The extended clinical phenotype of 64 patients with dedicator of cytokinesis 8 deficiency

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Background: Mutations in dedicator of cytokinesis 8 *(DOCK8)* cause a combined immunodeficiency (CID) also classified as autosomal recessive (AR) hyper-IgE syndrome (HIES). Recognizing patients with CID/HIES is of clinical importance because of the difference in prognosis and management.
Objectives: We sought to 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 AR-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 those from 10 patients with AR-HIES without a *DOCK8* mutation and 64 patients with signal transducer and activator of transcription 3 *(STAT3)* mutations.
Results: DOCK8-deficient patients had median IgE levels of 5201 IU, high eosinophil levels of usually at least 800/μL (92% of patients), and low IgM levels (62%). About 20% of patients were lymphopenic, mainly because of low CD4$^+$ and CD8$^+$ T-cell counts. 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 5 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. (J Allergy Clin Immunol 2015;136:402-12.)

Dedicator of cytokinesis 8 (DOCK8) deficiency is an autosomal recessive (AR) 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 (AD) form of hyper-IgE syndrome (HIES) caused by signal transducer and activator of transcription 3 *(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 IgE levels, and hypereosinophilia. However, patients with *STAT3* mutations might have pneumatoceles, which are rarely seen in DOCK8-deficient patients. Mutations in *STAT3* are often associated with nonimmune symptoms involving dentition, bone, and connective tissue. In contrast, DOCK8-deficient patients present frequently with allergies, severe and refractory cutaneous viral infections, and sometimes neurological symptoms. However, not all patients demonstrate the full spectrum of this syndrome, especially in early childhood; therefore it can sometimes be difficult to

Abbreviations used	
AD:	Autosomal dominant
AR:	Autosomal recessive
CID:	Combined immunodeficiency
CNS:	Central nervous system
DOCK8:	Dedicator of cytokinesis 8
HIES:	Hyper-IgE syndrome
HSCT:	Hematopoietic stem cell transplantation
NIH:	National Institutes of Health
NK:	Natural killer
PGM3:	Phosphoglucomutase 3
PML:	Progressive multifocal leukoencephalopathy
STAT3:	Signal transducer and activator of transcription 3
STK4:	Seronine/threonine kinase 4
SVM:	Support vector machine
TYK2:	Tyrosine kinase 2

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 (see Fig E1 in this article's Online Repository at www.jacionline.org) and to establish diagnostic measures that help distinguish patients with HIES with a *DOCK8* mutation from other patients with a combined immunodeficiency (CID) and from those with a *STAT3* mutation, thus helping to guide clinicians in their workup of patients and recognition of this primary immune deficiency as early as possible to avoid diagnostic delay.

METHODS
Patients and control subjects

We enrolled a cohort of 82 patients from 60 families in a worldwide collaboration. All patients fulfilled the following inclusion criteria for this study: signed informed consent forms, strong clinical suspicion of AR-HIES according to the referring immunologist, and available samples of genomic DNA or RNA. Of the 82 patients, 40 were male, and 42 were female. Forty-seven of the patients were also described by Aydin et al in a separate study (accepted for publication in the *Journal of Clinical Immunology*). 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 in this article's Online Repository at www.jacionline.org. The laboratory measurements of each DOCK8-deficient patient are shown in Table E2 in this article's Online Repository at www.jacionline.org.

All patients and control subjects or their parents or legal guardians provided written consent for the conducted studies, according to local ethics committee requirements. The study was approved by the ethics committee at University College London (protocols #04/Q0501/119_AM03 for affected patients and #07/H0720/182 for family members).

Genotyping and genetic linkage analysis

For many of the patients described here, microsatellite or single nucleotide polymorphism marker genotyping was performed, as described in the Methods section in this article's Online Repository at www.jacionline.org or as previously reported.[1]

PCR and sequence analysis

Genomic DNA and RNA of control subjects and patients were isolated from either whole blood or PBMCs. RNA was isolated with the RNeasy Kit

FIG 1. Schematic representation showing *DOCK8* mutations in 44 of 46 families. Mutations in 2 families (1 with a retained intronic sequence and 1 without *DOCK8*-specific mRNA expression despite wild-type exonic sequences) are not shown. *Straight lines* depict multiexon deletions with undetermined breakpoints (*gray*, heterozygous). With the exception of the compound heterozygous multiexon deletion, all mutations were homozygous. *DHR*, DOCK homology region.

