Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome

Karin R. Engelhardt, Dr,[a]* Sean McGhee, MD,[b]* Sabine Winkler, MSc,[a] Atfa Sassi, PhD,[c] Cristina Woellner, MSc,[a] Gabriela Lopez-Herrera, PhD,[a] Andrew Chen,[b] Hong Sook Kim, PhD,[b] Maria Garcia Lloret, MD,[b] Ilka Schulze, MD,[d] Stephan Ehl, MD,[d] Jens Thiel, MD,[d] Dietmar Pfeifer, Dr,[e] Hendrik Veelken, MD,[e] Tim Niehues, MD,[f] Kathrin Siepermann, MD,[f] Sebastian Weinspach, MD,[g] Ismail Reisli, MD,[h] Sevgi Keles, MD,[h] Ferah Genel, MD,[i] Necil Kutuculer, MD,[j] Yıldız Camcıoğlu, MD,[k] Ayper Somer, MD,[l] Elif Karakoc-Aydiner, MD,[m] Isil Barlan, MD,[m] Andrew Gennery, MD,[n] Ayse Metin, MD, PhD,[o] Aydan Degerliyurt, MD,[o] Maria C. Pietrogrande, MD,[p] Mehdi Yeganeh, MD,[q] Zeina Baz, MD,[r] Salem Al-Tamemi, MD,[s] Christoph Klein, MD, PhD,[t] Jennifer M. Puck, MD,[u] Steven M. Holland, MD,[v] Edward R. B. McCabe, MD, PhD,[w] Bodo Grimbacher, MD,[a] and Talal A. Chatila, MD, MSc[b]**** *London and Newcastle upon Tyne, UK, Los Angeles and San Francisco, Calif, Tunis, Tunisia, Freiburg, Krefeld, Dusseldorf, and Hannover, Germany, Izmir, Istanbul, and Ankara, Turkey, Milan, Italy, Tehran, Iran, Beirut, Lebanon, Muscat, Oman, and Bethesda, Md*

Background: The genetic etiologies of the hyper-IgE syndromes are diverse. Approximately 60% to 70% of patients with hyper-IgE syndrome have dominant mutations in STAT3, and a single patient was reported to have a homozygous *TYK2* mutation. In the remaining patients with hyper-IgE syndrome, the genetic etiology has not yet been identified.

Objectives: We aimed to identify a gene that is mutated or deleted in autosomal recessive hyper-IgE syndrome.

Methods: We performed genome-wide single nucleotide polymorphism analysis for 9 patients with autosomal-recessive hyper-IgE syndrome to locate copy number variations and homozygous haplotypes. Homozygosity mapping was performed with 12 patients from 7 additional families. The candidate gene was analyzed by genomic and cDNA sequencing to identify causative alleles in a total of 27 patients with autosomal-recessive hyper-IgE syndrome.

Results: Subtelomeric biallelic microdeletions were identified in 5 patients at the terminus of chromosome 9p. In all 5 patients, the deleted interval involved dedicator of cytokinesis 8 *(DOCK8)*, encoding a protein implicated in the regulation of the actin cytoskeleton. Sequencing of patients without large deletions revealed 16 patients from 9 unrelated families with distinct homozygous mutations in *DOCK8* causing premature termination, frameshift, splice site disruption, and single exon deletions and microdeletions. DOCK8 deficiency was associated with impaired activation of $CD4^+$ and $CD8^+$T cells.

Conclusion: Autosomal-recessive mutations in *DOCK8* are responsible for many, although not all, cases of autosomal-recessive hyper-IgE syndrome. *DOCK8* disruption is associated with a phenotype of severe cellular immunodeficiency characterized by susceptibility to viral infections, atopic eczema, defective T-cell activation and T_H17 cell differentiation, and impaired eosinophil homeostasis and dysregulation of IgE. (J Allergy Clin Immunol 2009;124:1289-302.)

From [a]the Department of Immunology and Molecular Pathology, Royal Free Hospital and University College London; [b]the Division of Immunology, Allergy and Rheumatology, Department of Pediatrics, David Geffen School of Medicine at the University of California at Los Angeles; [c]Laboratoire d'immunologie, vaccinologie et génétique moléculaire, Institut Pasteur de Tunis, Tunisia; [d]the Center of Chronic Immunodeficiency and [e]the Department of Hematology/Oncology, University Medical Center Freiburg; [f]HELIOS Klinikum Krefeld, Zentrum für Kinder-und Jugendmedizin; [g]the Department of Pediatric Oncology, Hematology and Clinical Immunology, Center of Child and Adolescent Medicine, Heinrich-Heine-University Dusseldorf; [h]Selcuk University, Division of Pediatric Allergy and Immunology, Konya; [i]Behcet Uz State Hospital Division of Pediatric Immunology, Izmir; [j]the Ege University Faculty of Medicine, Department of Pediatrics, Izmir; [k]the Division of Pediatric Allergy-Immunology and Infectious Diseases, Cerrahpasa Medical Faculty, and [l]the Division of Infectious Diseases and Immunology, Istanbul Medical Faculty, Istanbul University; [m]the Division of Pediatric Allergy and Immunology, Marmara University, Istanbul; [n]the Institute of Cellular Medicine, Child Health, University of Newcastle Upon Tyne; [o]the Pediatric Immunology Unit, SB Ankara Diskapi Children's Hospital; [p]the Department of Maternal and Pediatric Sciences, University of Milan, Fondazione IRCCS Policlinico Milano; [q]the Immunology Asthma and Allergy Research Institute, Children's Medical Center, Tehran University of Medical Sciences; [r]the Department of Pediatrics, St George Hospital University Medical Center, Beirut; [s]the Department of Pediatrics, Sultan Qaboos University, Muscat; [t]the Department of Pediatric Hematology/Oncology, Hannover Biomedical Research School; [u]the University of California, San Francisco; [v]the Laboratory of Clinical Infectious Diseases, NIAID, NIH, Bethesda; and [w]the Departments of Pediatrics, Human Genetics, and Bioengineering, and the Center for Society and Genetics, UCLA, Los Angeles.

*These authors contributed equally to this work.

**These authors contributed equally to this work.

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Reprint requests: Bodo Grimbacher, MD, Department of Immunology and Molecular Pathology, Royal Free Hospital and University College London, Pond Street, London NW3 2QG, United Kingdom. E-mail: b.grimbacher@ucl.ac.uk. Talal Chatila, MD, MSc, Division of Pediatric, Immunology, Allergy and Rheumatology, MDCC 12-430, Mail Code 175217, David Geffen School of Medicine, University of California at Los Angeles, 10833 Le Conte Avenue Los Angeles, CA 90095-1752. E-mail: tchatila@mednet.ucla.edu.

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Abbreviations used
AR: Autosomal-recessive
CFSE: Carboxyfluorescein succinimidyl ester
CNV: Copy number variation
DHR: Dedicator of cytokinesis homology region
DOCK: Dedicator of cytokinesis
GEF: Guanine nucleotide exchange factor
HIES: Hyper-IgE syndrome
NIH: National Institutes of Health
NK: Natural killer
STAT3: Signal transducer and activator of transcription 3
WASP: Wiskott-Aldrich syndrome protein

Key words: *Autosomal recessive hyper-IgE syndrome, human gene mutation, DOCK8, primary immunodeficiency, molluscum contagiosum, recurrent infection, T cells, T_H17 cells, eosinophils, IgE regulation, copy number variations, genomic deletions*

The hyper-IgE syndromes (HIES; also called Job syndrome; OMIM: Online Mendelian Inheritance in Man #147060 and #243700) are rare primary immunodeficiencies (estimated prevalence <1:1 million) characterized by the clinical triad of recurrent staphylococcal skin abscesses, recurrent pneumonia, and serum IgE levels >10 times the upper norm. HIESs usually manifest in childhood and have a highly variable expressivity.[1,2] Although most cases are sporadic, both autosomal-dominant and autosomal-recessive (AR) inheritance have been described. The predominant form is autosomal-dominant HIES caused by heterozygous, dominant-negative mutations in signal transducer and activator of transcription 3 *(STAT3)*.[3] In this form of HIES, extraimmune manifestations occur, including skeletal abnormalities such as retained primary teeth and a typical facial appearance. A scoring system for these findings has been developed at the National Institutes of Health (NIH)[4] and refined to a STAT3 score by Woellner et al.[5] In contrast, AR-HIES is characterized by recurrent viral and bacterial infections, extreme eosinophilia, and elevated IgE without skeletal or dental abnormalities.[6] The genetic causes of AR-HIES are largely unknown. Minegishi et al[7] reported a monogenetic defect in the cytoplasmatic tyrosine kinase Tyk2 in a single patient with clinical features of AR- HIES who had consanguineous parents. In a follow-up study, however, additional patients with AR-HIES did not have mutations in the *TYK2* gene.[8] Recently a second patient with a related phenotype has been found to be deficient in Tyk2 (J.-L. Casanova, personal communication, October, 2009).

