrics, David Geffen School of Medicine at the University of California at Los Angeles, Los Angeles, USA

[6]Konya Necmettin Erbakan University, Division of Pediatric Allergy and Immunology, Konya Turkey

[7]Division of Immunology, The Children's Hospital, Boston, USA

[8]Immmunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

[9]Laboratory of Immunology, Vaccinology, and Molecular Genetics, Pasteur Institute of Tunis and University of Tunis el Manar, Tunisia

[10]Department of Pediatrics, Bone Marrow Transplantation Center, Tunis, Tunisia

[11]Department of Pediatrics, Children's Hospital, Tunis, Tunisia

[12]Department of Pediatric Immunology, Faculty of Medicine, Uludag University, Bursa, Turkey

[13]Meyer's Children Hospital, Rambam Health Care Campus and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

[14]Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, USA

[15]Department of Pediatrics, Faculty of Medicine, Kuwait University and Allergy and Clinical Immunology Unit, Department of Pediatrics, Al-Sabah Hospital. Kuwait

[16]Pediatric Immunology Unit, SB Ankara Diskapi Children's Hospital, Ankara, Turkey

[17]Immunology Division, Hacettepe University, Children's Hospital, Ankara, Turkey

[18]Immunology Asthma and Allergy Research Institute, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

[19]HELIOS Klinikum Krefeld, Zentrum für Kinder- und Jugendmedizin, Krefeld, Germany

[20]Department of Pediatric Oncology, Hematology and Clinical Immunology, Center of Child and Adolescent Medicine, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

[21]Erciyes University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology and Immunology, Kayseri, Turkey

[22]Erciyes University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology and Oncology, Kayseri, Turkey

[23]Department of Pediatrics, University of Kansas Medical Center, Kansas City, USA

[24]Cukurova University, Adana, Turkey

[25]Behcet Uz State Hospital, Division of Pediatric Immunology, Izmir, Turkey

[26]Department of Pediatric Immunology, Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital, Ankara, Turkey

[27]Ege University Faculty of Medicine, Department of Pediatrics, Izmir, Turkey

[28]Division of Infectious Diseases and Immunology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

[29]Firat University, Elazig, Turkey

[30]Division of Pediatric Allergy-Immunology and Infectious Diseases, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

[31]Department of Immunology, Great Ormond Street Hospital, London, UK

[32]University of Manchester, Royal Manchester Children's Hospital, Manchester, UK

[33]Department of Pediatrics, University of Milan, Fondazione Policlinico IRCCS, Milan, Italy

[34]Department of Pediatrics, St George Hospital University Medical Center, Beirut, Lebanon

[35]Department of Pediatrics, Sultan Qaboos University, Muscat, Oman

[36]Pediatrics Clinic and Institute for Molecular Medicine A.Nocivelli. Department of Clinical and Experimental Sciences, University of Brescia, Spedali Civili di Brescia, Brescia, Italy

[37]University Montpellier 2 and CNRS Institute of Human Genetics, Montpellier, France

[38]Medical Genetics Unit, Saint Joseph University, Beirut, Lebanon

[39]Cairo University, Specialized Pediatric hospital, Primary Immunodeficiency Clinic, Cairo, Egypt

Acknowledgements

This study was supported by the German Federal Ministry of Education and Research (BMBF 01EO1303). The research was funded in part by the European Community's 7th Framework Programmes FP7/2007-2013 under grant agreement Health-F5-2008-223292 (Euro Gene Scan), by a Marie Curie Excellence Grant to B.G. (MEXT-CT-2006-042316, MC-PIAID), and by the National Institutes of Health grants 5R01AI065617 and 1R21AI087627 to T.A.C. This research was supported in part by a grant from the Scientific and Technological Research Council of Turkey (Tubitak, grant number 1059B191300622). This research was supported in part by the Intramural Research program of the National Institutes of Health, NLM and NIAID. Thanks to Judy Levin for administrative support.