(Qiagen, Hilden, Germany), according to the manufacturer's instructions. RNA was reverse transcribed with Omniscript reverse transcriptase (Qiagen). Coding genomic sequences and cDNA of *DOCK8* were amplified and purified by using the QIAquick PCR purification kit (Qiagen). Primer sequences are available on request. Purified PCR products were sequenced with the ABI PRISM BigDye Terminator Cycle Ready Reaction Kit V3.1 (Applied Biosystems, Foster City, Calif) by using the PCR primers as sequencing primers. Sequencing was performed on a 3130xl Applied Biosystems Genetic Analyzer, and data were analyzed with DNA Sequencing Analysis software version 5.2 (Applied Biosystems) and Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, Mich).

Statistical analysis

We investigated the significance of each of 20 features on the National Institutes of Health (NIH) score sheet by using logistic regression. We also used the machine-learning technique of support vector machines (SVMs) to reduce the number of features and produce a linear classifier that best distinguished this cohort of patients with DOCK8 deficiency from a previously published cohort of STAT3-deficient patients (see the Methods section in this article's Online Repository).

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

RESULTS
Identification of DOCK8 deficiency

Of the 82 subjects studied in 60 families, we diagnosed DOCK8 deficiency in 64 patients from 50 families (see Fig E1). For 60 patients from 46 unrelated families, a homozygous or compound heterozygous mutation was identified in *DOCK8* (Fig 1 and see Table E3 in this article's Online Repository at

www.jacionline.org), for a total of 40 distinct mutations. For 4 patients from 4 families (ARH018, ARH019, ARH006, and ARH007), the *DOCK8* mutation could not be identified by means of sequencing because of the unavailability of cDNA or additional genomic DNA. We summarize the evidence for DOCK8 deficiency in each of these 4 families in the Results section in this article's Online Repository at www.jacionline.org.

Mutations in *DOCK8*

Of the mutations identified in this cohort, 14 distinct mutations in 21 patients from 14 families were previously reported.[1] Any families appearing in both the study by Engelhardt et al[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 article, including 2 previously reported patients[1] whose *DOCK8* mutation detection was completed as part of this study.

Thirty-three (72%) of 46 families had insertions or deletions (indels): 1 homozygous 2-bp insertion; 1 homozygous 2-bp deletion; 6 homozygous single-exon deletions; 24 homozygous multiexon deletions spanning at least 2 exons to as much as nearly the whole gene, including neighboring genes; and 1 compound heterozygous multiexon deletion with an overlap of 27 deleted exons (Fig 1 and see 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 frameshift 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, 1 abrogates gene transcription, and 37 result in an mRNA that, if translated at all, would lead to a severely truncated DOCK8 protein. Only 2 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 2 DOCK homology region domains of DOCK8 (Fig 1).

Affected patients identified as unlikely to have DOCK8 deficiency

We excluded 14 patients from 8 consanguineous families from further *DOCK8* mutation detection after homozygosity mapping with microsatellite or single nucleotide polymorphism markers showed that they were heterozygous in a genetic interval including *DOCK8* (see the Methods and Results sections in this article's Online Repository). Some families also had other candidate loci excluded (see the Results section in this article's Online Repository). We did not investigate the possibility of compound heterozygous mutations in these patients because of parental consanguinity. Of these 14 patients, homozygous mutations in phosphoglucomutase 3 *(PGM3)* were subsequently found in 9 patients from 3 families[7]; 2 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 4 affected patients from 2 families did not have DOCK8 deficiency. One patient was sequenced from each of these 2 families. Neither person had exonic mutations or mutations in flanking splice sites. For both patients, *DOCK8* mRNA was expressed normally.

Clinical phenotype of DOCK8 deficiency

In our cohort of 64 DOCK8-deficient patients, 30 were male, and 34 were female. Of the 50 families with DOCK8 deficiency, 40 were consanguineous, and 10 were not known to be consanguineous. Among the 10 families without DOCK8 deficiency, 6 of 10 are also consanguineous (see Table E1), and therefore our results are primarily, although 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 the last evaluation. Thirty-nine (61%) patients were in their first decade of life, 21 (33%) were in their second decade of life, 2 were in their third decade of life, and 2 were in their fifth decade of life (Fig 2, A, and see Table E1). The 2 eldest patients are brothers (family ARH010) with a *DOCK8* splice site mutation, allowing for some residual protein expression.