Autosomal-recessive HIES has been described predominantly in consanguineous families from Turkey. We investigated, by genome-wide homozygosity mapping and copy number analysis, 16 patients from 14 families with AR-HIES, defined as a positive NIH HIES score, and absence of significant skeletal findings. Eleven additional patients from six families were analyzed after the candidate gene had been identified.

Homozygosity mapping is a method to localize a disease-associated recessive genotype by searching for homozygous haplotypes in consanguineous families; the underlying assumption is that the recessive mutant allele is identical by descent in affected subjects.[9] If the founder mutation is relatively recent, the causative mutation is likely to be found within the largest stretches of homozygosity. The genotyping required for this approach may be accomplished by using high-density oligonucleotide arrays, which have the additional benefit of providing data on copy number at each single nucleotide polymorphism locus on the array. Copy number variations (CNVs) occur as a result of deletions and insertions of variable size in the genome.[10] CNVs are common and may be polymorphic in the population or arise *de novo* in individuals.

Our analysis has demonstrated an AR-HIES genomic locus and made possible detection of mutations in the dedicator of cytokinesis *(DOCK)–8* as the major defect in AR-HIES.

METHODS

Patients and controls

The phenotypes of the patients with AR-HIES analyzed are shown in Table I. Whole blood samples were taken from patients, family members, and healthy volunteers with informed consent. We analyzed DNA from 20 families suspected of having AR-HIES on the basis of clinical assessment. Criteria for AR-HIES were defined as elevated IgE, eczema, hypereosinophilia, and significant infections (particularly with molluscum contagiosum and herpes family viruses; Fig 1). In three kindreds, there were five deceased affected siblings, but all parents were unaffected. There were 13 families from Turkey, 2 from Iran, and 1 each from Lebanon, Oman, Mexico, Italy, and Ireland. All affected individuals had an NIH HIES score[4] > 20. In family AHR019 an elder sibling died following seizures and developing a coma, both characteristic of AR-HIES. Clinical data on families ARH011 and ARH015 were previously published by Renner et al,[6] family ARH011 has also been reported by Zhang et al,[11] family ARH010 is family 18 in the report by Grimbacher et al,[4] and patients ARH001 to ARH004 and ARH006 to ARH009 were reported by Al Khatib et al.[12]

Methods

A detailed description of the methods can be found in this article's Methods section in the Online Repository at www.jacionline.org, including homozygosity mapping and copy number analysis, PCR and sequence analysis, immunoblotting, and proliferation and carboxyfluorescein succinimidyl ester (CFSE) dilution studies.

RESULTS

Search for copy number variations, microdeletions, and homozygosity

Representational oligonucleotide microarray analysis for CNVs was performed on 8 index patients with AR-HIES previously identified among a cohort of Turkish patients[12] and a singleton patient from Italy with HIES (Fig 2, *A*). In the subtelomeric region of chromosome 9p, homozygous deletions (copy number = 0) were identified in 4 patients and a large compound heterozygous deletion was identified in the Italian patient with AR-HIES. In 1 additional patient hemizygosity was identified at this locus. For most patients the deletion extended from the most terminal p locus to within the *DOCK8* gene (Fig 2, *B*). Homozygous deletions were confirmed by PCR of the affected segments of *DOCK8* (see this article's Fig E1 in the Online Repository at www.jacionline.org). The parental origin of the deletion was investigated and is shown in this article's Fig E2 in the Online Repository at www.jacionline.org.

To obtain additional evidence that the terminal region of chromosome 9p is associated with AR-HIES, homozygosity mapping was performed on the four patients that did not have biallelic deletions and on further seven affected subjects. All eleven subjects were identified to have homozygous regions on distal chromosome 9p (data not shown).

Positional identification of candidate genes

Taken together, all 16 affected probands had either deletions or extended homozygous haplotypes at 9p24.3, a region encoding at least 4 genes, the largest of which is *DOCK8*. The other known

genes in this interval include forkhead box D4 (*FoxD4*), COBW domain containing 1 (*CBWD1*), and family with sequence similarity 138, member C (*FAM138C*, a non-coding RNA gene). In addition, there are 4 hypothetical genes and 1 open reading frame present in this region. The majority of the deletions described in our families (ARH001-ARH006) deleted all of these genes as well as disrupting *DOCK8*. Because individuals ARH003 and ARH005 had only *DOCK8* involved in biallelic deletions, we focused on *DOCK8* for further analysis.

Mutation detection in *DOCK8*

Analysis of *DOCK8* genomic and cDNA sequences in individuals with no biallelic deletions detectable by CNV analysis revealed a number of mutations and smaller deletions in 16 patients from 9 unrelated families of 15 families sequenced.

The lack of exonic PCR products in families ARH014, ARH015, and ARH016 suggests deletions including parts of the *DOCK8* gene (Fig 3). The single affected individual in family ARH014 showed normal PCR amplification products for exons 3 to 10, a shorter PCR product for exon 11, and no amplification products for exons 12, 13, 15, 16, 26, and 46 to 48 (Fig 3, *A and B*). We conclude that a large homozygous genomic deletion begins in exon 11 and extends beyond the *DOCK8* gene. Likewise, patients from families ARH015 and ARH016 show a deletion up to and including exon 26 and 25, respectively (Fig 3, *C-F*), deleting the first half of the gene.

In family ARH010 (Fig 4, *A*), we found a homozygous splice donor site mutation (gta→tta) after exon 25 (Fig 4, *B*) that leads to skipping of exon 25 in the *DOCK8* cDNA (Fig 4, *C*). PCR amplification of exons 22 to 27 from cDNA revealed 2 products, 1 normal for wild-type individual ARH010.5 and 1 faster migrating because of a lack of exon 25 sequence for the patients, whereas both products were obtained for heterozygous family members (Fig 4, *C*). Sequence analysis confirmed that exon 25 was missing from mRNA transcribed from the mutated allele (Fig 4, *C*). The mutation perfectly segregated with the phenotype in this family (Fig 4, *A*), with all affected subjects homozygous, and the mother as well as the healthy sister of the affected girls heterozygous for the mutation. Family members heterozygous or homozygous for the wild-type allele had no signs of HIES. We analyzed this specific splice site mutation in DNA from 148 healthy controls and found no evidence of this sequence variant, indicating that it is not a polymorphism. Exon 25 is made up of 150 base pairs; hence its excision leads to an in-frame deletion of 50 amino acids within the DOCK8 protein that are not part of either of the 2 predicted functional domains (UniProtKB/Swiss-Prot: DOCK8_HUMAN, Q8NF50; Fig 5).

Sequence analysis of family ARH011 revealed a homozygous point mutation (A→G) at position 133 in exon 12 of *DOCK8* that segregated with HIES (Fig 4, *D*), resulting in substitution of arginine for lysine at position 473 (K473R; Fig 4, *E*). However, in the cDNA of patient ARH011.4, we found a 4-bp deletion directly after the point mutation, which was not seen on the genomic level (Fig 4, *F*). To explain this finding, the point mutation must create an upstream cryptic splice donor site, described as CAG/gc site (http://www.life.umd.edu/labs/mount/RNAinfo/consensus.html), which may be used instead of or in preference over the wild-type sequence CAG/gt at the 3′-exon/intron boundary (Fig 4, *G*) and causes the 4-bp deletion in the cDNA. The deletion creates a frameshift followed by a premature stop codon (Fig 4, *H*), predicted to result in the expression of a truncated protein lacking the end of the dedicator of cytokinesis homology region (DHR)–1 and the whole DHR-2 domain (Fig 5). The residual full-length protein expression reported in this family by Zhang et al[11] suggests that the cryptic splice site is used in preference over, rather than instead of, the wild-type splice site. We sequenced exon 12 of *DOCK8* in 91 healthy controls and found no evidence that this is a polymorphism.