ABBREVIATIONS

HIES	hyper-IgE syndrome
AR	autosomal recessive
AD	autosomal dominant
DOCK8	dedicator of cytokinesis 8
STAT3	signal transducer and activator of transcription 3
SNP	single nucleotide polymorphism
HSCT	hematopoietic stem cell transplantation
DHR-1	DOCK homology region-1
DHR-2	DOCK homology region-2
CNS	central nervous system
PML	progressive multifocal leukoencephalopathy
bp	base pairs
cDNA	complementary DNA
FACS	Fluorescence-activated cell sorting
TH17 cells	T-helper 17 cells
IgE	immunoglobulin E
GEF	guanine nucleotide exchange factor
PBMC	peripheral blood mononuclear cell

FCS	fetal calf serum
SVM	support vector machine
pts	patients

References

1. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, Lopez-Herrera G, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol. 2009; 124 1289-302.e4.
2. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, et al. Combined immunodeficiency associated with *DOCK8* mutations. N Engl J Med. 2009; 361:2046–2055. [PubMed: 19776401]
3. Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, et al. Hyper-IgE syndrome with recurrent infections--an autosomal dominant multisystem disorder. N Engl J Med. 1999; 340:692–702. [PubMed: 10053178]
4. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. *STAT3* mutations in the hyper-IgE syndrome. N Engl J Med. 2007; 357:1608–1619. [PubMed: 17881745]
5. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature. 2007; 448:1058–1062. [PubMed: 17676033]
6. Freeman, AF.; Davis, J.; Hsu, AP.; Holland, SM.; Puck, JM. Autosomal dominant hyper IgE syndrome. In: Pagon, RA.; Bird, TD.; Dolan, CR., et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993.
7. Sassi A, Lazaroski S, Wu G, Haslam SM, Fliegauf M, Mellouli F, et al. Hypomorphic homozygous utations in phosphoglycomutase 3 (*PGM3*) impair immunity and increase serum IgE levels. J Allergy Clin Immunol. 2014; 133:1410–1419. [PubMed: 24698316]
8. Zhang Y, Yu X, Ichikawa M, Lyons JJ, Datta S, Lamborn IT, et al. Autosomal recessive phosphoglycomutase 3 (*PGM3*) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive development. J Allergy Clin Immunol. 2014; 133:1400–1409. [PubMed: 24589341]
9. Stray-Pedersen A, Backe PH, Sorte HS, Mørkrid L, Chokshi NY, Erichsen HC, et al. *PGM3* mutations cause a congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia. Am J Hum Genet. 2014; 95:96–107. [PubMed: 24931394]
10. Grimbacher B, Schäffer AA, Holland SM, Davis J, Gallin JI, Malech HL, et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. Am J Hum Genet. 1999; 65:735–744. [PubMed: 10441580]
11. Bittner TC, Pannicke U, Renner ED, Notheis G, Hoffmann F, Belohradsky BH, et al. Successful long-term correction of autosomal recessive hyper-IgE syndrome due to DOCK8 deficiency by hematopoietic stem cell transplantation. Klin Padiatr. 2010; 222:351–355. [PubMed: 21058221]
12. McDonald DR, Massaad MJ, Johnston A, Keles S, Chatila T, Geha RS, et al. Successful engraftment of donor marrow after allogeneic hematopoietic cell transplantation in autosomal-recessive hyper-IgE syndrome caused by *dedicator of cytokinesis 8* deficiency. J Allergy Clin Immunol. 2010; 126 1304-5.e3.
13. Barlogis V, Galambrun C, Chambost H, Lamoureux-Toth S, Petit P, Stephan J-L, et al. Successful allogeneic hematopoietic stem cell transplantation for *DOCK8* deficiency. J Allergy Clin Immunol. 2011; 128 420-22.e2.
14. Gatz SA, Benninghoff U, Schütz C, Schulz A, Hönig M, Pannicke U, et al. Curative treatment of autosomal-recessive hyper-IgE syndrome by hematopoietic cell transplantation. Bone Marrow Transplant. 2011; 46:552–556. [PubMed: 20622910]