Clinical data were not complete for all of the patients because of the loss of patients during follow-up and lack of proper documentation. For example, mortality data were only available for 58 of the 64 patients. The mortality rate in our cohort was 34% (20/58 patients), with death occurring at a mean age of 9 years and 3 months (range, 1.5-19 years); 14 patients died in the first and 6 in the second decades of life (Fig 2, A). 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
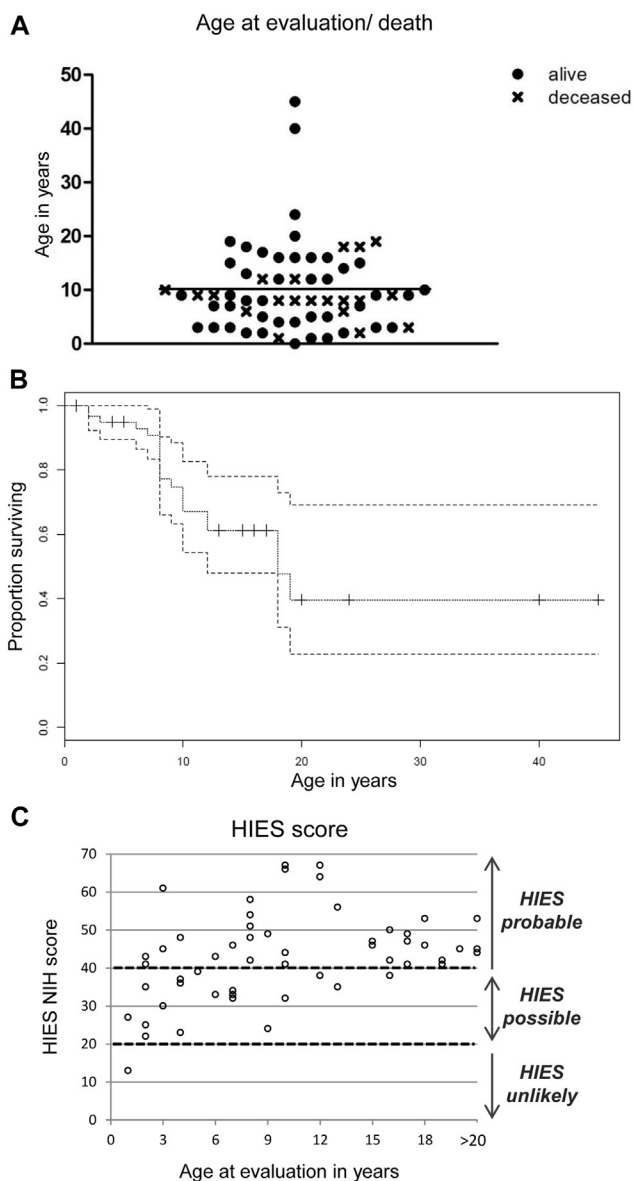
FIG 2. Characteristics of DOCK8-deficient patients. **A,** Age at evaluation is represented by *black dots*, and age at death is represented by *black crosses*. **B,** Kaplan-Meier survival curve, with the 95% CI indicated by *dotted lines*. **C,** NIH HIES score. All 57 patients with information about HIES scores were included.

(1 patient), and JC virus–negative progressive multifocal leukoencephalopathy (PML; 1 patient; see Table E1). Survival by the age of 10 years was 67% (95% CI, 54% to 83%), but by the age of 18 years, it decreased to 48% (95% CI, 31% to 73%; Fig 2, B).

Fifty-seven of 64 patients were evaluated with the NIH HIES scoring system,[10] and 46 of 57 of the score sheets were completed; 31 (67%) of 46 scored at least 40 points (highest score, 67 points), indicating that the diagnosis of HIES is probable, and 14 (30%) scored between 20 and 40 points, suggesting HIES is possible (Fig 2, C). Only 1 DOCK8-deficient patient had a low score of 13; he was the healthy 6-month-old brother of a patient and was given a diagnosis of DOCK8 deficiency based on sequencing only because of his sibling's diagnosis.

TABLE I. Skin and lung disease, atopy, and autoimmunity

	No. of patients	Percentage 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 (>5)	34/60 (21/60)	57% (35%)
No. of episodes unspecified	3/60	5%
Other LRTI (bronchitis and 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%
Atopy		
Eczema†		
Asthma	17/56	30%
Allergies	41/56	73%
Food	36/56	64%
Environmental‡ (of these 16 have both food and environmental allergies)	18/56	32%
Drugs	4/56	7%
Latex	3/56	5%
Unspecified	2/56	4%
Autoimmunity		
Autoimmune hemolytic anemia	2/58	3%

LRTI, Lower respiratory tract infection.

*For 7 patients, the type of herpes simplex virus 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.