In the only patient of family ARH012, genomic DNA sequencing revealed a homozygous point mutation (A→T) at position 70 in exon 7 of *DOCK8* that segregated with HIES (Fig 4, *I*), resulting in the premature stop codon TAG at amino acid position 271 (Fig 4, *J and K*). Thus, the mutation will cause a truncated form of the DOCK8 protein lacking both DHR domains (Fig 5). We sequenced exon 7 of *DOCK8* in 115 healthy controls and found no evidence that this is a polymorphism.

In family ARH013, exon 46 of *DOCK8* was absent in the cDNA of both patients (Fig 4, *M*). In contrast with the parents' DNA, it was impossible to amplify exon 46 with specific primers by PCR from the patients' genomic DNA (Fig 4, *N*). Sequence analysis of the parents' PCR products showed no mutation within exon 46 or the flanking splice sites (data not shown). We therefore conclude that the patients harbor a homozygous genomic deletion of exon 46. The lack of exon 46 with its 107 base pairs will lead to a frameshift and cause a truncated protein (Fig 5).

In patient ARH008.3, a homozygous missense mutation replaced the canonical G in the exon 17 splice acceptor site to a C (IVS16-1 G→C; Fig 4, *P*). The mutation segregated with HIES (Fig 4, *O*). The failure to amplify cDNA beyond exon 11 (data not shown) suggested that only proximal shorter messages were expressed. Longer messages would probably be rendered unstable because of exon skipping, leading to out-of-frame sequences and premature termination, which in turn would trigger nonsense mediated degradation. Consistent with this view, the parental cDNA sequences were normal, suggesting that abnormal messages directed by the mutant allele were lost.

Sequencing of *DOCK8* cDNA in the proband ARH009 revealed skipping of exon 37 (Fig 4, *S*). This deletion is predicted to result in an in-frame deletion of 53 amino acids. Although genomic sequences of exon 36 and 38 and their surrounding intronic junctions were normal in the proband, attempts to amplify exon 37 and its associated splice junctions were unsuccessful (Fig 4, *R*). Normal genomic exon 37 sequences were amplified from both parents, but cDNA analysis revealed 2 species, 1 normal and 1 faster migrating because of a lack of exon 37 sequence. These results are consistent with homozygous deletion of exon 37 in the proband, inherited from parents heterozygous for the same deletion (Fig 4, *Q*).

Finally, sequencing of genomic DNA of proband ARH006, who had a heterozygous deletion in the *DOCK8* locus, failed to reveal exonic mutations, suggesting the presence of a cryptic mutation in the undeleted allele.

DOCK8 deficiency impairs T-cell activation

To establish the functional consequences of *DOCK8* mutations, we first analyzed the expression of DOCK8 protein in PBMCs of probands, their family members, and control subjects. Immunoblot analysis of PBMC lysates of control subjects and family members, carried out by using an anti-human DOCK8 antibody, revealed a major immunoreactive band at about 180 kilo Dalton (kDa) (Fig 6, *A, arrowheads*) and several less intensely staining

TABLE I. Characteristics of patients with AR-HIES with and without detected *DOCK8* mutations

	ARH001	ARH002	ARH003	ARH004	ARH005	ARH006
Ethnicity	Turkish	Turkish	Turkish	Turkish	Italian	Turkish
Consanguinity	+	—	+	—	—	—
Age (y)	3.8	6,9*	8,2*	8.1	8*	9,8*
Sex	Female	Male	Male	Male	Male	Male
DOCK8 mutation	Homozygous deletion	Homozygous deletion	Homozygous deletion	Homozygous deletion	Compound heterozygous deletion	Heterozygous deletion
HIES score	47	48	42	58	49	61
IgE (IU/mL)	5,000	30,000	4,970	5,000	5,400	1,163
Eosinophil count (cells/mL) or (%)	10,600	7,400	37,880	1,400	NA	2,100
Upper respiratory infections	Recurrent upper respiratory infections	Recurrent otitis	Recurrent otitis	Recurrent otitis	Sinusitis and otitis	Recurrent upper respiratory infections
Lower respiratory infections	1 Pneumonia	2 Pneumonia	2 Pneumonia	>5 Pneumonia and recurrent bronchitis	>3 Pneumonia	>5 Pneumonia
Lung abnormalities	—	—	—	Bronchiectasis	Bronchiectasis	Bronchiectasis
Abscesses	+	+	+	+	+	—
Viral infections	Molluscum contagiosum, recurrent HSV: herpes simplex virus	Herpetic keratitis	—	—	JC virus-associated PML	Molluscum contagiosum, chronic hepatitis B virus
Other infections	Candidiasis	Candidiasis	Candidiasis	Candidiasis	Candidiasis	NA
Atopy	Eczema	Eczema, newborn rash	Eczema	Eczema	Eczema	Eczema, newborn rash
CNS	—	CNS vasculitis	+	—	Severe neurological disease (PML)	Fatal encephalitis
Skeletal/dental features	Characteristic facial features, high palate	Characteristic facial features	Characteristic facial features, high palate	Characteristic facial features, high palate	Retained primary teeth, increased nasal width	Increased nasal width, high palate, retained primary teeth
Malignancies	—	—	—	—	—	—
Autoimmunity	—	—	—	—	—	—
Other features	—	—	—	—	—	—
Immunologic analysis						
WBC, white blood cells (cells/mL)	29,400	13,700	51,200	11,180	10,300	19,100
ANC, absolute neutrophil count (cells/mL)	7,200	3,100	4,096		7,210	10,900
ALC, absolute lymphocyte count (cells/mL)	6,800	2,300	4,096	2,158	2,266	5,340
PLT, platelets count (cells/mL)	596,000	413,000	350,000	49,000	263,000	684,000
IgA (mg/dL)	103 (44-244)	174 (57-282)	215 (70-303)	484 (70-303)	387	468 (62-390)
IgM (mg/dL)	72 (52-297)	46.1 (78-261)	28 (69-387)	29.2 (69-387)	35	31 (54-392)
IgG (mg/dL)	1,930 (640-2,010)	1,740 (745-1,804)	1,400 (764-2,314)	2,460 (764-2,134)	1,153	1,190 (842-1,943)
CD3 (%)	69 (43-76)	51 (55-78)	32,5 (55-78)	52,7 (55-78)	29	25 (55-78)
CD4 (%)	18 (23-48)	15 (27-53)	14.9 (27-53)	19 (27-53)	12	13 (27-53)
CD8 (%)	33 (14-33)	36 (19-34)	8,4 (19-34)	31 (19-34)	15	12 (19-34)
CD19 (%)	18 (10-31)	41.6 (10-31)	20 (10-31)	29 (10-31)	55	8 (10-31)
CD16/CD56 (%)	8 (4-23)	4 (4-26)	13 (4-26)	13 (4-26)	NA	54 (4-26)

CMV, Cytomegalovirus; *CNS,* central nervous system; *HSV,* herpes simplex virus; *HPV,* human papilloma virus; *NA,* not available; *PML,* progressive multifocal leukoencephalopathy.
*Deceased individuals.