15. Metin A, Tavil B, Azık F, Azkur D, Ok-Bozkaya I, Kocabas C, et al. Successful bone marrow transplantation for DOCK8 deficient hyper IgE syndrome. Pediatr Transplant. 2012; 16:398–399. [PubMed: 22248482]
16. Woellner C, Gertz EM, Schäffer AA, Lagos M, Perro M, Glocker E-O, et al. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. J Allergy Clin Immunol. 2010; 125:424–432. [PubMed: 20159255]
17. Gennery AR, Flood TJ, Abinum M, Cant AJ. Bone marrow transplantation does not correct the hyper IgE syndrome. Bone Marrow Trasplant. 2000; 25:1303–1305.
18. Goussetis E, Peristeri I, Kitra V, Traeger-Synodinos J, Theodosaki M, Psarra K, et al. Successful long-term immunologic reconstitution by allogeneic hematopoietic stem cell transplantation cures patients with autosomal dominant hyper-IgE syndrome. J Allergy Clin Immunol. 2010; 126:392–394. [PubMed: 20584545]
19. Chu EY, Freeman AF, Jing H, Cowen EW, Davis J, Su HC, et al. Cutaneous manifestations of DOCK8 deficiency syndrome. Arch Dermatol. 2012; 148:79–84. [PubMed: 21931011]
20. Sanal O, Jing H, Ozgur T, Ayvaz D, Strauss-Albee DM, Ersoy-Evans S, et al. Additional diverse findings expand the clinical presentation of DOCK8 deficiency. J Clin Immunol. 2012; 32:698–708. [PubMed: 22476911]
21. Pai S-Y, de Boer H, Massaad MJ, Chatila TA, Keles S, et al. Flow cytometry diagnosis of dedicator of cytokinesis 8 (DOCK8) deficiency. J Allergy Clin Immunol. 2014; 134 221-223.e7.
22. Jing H, Zhang Q, Zhang Y, Hill BJ, Dove CG, Gelfand EW, et al. Somatic reversion in dedicator of cytokinesis 8 immunodeficiency modulates disease phenotype. JAllergy Clin Immunol. 2014; 133:1667–1675. [PubMed: 24797421]
23. Vowells SJ, Fleisher TA, Sekhsaria S, Alling DW, Maguire TE, Malech HL. Genotype-dependent variability in flow cytometric evaluation of reduced nicotinamide adenine dinucleotide phosphate oxidase function in patients with chronic granulomatous disease. J Pediatr. 1996; 128:104–107. [PubMed: 8551399]
24. Harada Y, Tanaka Y, Terasawa M, Pieczyk M, Habiro K, Katakai T, et al. DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. Blood. 2012; 119:4451–4461. [PubMed: 22461490]
25. Dasouki M, Okonkwo KC, Ray A, Folmsbeel CK, Gonzalez D, Keles S, et al. Deficient T cell receptor excision circles (TRECs) in autosomal recessive hyper IgE syndrome caused by DOCK8 mutation; implications for pathogenesis and potential detection by newborn screening. Clin Immunol. 2011; 141:128–132. [PubMed: 21763205]
26. Jabara HH, McDonald DR, Janssen E, Massaad MJ, Ramesh N, Borzutzky A, et al. DOCK8 functions as an adaptor that links TLR-MyD88 signaling to B cell activation. Nat Immunol. 2012; 13:612–620. [PubMed: 22581261]
27. Randall KL, Lambe T, Johnson AL, Treanor B, Kucharska E, Domaschenz H, et al. *Dock8* mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. Nat Immunol. 2009; 10:1283–1291. [PubMed: 19898472]
28. Janssen E, Tsitsikov E, Al-Herz W, Lefranc G, Megarbane A, Dasouki M, et al. Flow cytometry biomarkers distinguish DOCK8 deficiency from severe atopic dermatitis. Clin Immunol. 2014; 150:220–224. [PubMed: 24440647]
29. Lambe T, Crawford G, Johnson AL, Crockford TL, Bouriez-Jones T, Smyth AM, et al. DOCK8 is essential for T-cell Survival and the maintenance of $CD8^+$ T-cell memory. Eur J Immunol. 2011; 41:3423–3435. [PubMed: 21969276]
30. Randall KL, Chan SS-Y, Ma CS, Fung I, Mei Y, Yabas M, et al. DOCK8 deficiency impairs CD8 T cell survival and function in humans and mice. J Exp Med. 2011; 288:2305–2320. [PubMed: 22006977]
31. Al-Herz W, Ragupathy R, Massaad MJ, Al-Attiyah R, Nanda A, Engelhardt KR, et al. Clinical, immunologic and genetic profiles of DOCK8-deficient patients in Kuwait. Clin Immunol. 2012; 143:266–272. [PubMed: 22534316]
32. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WCJ, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr. 1997; 130:388–393. [PubMed: 9063413]