All but 2 (59/61 patients) patients had eczema, and 16 (35%) patients presented with a newborn rash (Table I). Skin abscesses were common (34/57 [60%] patients). Three patients had abscesses in organs, such as the liver, kidney, lung, and brain. In 1 patient *Staphylococcus aureus* was isolated from a renal abscess, and in another patient a brain abscess was positive for *Aspergillus* species.

Mucocutaneous infections with *Candida* species (37/58 [64%] patients) and viruses (41/60 [68%] patients) were common. Severe and refractory skin infections with herpes simplex virus (22/58 [38%] patients) and varicella-zoster virus (11/58 [19%] patients), molluscum contagiosum virus (21/56 [38%] patients), or human papilloma virus (16/55 [29%] patients) were frequent findings (Table I). Noncutaneous viral infections included the fatal JC virus–associated PML in 2 patients; pneumonia, meningitis, encephalitis, retinitis, keratitis, and/or conjunctivitis caused by herpes family viruses in 9 patients; rotavirus enteritis in 1 patient; and viral hepatitis (caused by HAV, HBV, and HCV, respectively) in 3 patients (Table II). Two patients had systemic *Candida* species infections, of whom 1 had pneumonia and 1 had sepsis. Lung colonization, sinusitis, or chronic infection with the fungus *Aspergillus* species occurred in 3 patients, and 1 other patient was given a diagnosis of allergic bronchopulmonary aspergillosis. Other fungal infections were rare: among them, 1 patient presented with tinea cruris and 2 presented with *Cryptococcus neoformans* infection (1 central nervous system [CNS] infection and 1 in a skin abscess). Three Turkish patients had infections with the parasite *Entamoeba histolytica*, and in 1 patient the protozoan parasite *Cryptosporidium* species was found. Eighty-four percent (43/51) of patients had infections with bacteria, mainly with gram-positive cocci (41/51 [80%] patients), 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 II).

Upper and/or lower respiratory tract infections occurred in all but 1 (59/60) patient (Table I). Ninety percent (54/60) of patients had at least 1 episode of pneumonia, with 35% (21/60) having had more than 5 such episodes. Infections could result in abnormalities of the lung; 20 patients had bronchiectasis, and 2 had pneumatoceles (see Table E1 and Fig E2 in this article's Online Repository at www.jacionline.org). Seventeen (30%) of 56 patients presented with asthma, which was sometimes linked to allergies.

Allergies are another feature of DOCK8 deficiency, with 73% (41/56) of patients affected, mostly by food allergies (36 patients, Table I). Eighteen patients reacted to environmental and inhaled allergens, 3 to latex, and 4 to drugs. Poor growth and failure to thrive were present in 59% (32/54) of patients (see 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 patients), CNS lymphoma (2 patients), JC virus–associated PML (2 patients), and non-JC viral encephalopathy (1 patient, Table III). In total, 20 patients had CNS involvement, including CNS vasculitis (3 patients), a vascular aneurysm (1 patient), meningitis (4 patients), brain abscesses (4 patients), or a brain infarct/stroke (3 patients). Apart from the 2 patients with CNS lymphoma (Burkitt and non-Hodgkin lymphoma), 1 other patients had a retropharyngeal Burkitt lymphoma, and 2 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 III). Rare or unusual features observed in the cohort are listed in Table E4 in this article's Online Repository at www.jacionline.org.

DOCK8-deficient patients had a median IgE level of approximately 5,201 IU. Nearly all patients (54/59 [92%] patients) presented with hypereosinophilia that was characterized