ARH007	ARH008	ARH009	ARH0010.2	ARH0010.4	ARH0010.8	ARH0010.9
Turkish	Turkish	Turkish	Turkish	Turkish	Turkish	Turkish
+	+	+	+	+	+	+
9.3	8	12.9	45.4	40.8	15.9	14.8
Female	Female	Female	Male	Male	Female	Female
None detected	Homozygous point mutation in splice site	Homozygous single exon deletion (37)	Homozygous point mutation in splice site	Homozygous point mutation in splice site	Homozygous point mutation in splice site	Homozygous point mutation in splice site
49	65	61	40	52	42	49
400	10,500	19,100	4,074	90,910	74,688	25,987
8,100	5,100	1,000	544	1,330	1,527	932
Recurrent otitis	Recurrent otitis, sinusitis	Recurrent suppurative otitis	Sinusitis, recurrent upper respiratory tract infections	Recurrent otitis media and sinusitis	Mild recurrent upper respiratory infections	Mild recurrent upper respiratory infections
>5 Pneumonia	>5 Pneumonia	>5 Pneumonia	>20 Pneumonia, recurrent bronchitis	>20 Pneumonia	Pneumonia	Frequent pneumonia
Pneumatoceles	—	—	Bronchiectasis	Bronchiectasis	NA	NA
+	+	+	+	+	+	+
—	Recurrent HSV	Heck disease associated with chronic oral and vulvar HPV infection	HSV	Molluscum contagiosum	Recurrent HSV	Recurrent HSV, herpes zoster
Candidiasis	Candidiasis, *Listeria* meningitis	Candidiasis	Candidiasis	Candidiasis, onychomycosis	Candidiasis	Candidiasis, sepsis
Eczema	Eczema	Eczema	Severe eczema, asthma, multiple food allergies	Eczema, newborn rash, asthma	Severe eczema, asthma, multiple food dust mite and latex allergies	Severe eczema, asthma, multiple food dust mite and latex allergies
—	Brain infarct, meningitis	—	—	—	—	CNS vasculitis
Retained primary teeth	Characteristic facial features	Increased nasal width, high palate, retained primary teeth	—	—	—	—
Burkitt lymphoma	—	Squamous cell carcinoma	—	—	—	—
—	Autoimmune hemolytic anemia	—	—	—	—	—
—	—	—	Thrombopenia	Dupuytren contraction	Paronychia	—
12,200	13,600	8,900	6,800	9,500	10,300	8,400
2,000	7,800	3,750	4,692	6,460	4,635	4,956
5,100	3,200	1,500	952	1,243	3,811	1,596
172,000	393,000	327,000	188,000	282,000	371,000	211,000
428 (62-390)	239 (70-303)	20 (67-433)	165 (40-238)	94	156	162
93 (54-392)	62 (69-387)	26 (47-484)	31 (48-228)	42	59	30
778 (842-1,943)	1,500 (764-2,134)	1,568 (835-2,094)	1,050 (672-1,536)	1,050	2,150	1,440
70 (55-78)	46 (55-78)	54 (52-78)	60	71 (55-83)	79	70
16 (27-53)	32 (27-53)	24 (25-48)	64 (% of $CD3^+$)	35 (28-57)	55 (% of $CD3^+$)	62 (% of $CD3^+$)
49 (19-34)	N/A	27 (9-35)	35 (% of $CD3^+$)	29 (10-39)	43 (% of $CD3^+$)	35 (% of $CD3^+$)
20 (10-31)	58 (10-31)	20 (8-24)	30	21 (6-19)	16	25
6 (4-26)	NA	15 (6-27)	10	7 (7-31)	5	5

(Continued)

	ARH011.3	ARH011.4	ARH011.5	ARH012	ARH013.3	ARH013.4	ARH014
Ethnicity	Mexican	Mexican	Mexican	Irish	Turkish	Turkish	Turkish
Consanguinity	+	+	+	+	+	+	+
Age (y)	24	18*	16	9.5	9.6	4.4	16.7
Sex	Male	Female	Male	Female	Female	Female	Male
DOCK8 mutation	Homozygous point mutation, cryptic splice site	Homozygous point mutation, cryptic splice site	Homozygous point mutation, cryptic splice site	Homozygous point mutation, premature termination	Homozygous single exon deletion (exon 46)	Homozygous single exon deletion (exon 46)	Homozygous deletion exons 11-48
HIES score	44	54	42	24	41	23	51
IgE (IU/mL)	5,992	39,669	31,348	10,030	11,140	2,830	8,080
Eosinophil count (cells/mL) or (%)	13,600	4,730	2,610	1,069	290	1,965	18% (2% to 4%)
Upper respiratory infections	Recurrent upper respiratory infections	Otitis media, sinusitis	Recurrent upper respiratory infections	—	Chronic otitis media	Recurrent otitis media	—
Lower respiratory infections	Pneumonia	Pneumonia	Pneumonia	Pneumonia	2 Pneumonia, bronchitis	—	>3 Pneumonia, bronchitis
Lung abnormalities	—	—	—	—	—	—	—
Abscesses	+	+	+	—	+	—	+
Viral infections	Papilloma virus	Molluscum contagiosum, HSV, herpes zoster, papilloma virus	Molluscum contagiosum, recurrent HSV	Severe and refractory molluscum contagiosum	Recurrent HSV, herpetic keratitis, CMV meningitis, papillitis and retinitis	—	Severe and refractory molluscum contagiosum, HSV
Other Infections	Candidiasis, *Tinea cruris*	Candidiasis, *Haemophilus influenza* and cryptococcal meningitis, sepsis	Candidiasis	Pneumococcal meningitis	—	—	Candidiasis, *Klebsiella* sepsis
Atopy	Eczema	Eczema, multiple food and drug allergies	Eczema, multiple food and environmental allergies	Eczema, asthma	Eczema, asthma, multiple food, and drug allergies	Eczema	Eczema, asthma, multiple food and animal hair allergies
CNS	—	Meningitis, post-cryptococcal lesions in brain	—	Meningitis	Meningitis	—	—
Skeletal/dental features	—	—	Retained primary teeth	—	Clavicular fracture	—	Characteristic facial features, fractures
Malignancies	—	—	—	—	Burkitt lymphoma	—	—
Autoimmunity	—	—	—	—	—	—	—
Other features	Osteomyelitis of the femur	Malabsorption and diarrhea	—	—	—	Microcytic anemia	Retinitis, osteomyelitis
Immunologic analysis							
WBC white blood cells (cells/mL)	6,900	19,200	10,300	8,400	5,800	13,100	15,900
ANC absolute neutrophil count (cells/mL)	3,531	6,509	3,955	4,180	NA	NA	10,176
ALC absolute lymphocyte count (cells/mL)	963	2,054	2,225	2,332	4,290	6,943	1,908
PLT platelets count (cells/mL)	263,000	567,000	370,000	601,000	250,000	420,000	407,000
IgA (mg/dL)	175	630	362	211 (40-2,180)	29 (70-230)	90 (40-180)	151 (67-310)
IgM (mg/dL)	24	53	44	<22 (43-190)	5 (40-150)	49 (40-180)	17 (50-190)
IgG (mg/dL)	1,710	2,530	3,310	2,500 (360-1,520)	1,090 (700-1,400)	1,250 (500-1,300)	1,300 (720-1,560)
CD3 (%)	58 (57-86)	65 (57-86)	59 (57-86)	47	64 (55-78)	63 (43-76)	36.2 (52-78)
CD4 (%)	28 (29-57)	39 (29-57)	32 (29-57)	21	28 (27-53)	31 (23-48)	24 (25-48)
CD8 (%)	39 (25-51)	23 (25-51)	25 (25-51)	24	35 (19-34)	29 (14-33)	5 (9-35)
CD19 (%)	22 (4-16)	30 (4-16)	36 (4-16)	38	27 (10-31)	22 (14-44)	41 (8-24)
CD16/CD56 (%)	20 (5-30)	5 (5-30)	6 (5-30)	13	2 (4-26)	7 (4-23)	19.4 (6-27)

smaller bands. In contrast, these bands were missing in 2 patients with documented mutations in DOCK8, including 1 with a splice junction mutation leading to early premature termination (ARH008) and another with an in-frame exon 37 deletion (ARH009). These results demonstrate a deleterious effect of these DOCK8 mutations on protein expression. Interestingly, another