33. “Eosinophilic disorders” Merck & Co., Inc. www.merckmanuals.com
34. The Harriet Lane handbook: a manual for pediatric house officers. Philadelphia: Elsevier Mosby; 2009. p. 18

CLINICAL IMPLICATIONS

The detailed clinical description of DOCK8 deficiency may help in the early diagnosis of DOCK8 deficiency. As this disease has a bad prognosis, patients diagnosed with DOCK8 deficiency may be evaluated for bone marrow transplantation.

CAPSULE SUMMARY

Sixty-four patients with DOCK8 deficiency, which is a severe form of combined immune deficiency, were phenotypically characterized. Clinically distinguishing features are put forward to help distinguish between DOCK8-deficient recessive HIES and STAT3-deficient dominant HIES.

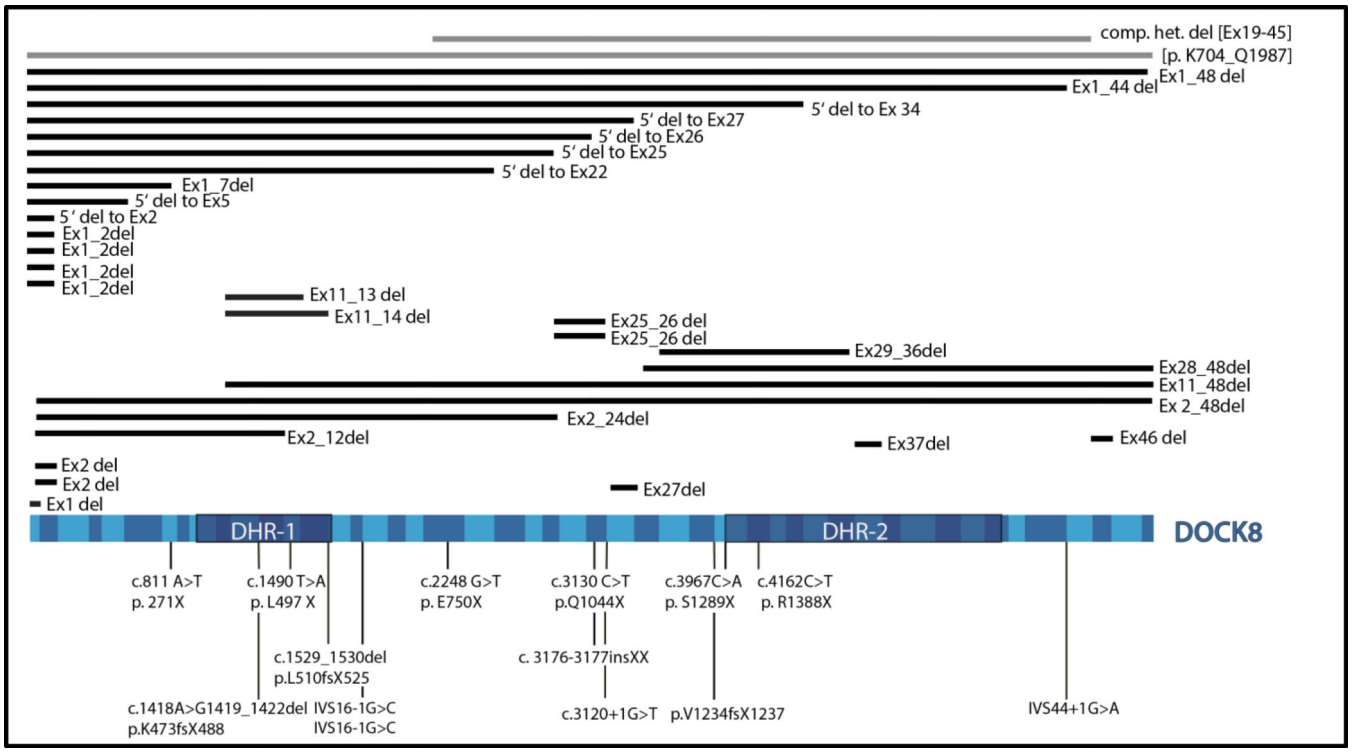

Figure 1. Schematic representation showing *DOCK8* mutations in 44 of 46 families