TABLE II. Microbiological infections in patients with DOCK8 deficiency

Infections	x/y Patients	Percentage of patients	Manifestation
Bacterial	43/51	84%	
Gram-positive cocci	41/51	80%	
Staphylococcus species	33		
S aureus	25		Skin, mucosal, abscesses, eye, lung, otitis, septicemia
S chromogenes	1		Sepsis
S epidermidis	1		Skin
S haemolyticus	1		Abscess
Streptococcus species	8		
S pneumoniae	5		Pneumonia, bacteremia, meningitis, bronchial infection
S pyogenes	1		Wound culture
Enterococcus species	4		Sepsis, wound culture, bacteremia, pneumonia
Gram-positive cocci	2/44	5%	
Moraxella catarrhalis	2		Bronchial infection
Gram-positive bacilli	2/41	5%	
Listeria monocytogenes	1		Meningitis
Corynebacterium species	1		Otitis
Gram-negative bacilli	15/46	33%	
Klebsiella species	4		Pneumonia, bacteremia, sepsis
Proteus mirabilis	4		Skin, nasal smear, wound culture, otitis
Escherichia coli	4		Bacteremia, otitis
Haemophilus influenza B	3		Meningitis
Pseudomonas species	4		Sepsis
Proteus vulgaris	1		Otitis
Achromobacter species	1		Otitis
Acinetobacter species	1		Sepsis
Others	4/51	8%	
Mycobacterium tuberculosis	2		Tuberculosis
Mycoplasma pneumoniae	1		
Viral	46/59	78%	
Herpesviridae	31/52	60%	
Herpes simplex virus	28		Skin infection, eczema herpeticum (2 patients), herpetic keratitis (4 patients), pneumonia (1 patient), encephalitis (1 patient), conjunctivitis (2 patients)
Varicella zoster virus	11		Severe primary chickenpox, herpes zoster
Cytomegalovirus	3		Retinitis, meningitis, pneumonia
EBV	2		pneumonia
Molluscum contagiosum	21/56	38%	Skin disease (mollusca)
Papovaviridae	18/55	33%	
Papilloma virus	16		Warts, Heck's disease
JC virus	2		PML
Others	4/49	8%	Hepatitis caused by HAV, HBV or HCV; rotavirus enteritis
Fungal	40/57	70%	
Candida species	39		Skin, nail (15 pts); oral, vaginal (15 pts); otitis (2 pts); systemic (5 pts)
Aspergillus species	5		ABPA; lung; nasal and ear wound; sinusitis
Dermatophyte	1		Tinea cruris
Cryptococcus species	2		Meningitis; abscess
Parasitic	4/47	9%	
Entamoeba histolytica	3		
Cryptosporidium species	1		

Denominators for numbers of DOCK8-deficient patients other than 64 are shown for those categories, where data reporting is incomplete.

ABPA, Allergic bronchopulmonary aspergillosis; *HAV*, hepatitis A virus; *HBV*, hepatitis B virus; *HCV*, hepatitis C virus.

by increased levels of greater than 800 cells/μL (range, 245-37,880 cells/μL; Fig 3, B).[11-13] Total numbers of lymphocytes were normal in 45 of 58 (78%) patients, despite an increased white blood cell count in 17 (32%) of 53 patients. Nineteen percent (11/58) of patients were lymphopenic, which mainly affected absolute T-cell counts (Fig 3, A, and Table IV[14,15]). Within the T-cell compartment, low absolute levels were detected in CD4$^+$ and CD8$^+$ T cells (16/56 [29%] patients and 16/55 [29%] patients, respectively, of whom 9 patients had low levels of both T-cell subtypes), but only CD8$^+$ T-cell counts were increased in 7 (13%) of 55 patients. One patient had highly increased natural killer (NK) cell counts (Fig 3, B), which were not due to a general increase in leukocyte counts. Apart from the symptom-free, DOCK8-deficient, 6-month-old infant, all patients with reported serum immunoglobulin levels had increased serum IgE levels, ranging from 400 to 90,910 IU/mL (average, 12,893 IU/mL; median, 5,201 IU/mL; Fig 3, B, and Table IV). Twenty-four (39%) of 62 patients had levels of more

TABLE III. Neurological complications, malignancies, and nonimmune features in DOCK8-deficient patients

	No. of patients	Percentage 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	
Nonimmune features typically seen in patients with 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	
No. unspecified	3	
High palate	12/51	24%
Hyperflexibility	6/59	10%
Fractures on minor trauma (1-2)	2/59	3%
Scoliosis	1/58	2%
Midline anomaly	1/51	2%

than 10,000 IU/mL. In the majority of patients, serum IgM levels were low (36/58 [62%] patients; Fig 3, C). Low or absent specific antibody responses to recall antigens, such as pneumococcus, diphtheria, tetanus, and *Candida* species were documented in 16 (52%) of 31 patients, and low isohemagglutinin titers were documented in 10 (32%) of 31 patients (see Table E1).

In 4 patients from 1 family investigated, cytotoxic T-cell cytotoxicity and degranulation were normal (see Fig E3 in this article's Online Repository at www.jacionline.org), as was NK cell degranulation (see Fig E3). In 1 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-cell numbers, T-cell numbers, or both. There was a reduction in memory B-cell numbers and switched memory B-cell numbers down to near absence (see Table E2). T-cell memory was more variable, with either normal or decreased levels of CD45RO$^+$ memory T cells (see Table E2). In 1 patient CD8$^+$ naive T-cell numbers were higher than the corresponding numbers of memory cells (see Table E2).