ARH015	ARH016.6	ARH016.7	ARH017	ARH018	ARH019	ARH020
Turkish	Turkish	Turkish	Iranian	Iranian	Omani	Lebanese
+	+	+	+	+	+	+
19	6	3	17	18	9.8	23
Female	Male	Female	Female	Male	Female	Female
Deletion up to exon 26	Deletion up to exon 25	Deletion up to exon 25	None detected	None detected	None detected	None detected
40	38	34	49	53	30	36
21,000	16,000	3,000	2,291	>1,000	3,325	3,703
4,500	25%	42%	1,368	5,513	1,400	1,026
Recurrent otitis media, sinusitis	Recurrent pulmonary infections	Recurrent pulmonary infections	Recurrent otitis media	Recurrent upper respiratory infections	Recurrent upper respiratory infections	—
>10 Pneumonia, bronchitis	Pneumonia (2-3/y)	3-4/y	>3 Pneumonia	>3 Pneumonia	—	Pneumonia
—	—	NA	Bronchiectasis	Bronchiectasis	—	Bronchiectasis
+	—	—	+	+	—	+
Severe molluscum contagiosum, recurrent herpes zoster, rotavirus enteritis	JC virus-associated PML	—	HSV	Molluscum contagiosum	Viral skin infections, oozing ear infections with ulcer	Molluscum contagiosum, HSV
Candidiasis	Candidiasis	—	—	—	Severe fungal nail bed infections, persistent mastoiditis	Candidiasis
Eczema, multiple food allergies	Pustular neonatal rash, severe multiple food allergies	Eczema, severe multiple food allergies	Eczema	Eczema	Eczema	Eczema, multiple food allergies
—	Severe neurological disease (PML)	—	—	—	Headache, diplegia, chorioform and dystonic movements	—
—	Coarse facies, deep-set eyes	—	Hyperflexibility, increased nasal width	Hyperflexibility, increased nasal width	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	Hypertension, parenchymal kidney disease	—
4,100	13,200	16,100	7,200	14,900	9,400	14,700
NA	9,500	5,600	4,104	6,556	4,700	9,408
1,066	1,400	3,000	1,656	1,926	1,900	3,381
NA	423	423,000	216,000	466,000	342,000	474,000
220	8.74 (70-303)	8 (26-296)	153 (70-312)	115 (70-312)	20.67 (8.2- 45.3)	351
127	70.8 (69-387)	70 (71-235)	37 (56-352)	<20 (56-352)	2.6 (4.6-30.4)	49
1,450	1,580 (764-2,134)	1,600 (604-1,941)	3,129 (639-1,349)	3,150 (639-1,349)	184 (75-156)	1,928
53	25	42	50.4 (52-78)	63 (52-78)	33	NA
16	11.7	15	20.9 (25-48)	56 (25-48)	11	NA
26	13.6	27	53.9 (9-35)	18 (9-35)	18	NA
22	52	41.4	28 (8-24)	28 (8-24)	7	NA
14	2	5	NA	NA	59	NA

proband (ARH007) with no identified *DOCK8* mutations also had absent DOCK8 immunoreactive bands, consistent with absent protein expression. This may be indicative of a hitherto undetected deleterious mutation affecting *DOCK8* in the non-exonic regions of the gene or a mutation in another gene that regulates DOCK8 expression and/or stability.

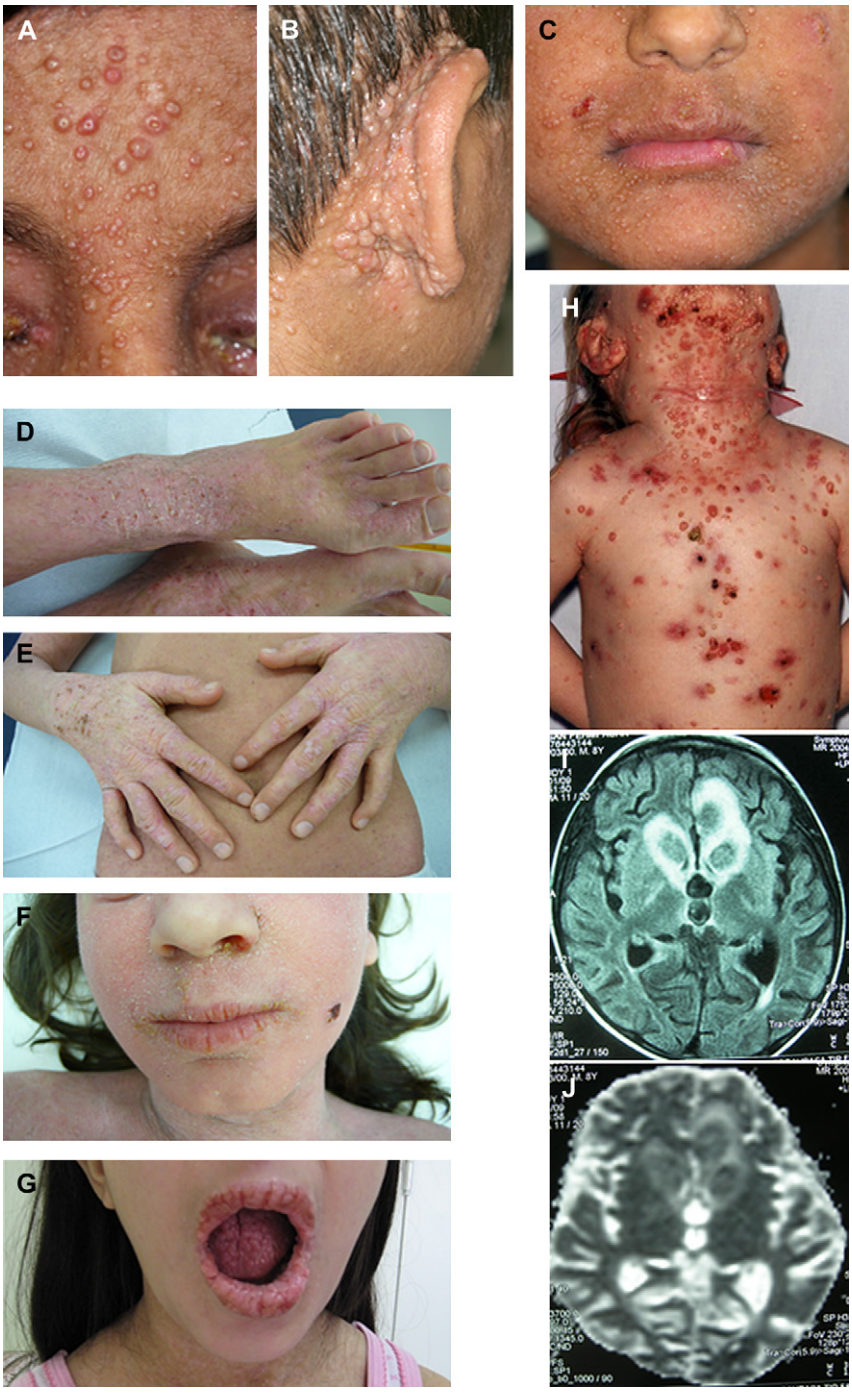

FIG 1. Clinical findings in patients with *DOCK8* mutations. **A-C,** Severe molluscum contagiosum burden of patient ARH014. **H,** Molluscum infection of patient ARH012. **D-F,** Severe dermatitis in patients ARH010.8 and ARH010.9. **G,** Severe oral papilloma virus infection of patient ARH009. **I** (MRI) and **J** (diffusion scan) document the cause of death in patient ARH003, who developed an undefined form of encephalitis.

We next analyzed T-cell proliferative responses in probands with documented DOCK8 defects compared with those of control subjects. Results revealed a profound defect in anti-CD3 mAb–induced lymphoproliferation in the probands when compared with controls as measured by ^{3}H-thymidine incorporation into proliferating cells (Fig 6, *B*). Similarly, proband T cells that had been loaded with the chromophore precursor CFSE before stimulation with anti-CD3 + anti-CD28 mAbs failed to dilute their immunofluorescence signal. Such a dilution is a marker of cell proliferation and was readily detected in similarly treated control T cells (Fig 6, *C*). The proliferative defect affected both the $CD4^{+}$ and $CD8^{+}$ subsets, consistent with a prominent role for DOCK8 in T-cell activation.