Mutations in two families (one with a retained intronic sequence and one without *DOCK8*-specific mRNA expression despite wild-type exonic sequences) are not shown. Straight lines depict multi-exon deletions with undetermined breakpoints (gray: heterozygous). With the exception of the compound heterozygous multi-exon deletion, all mutations were homozygous. DHR; DOCK homology region.

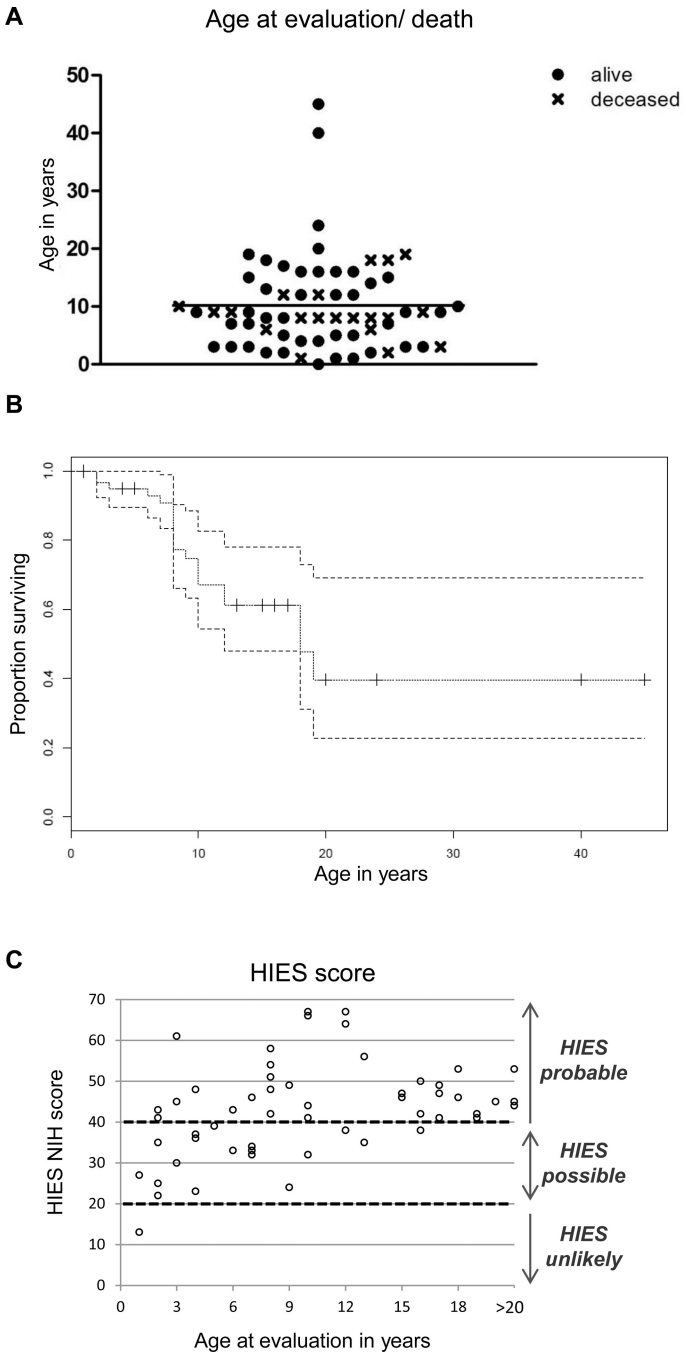

Figure 2. Characteristics of DOCK8-deficient patients

(A) Age at evaluation, depicted as black dots, and age at death, represented by black crosses, (B) Kaplan-Meier survival curve with the 95% confidence interval indicated by dotted lines, and (C) NIH HIES score. All 57 patients with information about the HIES score were included.