Statistical analysis

We performed logistic regression (see Table E5 in this article's Online Repository at www.jacionline.org) and SVM analysis to select 5 features and create a linear classifier that attempts to distinguish DOCK8-deficient patients from STAT3-deficient patients (see the Methods and Results sections in this article's Online Repository). The 5 features chosen were lung abnormalities, eosinophilia, upper respiratory tract infections, retained primary teeth, and fractures with minimal trauma; the new SVM scoring system is shown in Table E6 in this article's Online Repository at www.jacionline.org.

The leave-one-out error rate (see the Methods section in this article's Online Repository) for the chosen set was 11.1%, with sensitivity for predicting a *DOCK8* mutation of 91.4% and specificity of 87.5%. By using the Wilcoxon rank sum test, the generated linear classifier is significantly predictive of a *DOCK8* mutation (2-sided $P = 3.6 \times 10^{-13}$). However, it should be emphasized 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 in 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 patients with DOCK8 deficiency. Early diagnosis of DOCK8 deficiency is important to facilitate an adequate treatment, such as hematopoietic stem cell transplantation (HSCT).[16-20]

DOCK8 deficiency has a high mortality at a young age, with more frequent severe infections and malignancy, and therefore HSCT should be considered. In contrast, conflicting results have been reported for HSCT as an effective treatment for AD-HIES because of *STAT3* mutations, the most common cause of HIES.[4,21] One patient with AD-HIES had a relapse of HIES symptoms 4 years after transplantation[22]; however, long-term follow up of this patient revealed no further infectious damage (unpublished data). Two other STAT3-deficient patients who underwent transplantation were considered cured 10 and 14 years later, respectively.[23] Because of its risks, HSCT would be considered only for STAT3 deficiency with severe complications, such as lymphoproliferative disease, whereas in patients with DOCK8 deficiency, HSCT will probably be considered in the majority of patients. Because HSCT is best done as early as possible, early identification of patients with HIES presenting with characteristics of 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.[24,25] Some of these rare symptoms (gastrointestinal tract problems, sclerosing cholangitis, and CNS lymphoma) have also been reported in singleton patients by Sanal et al,[25] suggesting that they might be associated with the lack of DOCK8. However, because most of the patients are born to consanguineous parents (40/50 families), additional homozygous defects might be present. We also have to caution that clinical findings very specific to STAT3 deficiency, such as pneumatoceles, can also occur in DOCK8-deficient patients (see Fig E2). Our study included some nonconsanguineous patients (10/50 families with DOCK8 deficiency and 4/10 families without), but the frequencies of
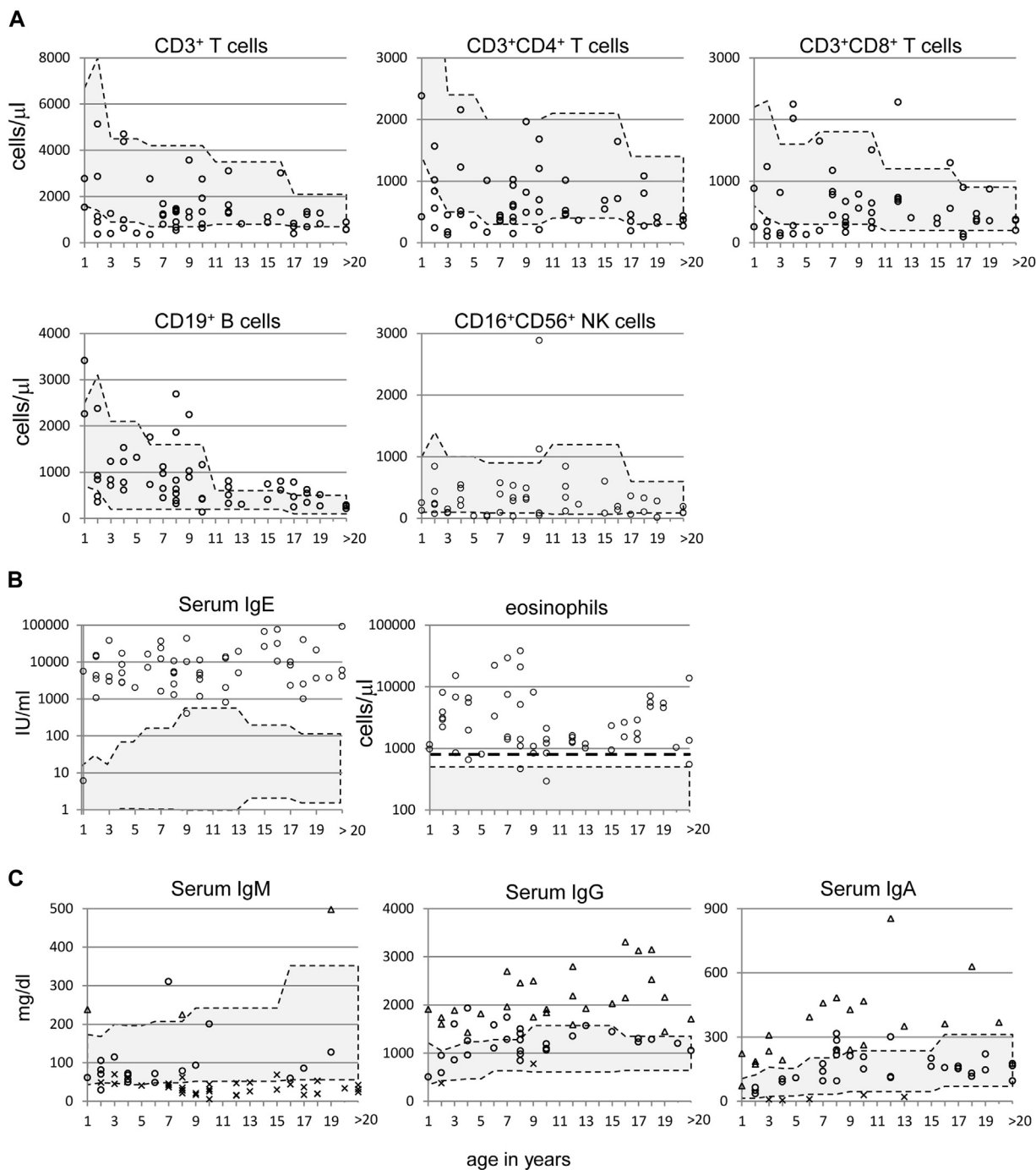
FIG 3. Eosinophil and lymphocyte counts and serum immunoglobulin levels in DOCK8-deficient patients.
A, Counts of several lymphocyte subtypes in blood. *Gray areas* represent age-adjusted normal ranges.[11]
B, IgE levels and eosinophil counts (normal, 100-500 cells/μL[12]; highly increased, >800 cells/μL[10]). *Heavy dotted black line*, 800 cells/μL. **C,** Patients' IgM, IgA, and IgG levels. *Gray areas* represent published normal ranges.[13] *Triangles* depict high values, *circles* depict normal values, and *crosses* depict low values, according to the laboratories' own normal ranges.