Clinical phenotype of DOCK8 deficiency

All but 2 patients with mutations in *DOCK8* had upper respiratory tract infections, and all but 3 had recurrent pneumonia on as

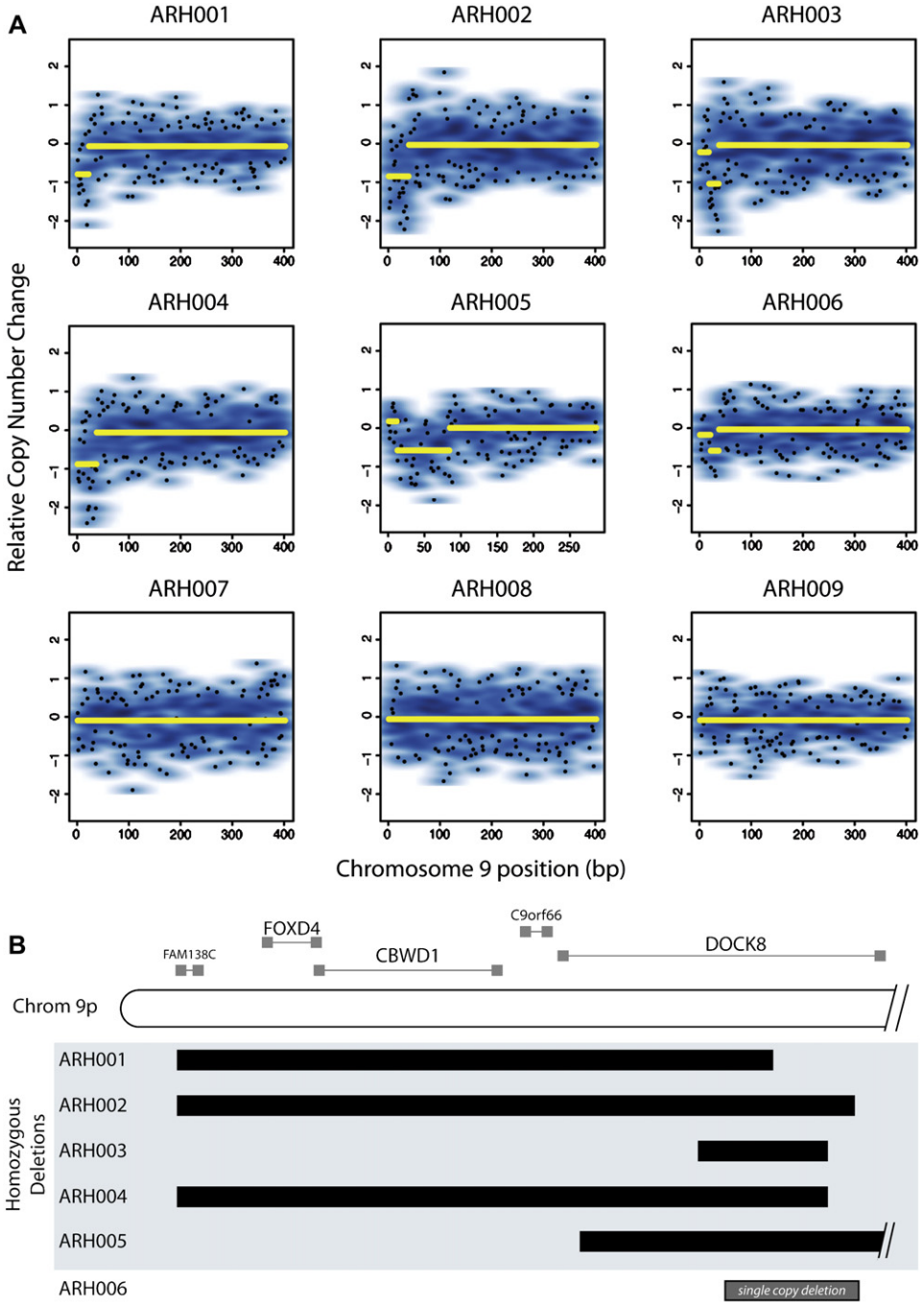

FIG 2. A, Representational oligonucleotide microarray analysis data demonstrating copy number abnormalities consistent with subtelomeric deletions of 9p in AR-HIES. Individuals ARH001 to ARH004 have homozygous deletions, ARH005 has a compound heterozygous deletion, and ARH006 has a heterozygous deletion. The remaining subjects do not have demonstrable deletions. Genome-wide single nucleotide polymorphism Nsp 250k arrays were used for subjects ARH001 to ARH009. **B,** Deletions and homozygous intervals and known and predicted genes at the terminus of chromosome 9p for patients with HIES. *C9orf66* is an open reading frame, and *FAM138C* is a noncoding RNA gene. *FOXD4* is a transcription factor, and *CBWD* has a cobalamin binding domain and nuclease function. *DOCK8* is described in the text.

many as 20 occasions (Table I). Four patients developed bronchiectasis. Seventeen of 21 (81%) patients had skin abscesses, and 17 of 21 (81%) patients had a severe susceptibility to recurrent and partially mutilating viral infections mainly caused by herpes simplex virus or molluscum contagiosum. Candidiasis was also very prevalent, with 17 of 21 (81%) patients affected. A very severe form of atopic dermatitis, often colonized with *Staphylococcus aureus* was seen in all 21 patients. Seven of 21 patients had (33%) asthma, and 10 of 21 (48%) patients had multiple food allergies, with 6 patients also having environmental allergies. Cerebral features associated with *DOCK8* mutations were central nervous system vasculitis (2 patients), brain infarction

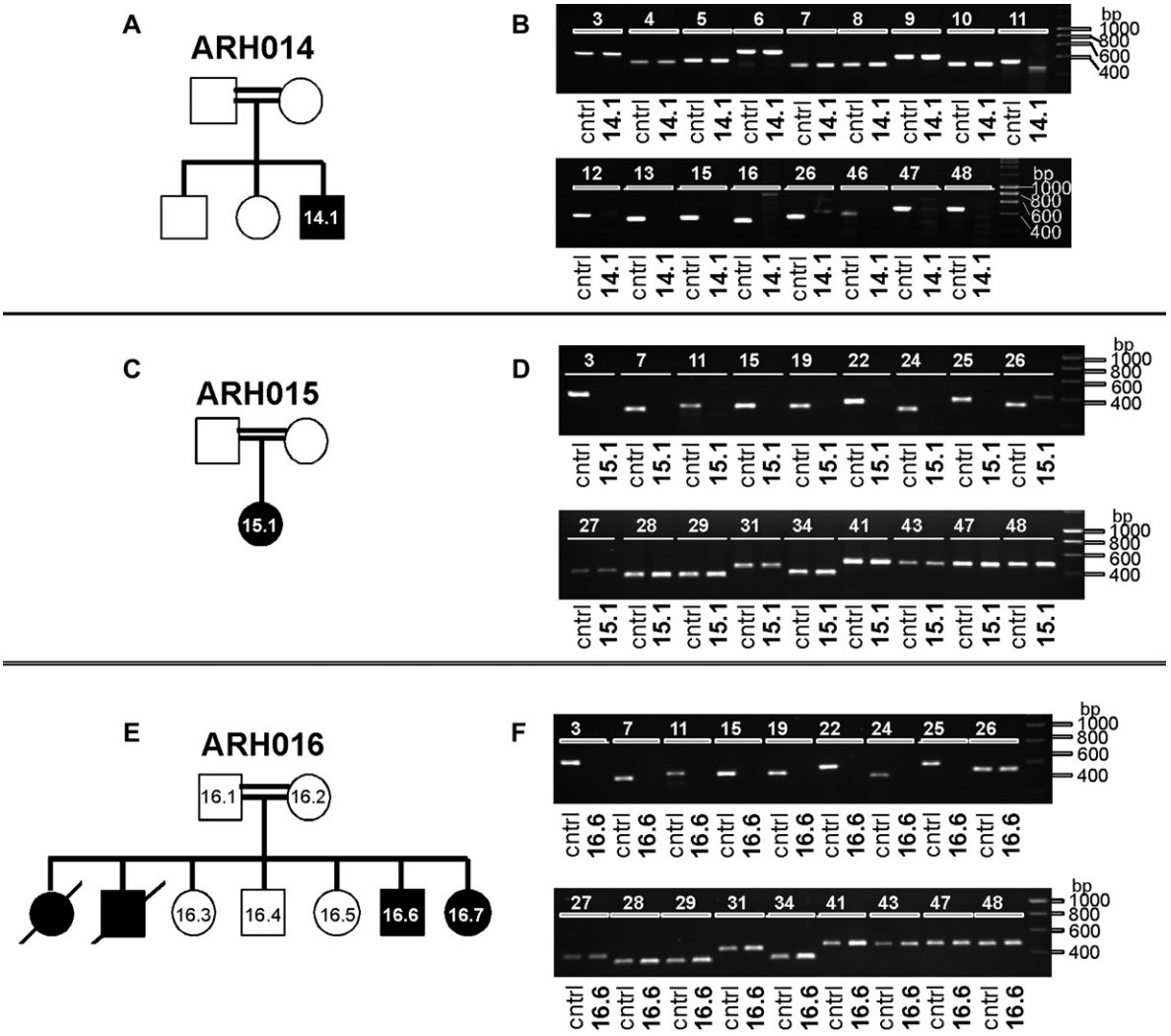

FIG 3. Exonic deletions in *DOCK8.* Pedigrees **(A, C, E)**. *Squares,* males; *circles,* females. *Filled symbols,* patients; *slashes,* deceased individuals. The lack of PCR products from patients' DNA compared with control DNA suggests exonic deletions **(B, D, F)**. *cntrl,* Control.