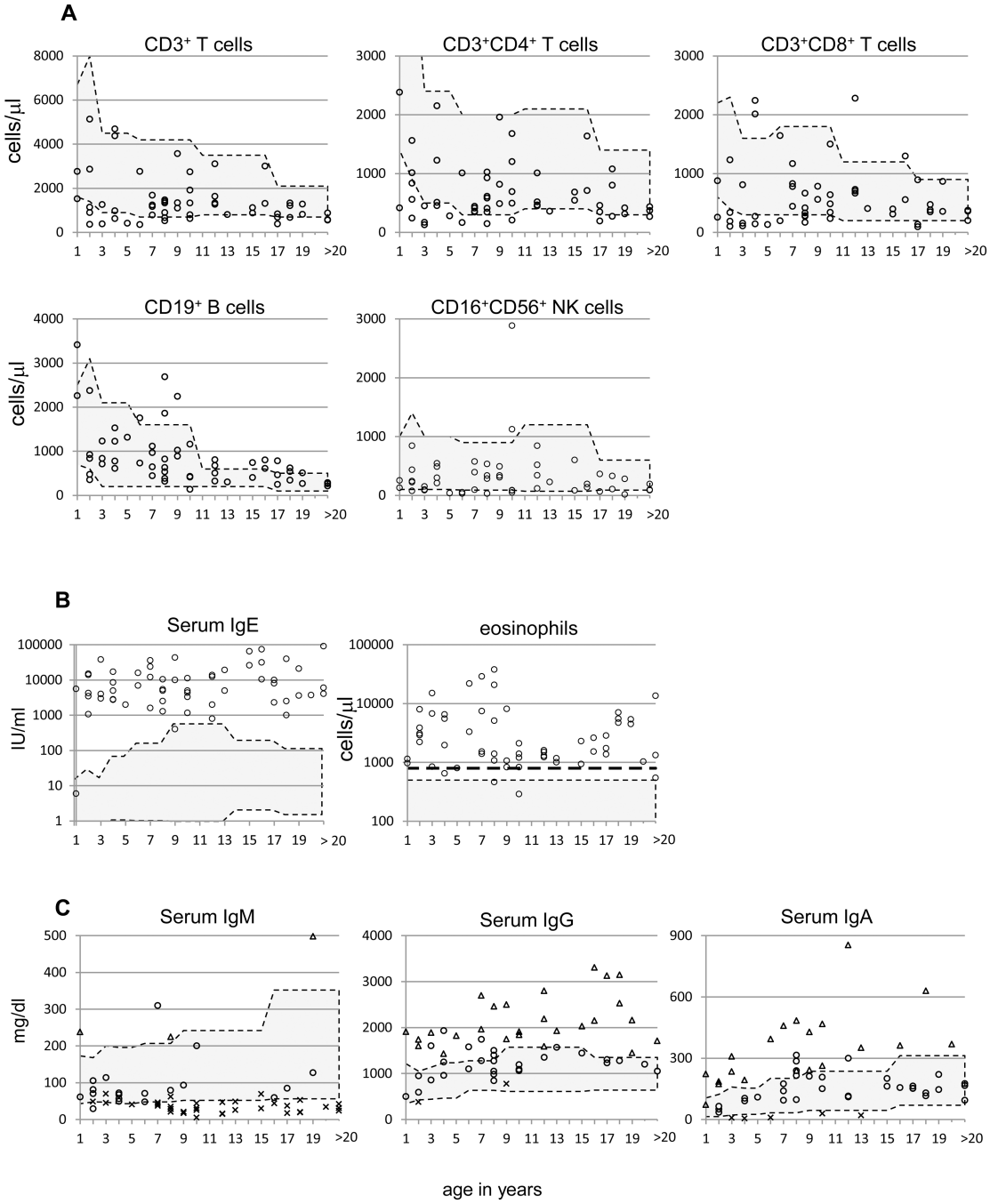

Figure 3. Eosinophil and lymphocyte counts and serum immunoglobulin levels in DOCK8-deficient patients

(A) Counts of several lymphocyte subtypes in blood; gray areas represent age-adjusted normal ranges.[32] (B) IgE level, and eosinophil counts (normal: 100–500 cells/µl[33]; highly elevated: above 800 cells/µl[10]; the heavy dotted black line marks 800 cells/µl). (C) Patient's IgM, IgA and IgG; gray areas represent published normal ranges[34]. Triangles depict values that were high, circles are values that were normal and crosses are values that were low when laboratories' own normal ranges were used.