various symptoms of DOCK8 deficiency could be significantly different in a sample with a lower rate of consanguineous parents.

In the 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 1 compound heterozygous patient carrying 2 overlapping multiexon deletions. Twenty-five of these mutations have not been previously reported. Although the majority of mutations in our cohort are insertions and deletions, there are nonsense and splice junction point mutations. We did not find any missense mutations. To date, only 2 missense mutations in DOCK8 have been described:

TABLE IV. Serum immunoglobulin levels and absolute lymphocyte subpopulation counts in DOCK8-deficient patients

	Increased (no. of patients)	Normal (no. of patients)	Decreased (no. of patients)	Unknown (no. 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$^+$ cells	0/56 (0%)	40/56 (71%)	16/56 (29%)	8
CD8$^+$ cells	7/55 (13%)	32/55 (58%)	16/55 (29%)	9
NK cells	2/50 (4%)	35/50 (70%)	13/50 (26%)	13

When available, normal ranges for healthy control subjects were used as provided by the respective laboratories. Otherwise, published ranges were applied for comparison.[14,15]

ALC, Absolute lymphocyte count; *WBC*, white blood cells.

p.C1447R and p. V797M.[25] The *DOCK8* mutation spectrum is quite different from that of *STAT3*, the latter being characterized by dominant negative point mutations in the 2 important functional domains of STAT3.[4-6] Differences in the mutation spectra of the 2 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 the presence of which is often initially identified by using fluorescence-activated cell sorting or Western blotting 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 through flow cytometry[26] or immunoblotting.