(1 patient), meningitis (4 patients), and JC virus-associated progressive multifocal leukoencephalopathy (2 patients). One patient had Burkitt lymphoma, and 1 patient had autoimmune hemolytic anemia.

DOCK8 deficiency was associated with elevated serum IgE (range, 2830-90,910 IU/mL; median, 20,225 IU/mL; normal < 100 IU/mL) and eosinophilia (range, 290-37,880 cells/μL; median, 5675 cells/μL). The remainder of the differential blood count was normal in most of the patients. Total T-cell numbers were within the normal range in 8 of 13 patients (62%) and decreased in 5 of 13 patients (38%). $CD4^+$ T cells were decreased in 7 of 13 patients (54%), whereas $CD8^+$ T cells were decreased in 3 of 12 patients (25%) and within the normal range in 7 of 12 patients (58%). In contrast with the data reported by Zhang et al,[11] B cells were within the normal range in 6 of 13 patients (46%) and increased in 7 of 13 patients (54%). Natural killer (NK) cells were within the normal range in most (11/12; 92%) patients. IgG serum levels were within the normal range in 11 of 13 (85%) of patients with mutations in *DOCK8*. Eight of 13 patients (62%) had normal IgA. In contrast, 10 of 13 (77%) of patients had decreased levels of IgM.

DISCUSSION

In 20 families with 27 patients with AR-HIES, a total of 21 patients had biallelic deletions or intragenic small mutations involving *DOCK8*. Four patients harbored large homozygous deletions, 1 had a large compound heterozygous deletion, 7 had exonic deletions, and 9 had homozygous point mutations predicted to impair DOCK8 protein expression or function.

The fact that we were not able to demonstrate exonic mutations in the remaining 6 AR-HIES families may be a result of difficulties in detecting e.g. compound heterozygous mutations in a 48-exon gene. Moreover, mutations may lie in intronic regions or in the regulatory regions of the gene. We will pursue this by in-depth analysis of patients' cDNA and protein expression.

By genomic sequence analysis, Griggs et al[13] had mapped *DOCK8* to chromosome 9p24. They determined that *DOCK8* contains 47 exons spanning 190 kb; more recently, however, the Ensembl database (http://www.ensembl.org) provides evidence for 10 different transcripts and 48 exons. Northern blot analysis detected *DOCK8* expression in human placenta, lung, kidney, and pancreas and to a lesser degree in brain, heart, and skeletal muscle.[14]

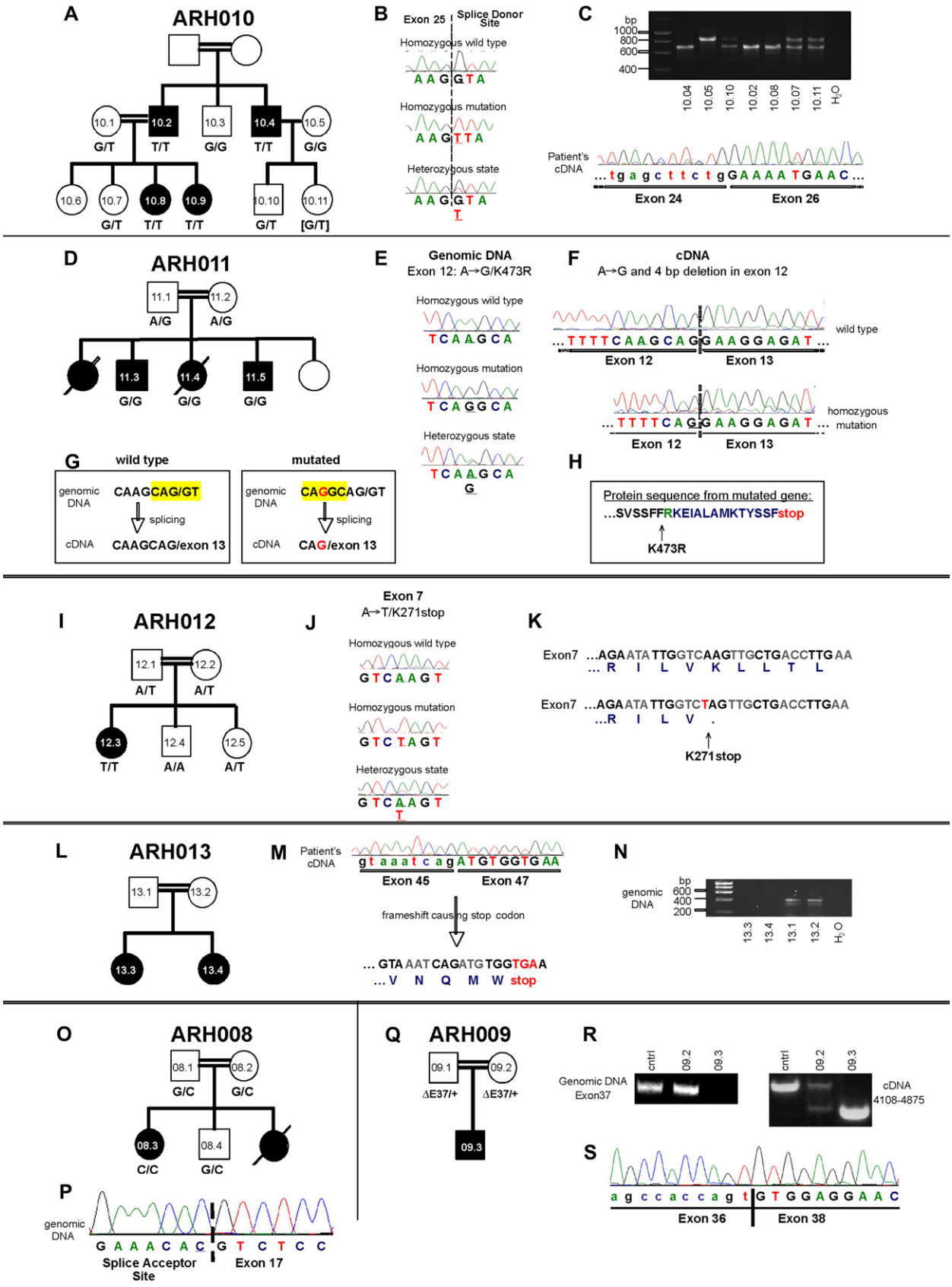

FIG 4. Mutations in *DOCK8*. Pedigrees **(A, D, I, L, O, Q)**. *Squares,* males; *circles,* females. *Filled symbols,* patients; *slashes,* deceased individuals. Point mutations in the splice site **(B, P)** or within exons **(E, J)**. Stop codon caused by point mutation **(K)** or frameshift **(H, M)**. Generation of a cryptic splice site leading to a 4-bp deletion **(F, G)**. Exon skipping shown by cDNA sequencing **(C, M, S)** and PCR **(C, R)**. Lack of PCR products from genomic DNA suggesting exonic deletions **(N, R)**.