Table 1

Skin and lung disease, atopy, and autoimmunity

	# of patients	% of patients
Skin disease		
Newborn rash	16/46	35%
Eczema	59/61	97%
• severe	42/61	69%
• moderate	8/61	13%
• mild	6/61	10%
• severity not determined	3/61	5%
Abscesses	34/57	60%
• "cold"	9/57	16%
• with inflammation *(of these 2 have both, abscesses with and without inflammation)*	15/57	26%
• inflammation status not determined	12/57	21%
Cutaneous viral infections	41/60	68%
• *Herpes simplex* virus*	22/58	38%
• *Varicella zoster* virus	11/58	19%
• *Human papilloma* virus	16/55	29%
• *Molluscum contagiosum* virus	21/56	38%
Mucocutaneous candidiasis	37/58	64%
Lung disease/abnormalities		
Pneumonia	54/60	90%
• 1	5/60	8%
• 2–3	12/60	20%
• >3 (of whom >5)	34/60 (21/60)	57% (35%)
• Number of episodes unspecified	3/60	5%
Other LRTI (bronchitis, chronic cough)	12/59	20%
Bronchiectasis	20/54	37%
Pneumatoceles	2/54	4%
Other lung changes	5/54	9%
• Chronic changes	3/54	6%
• Allergic bronchopulmonary aspergillosis	1/54	2%
• Interlobular septal thickening	1/54	2%

	# of patients	% of patients
Atopay		
Eczema**		
Asthma	17/56	30%
Allergies	41/56	73%
• Food	36/56	64%
• Environmental***	18/56	32%
(of these 16 have both, food and environmental allergies)		
• Drugs	4/56	7%
• Latex	3/56	5%
• unspecified	2/56	4%
Autoimmunity		
Autoimmune hemolytic anemia	2/58	3%

* for 7 patients the type of HSV infection was not specified, but was assumed to be skin;

** see above under skin disease;

*** environmental allergens include: animal hair and dander, dust mites, grass, inhalation allergens, and fungi;

LRTI, lower respiratory tract infection.

Table 2

Microbiological infections in DOCK8 deficiency

Infections	x/y patients	% of patients	manifestation
Bacterial	43/51	84%	
Gram +ve cocci	***41/51***	***80%***	
• *Staphylococcus* spp.	33		
- *S. aureus*	25		Skin, mucosal, abscesses, eye, lung, otitis, septicemia
- *S. chromogenes*	1		Sepsis
- *S. epidermidis*	1		Skin
- *S. haemolyticus*	1		Abscess
• *Streptococcus* spp.	8		
- *S. pneumoniae*	5		Pneumonia, bacteraemia, meningitis, bronchial infection
- *S. pyogenes*	1		Wound culture
• *Enterococcus* spp.	4		Sepsis, wound culture, bacteraemia, pneumonia
Gram −ve cocci	**2/44**	**5%**	
• *Moraxella catarrhalis*	2		Bronchial infection
Gram +ve bacilli	**2/41**	**5%**	
• *Listeria monocytogenes*	1		Meningitis
• *Corynebacterium* spp.	1		Otitis
Gram −ve bacilli	**15/46**	**33%**	
• *Klebsiella* spp.	4		Pneumonia, bacteraemia, sepsis
• *Proteus mirabilis*	4		Skin, nasal smear, wound culture, otitis
• *E. coli*	4		Bacteraemia, otitis
• *Haemophilus influenza B*	3		Meningitis
• *Pseudomonas* spp.	4		Sepsis
• *Proteus vulgaris*	1		Otitis
• *Achromobacter* spp.	1		Otitis
• *Acinetobacter spp*.	1		Sepsis
Others	**4/51**	**8%**	
• *Mycobacterium tuberculosis*	2		Tuberculosis
• *Mycoplasma pneumoniae*	1		