At the Center for Chronic Immunodeficiency, Freiburg, Germany, DOCK8 deficiency is typically diagnosed by means of protein analysis through fluorescence-activated cell sorting or Western blotting and genetically confirmed by means of targeted gene panel resequencing (including 16 genes involved in similar phenotypes), followed by copy number variation detection, PCR, or Sanger sequencing. Because *DOCK8* is a large gene, it is important to reduce costs, where possible. First, we show that there is a nonnegligible proportion of patients (18/82 [22%] patients) given a diagnosis of AR-HIES who do not have DOCK8 deficiency. Thus if a clinician receives from molecular diagnostic laboratory report indicating that the *DOCK8* sequence is wild-type, this is a plausible result. However, a possible somatic reversion of the germline mutation might be present.[27] 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 Methods and Results sections in this article's 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[27] has demonstrated that in some patients DOCK8 gene expression can be re-established in 1 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 might 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 3 have PGM3 deficiency and 7 are not yet explained genetically. It would be clinically useful to distinguish DOCK8 deficiency from PGM3 deficiency, seronine/threonine kinase 4 (STK4) deficiency, and tyrosine kinase 2 (TYK2) deficiency. However, such a distinction cannot be made statistically because the clinical presentations of these 3 other immunodeficiencies are too heterogeneous given the small number of patients described to date. Moreover, our cohort did not include STK4- or TYK2-deficient patients (see the Methods and Results sections in this article's 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, which can be readily diagnosed by using a test termed the Dihydrorhodamine test.[28]

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 the Methods and Results sections in this article's Online Repository, 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 and DOCK8 deficiency can be correctly distinguished by a linear classifier by using 5 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 (2-sided $P = 3.6 \times 10^{-13}$). It performs substantially better in leave-one-out testing than the NIH score or the STAT3 score (see the Methods and Results sections and Table E7 in this article's Online Repository at www.jacionline.org). The NIH score, although certainly indicative of the presence of disease, performed poorly at distinguishing patients with DOCK8 deficiency from those with STAT3 deficiency (see the Results section in this article's Online Repository). However, the usefulness of the DOCK8 score has not been confirmed in a prospective cohort of patients with immunodeficiencies that present with high IgE levels and a strong clinical suspicion of HIES. Thus the authors call for a validation on an independent cohort.

Because the NIH score and HIES clinical sheet were developed by using a cohort of STAT3-deficient patients,[10] it is interesting to note that 2 of the features in the DOCK8 score, eosinophilia and upper respiratory tract infections, have positive coefficients indicating that they are more prevalent in patients with 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 might eventually be found by investigating the cellular mechanisms of this peculiar disease. Some progress toward understanding the mechanisms of DOCK8 deficiency has been made in functional studies of Dock8-deficient mice. DOCK8 is a Cdc42-specific guanine nucleotide exchange factor at the plasma membrane needed for spatial activation of Cdc42 at the leading edge of dendritic cells during interstitial migration. Absence of DOCK8 results in failure of dendritic cell migration to lymph nodes and in defective CD4^{+} T-cell priming.[29] In that regard the decreased presence of T-cell recombination circles observed in the peripheral blood of DOCK8-deficient subjects might reflect impaired migration of mature thymocytes to the periphery.[30] In this context it will be interesting to see whether infants with biallelic *DOCK8* mutations will be detected in the T-cell recombination circle–based severe combined immunodeficiency newborn screening program. In B cells DOCK8 functions as an adaptor protein downstream of Toll-like receptor 9 and upstream of STAT3,[31] possibly explaining the interesting clinical overlap between these 2 forms of HIES. Moreover, Dock8-deficient mice do not form germinal centers and have a deficit of marginal zone B cells.[32] DOCK8 deficiency affects long-term memory of B cells, as well as of virus-specific CD8^{+} T cells,[14,31,33,34] which might explain the susceptibility to bacterial and viral infections. In line with the mouse data, we also found a reduction in memory B-cell and switched memory B-cell numbers in our patients.

Because B-cell function is compromised in patients with DOCK8 deficiency, Jabara et al[31] provided evidence for a mechanism of defective Toll-like receptor 9 signaling, interestingly involving DOCK8 and STAT3. Such studies of B-cell dysfunction have direct clinical relevance in the clinical management of DOCK8-deficient patients because they raise the question of whether immunoglobulin substitution is necessary and whether vaccination is effective in these patients. The published reports on vaccination are contradictory and further investigations are needed. Al-Herz et al[15] reported that antibody responses to vaccines were normal in patients with DOCK8 deficiency, whereas Jabara et al[31] 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, among whom 64 have DOCK8 deficiency, 9 have PGM3 deficiency, and 9 are genetically unexplained. We also compared DOCK8 deficiency with STAT3 deficiency using statistical analysis. Our quantification of how common the well-known symptoms of DOCK8 deficiency are and our compilation of dozens of rare symptoms of DOCK8 deficiency should aid clinicians in recognizing and managing this life-threatening immunodeficiency.

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

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