Infections	x/y patients	% of patients	manifestation
Viral	46/59	78%	
Herpesviridae	**31/52**	**60%**	
• *Herpes simplex* virus	28		Skin infection, eczema herpeticum (2 pts), herpetic keratitis (4 pts), pneumonia (1 pt), encephalitis (1 pt), conjunctivitis (2 pts)
• *Varicella zoster* virus	11		Severe primary chickenpox, herpes zoster
• *Cytomegalovirus*	3		Retinitis, meningitis, pneumonia
• Epstein-Barr virus	2		pneumonia
Molluscum contagiosum	**21/56**	**38%**	Skin disease (mollusca)
Papovaviridae	**18/55**	**33%**	
• *Papilloma* virus	16		Warts, Heck's disease
• JC virus	2		Progressive multifocal leukoencephalopathy (PML)
Others	**4/49**	**8%**	Hepatitis caused by HAV, HBV or HCV; rotavirus enteritis
Fungal	40/57	70%	
• *Candida* spp.	39		Skin, nail (15 pts); oral, vaginal (15 pts); otitis (2 pts); systemic (5 pts)
• *Aspergillus* spp.	5		ABPA; lung; nasal and ear wound; sinusitis
• Dermatophyte	1		Tinea cruris
• *Cryptococcus* spp.	2		Meningitis; abscess
Parasitic	4/47	9%	
• *Entamoeba histolytica*	3		
• *Cryptosporidium*	1		

HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; ABPA: allergic bronchopulmonary aspergillosis. Denominators for numbers of DOCK8-deficient patients other than 58 are shown for those categories where data reporting is incomplete.

Table 3

Neurological complications, malignancies, and non-immune features in DOCK8-deficient patients.

	# of patients	% of patients
Neurological complications	20/55	36%
• Encephalitis	3	
• Meningitis	4	
• encephalopathy	3	
• Lymphoma	2	
• Vasculitis	3	
• Vascular aneurysm	1	
• Abscess	4	
• Brain infarct/stroke	3	
• Hemiparesis and diplegia	2	
Malignancies	5/62	8%
• Burkitt lymphoma	2	
• Squamous cell carcinoma	2	
• Primary non-Hodgkin lymphoma of the brain	1	
Non-immune features typically seen in AD-HIES		
Characteristic face	17/58	29%
• mild	12	
• present	3	
• unspecified	2	
Increased nose width	13/51	25%
• 1–2 SD interalar distance	10	
• >2 SD interalar distance	3	
Retained primary teeth	10/56	18%
• 2 teeth	3	
• 3 teeth	1	
• > 3 teeth	3	
• number unspecified	3	
High Palate	12/51	24%
Hyperflexibility	6/59	10%
Fractures upon minor trauma (1–2)	2/59	3%
Scoliosis	1/58	2%
Midline anomaly	1/51	2%

Table 4

Serum immunoglobulin levels and absolute lymphocyte subpopulation counts in DOCK8-deficient patients. When available, normal ranges for healthy controls were used as provided by the respective laboratories.

	Increased (Number of patients)	Normal (Number of patients)	Decreased (Number of patients)	Unknown (Number of patients)
Immunoglobulin serum levels				
IgE	61/62 (98%)	1/62 (2%)	0	2
IgM	3/58 (5%)	19/58 (33%)	36/58 (62%)	6
IgG	25/58 (43%)	31/58 (53%)	2/58 (3%)	6
IgA	20/58 (34%)	33/58 (57%)	5/58 (9%)	6
Absolute lymphocyte subpopulation counts				
WBC	17/53 (32%)	33/53 (62%)	3/53 (6%)	5
ALC	1/58 (2%)	45/58 (78%)	11/58 (19%)	6
B cells	14/55 (25%)	38/55 (69%)	3/55 (5%)	9
T cells	1/55 (2%)	39/55 (71%)	15/55 (27%)	9
CD4+	0/56 (0%)	40/56 (71%)	16/56 (29%)	8
CD8+	7/55 (13%)	32/55 (58%)	16/55 (29%)	9
NK cells	2/50 (4%)	35/50 (70%)	13/50 (26%)	13

Otherwise, published ranges were applied for comparison [references 29 and 31]. WBC, white blood cells; ALC, absolute lymphocyte count; NK cells, natural killer cells.