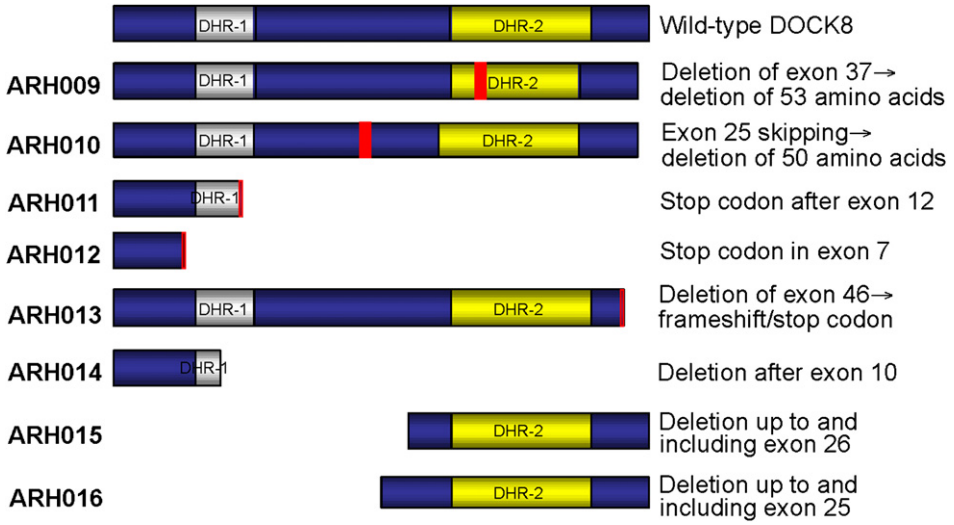

FIG 5. Cartoon showing the predicted impact of the mutations on DOCK8 protein expression. In 3 families, the mutation results in a truncated protein affecting both DHR domains, whereas in 2 families, the truncated protein lacks the DHR-1 domain. In family ARH009, 53 amino acids are missing within the DHR-2 domain, and in family ARH0010, 50 amino acids are missing in between the 2 DHR domains. In family ARH013, DOCK8 is truncated at the C-terminus.

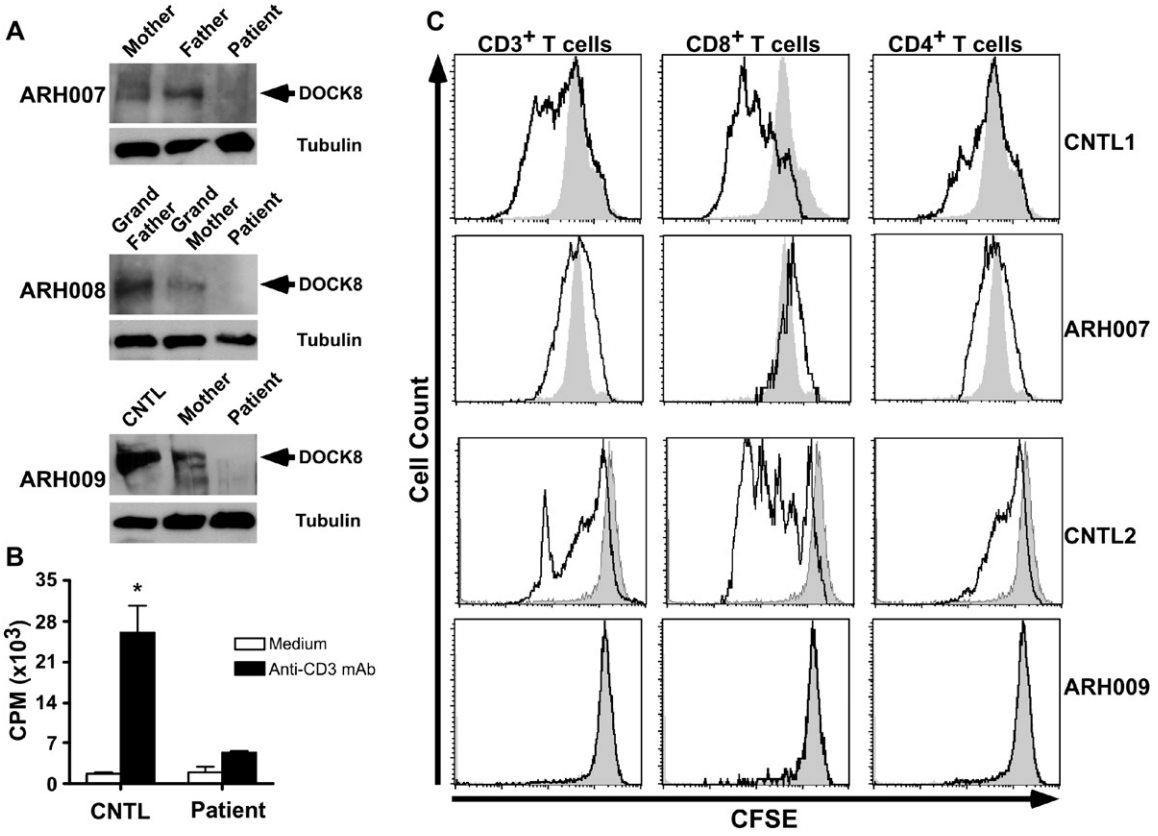

FIG 6. DOCK8 deficiency impairs T-cell activation. **A,** DOCK8 protein expression in probands, family members, and control samples. Lysates from PBMCs, normalized for protein content, were analyzed by immunoblotting with an anti-human DOCK8 antibody. A dominant band of about 180 Kilo Dalton *(arrowhead)* and several smaller isoforms were detected in control and family members samples but not in those of the probands. As loading control, the blots were re-probed with an anti-human Tubulin antibody. **B,** Proliferative responses of PBMCs to anti-CD3 mAb treatment (n = 2-5/group; $*P = .02$). **C,** DOCK8 deficiency impairs the activation of both $CD4^+$ and $CD8^+$ T cells. PBMCs were loaded with CFSE and stimulated with anti-CD3 + anti-CD28 mAb for 3 days. Gated populations of mAb-stimulated $CD3^+$, $CD4^+$, and $CD8^+$ T cells were analyzed for CFSE fluorescence intensity *(solid lines)* and compared with the respective unstimulated cell population *(shaded area). CNTL,* Control.

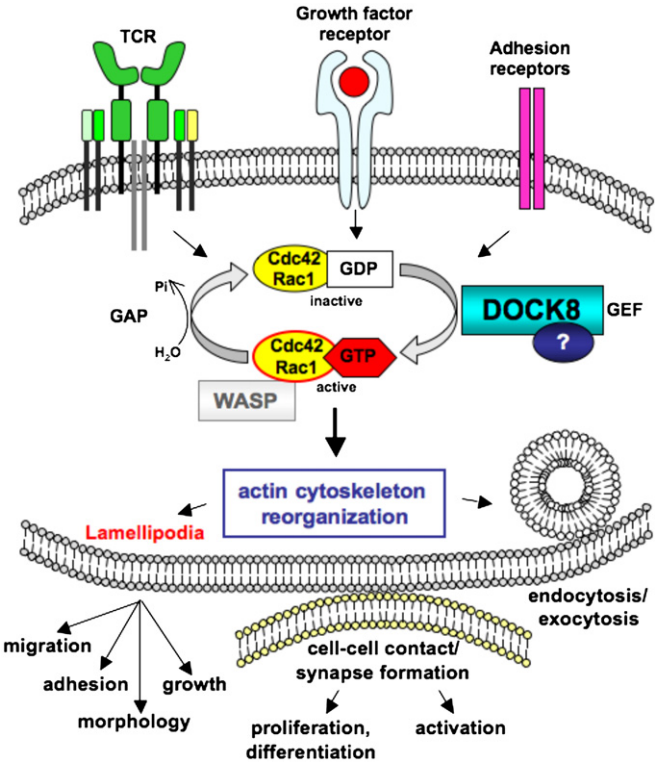

FIG 7. Hypothetical function of DOCK8. DOCK8 is likely to function as a GEF for the Rho-GTPases Cdc42 and Rac1, turning them into the active, GTP-bound form on receptor engagement (eg, receptor tyrosine kinases, antigen receptors, and adhesion receptors). An unknown protein possibly stabilizes the interaction of DOCK8 with Cdc42 and Rac1. GTPase activation induces dynamic filamentous actin rearrangements and lamellipodia formation, possibly *via* WASP, leading to cell growth, migration, and adhesion. Given the clinical phenotype of the AR-HIES patients with DOCK8 deficiency, we propose an important role of DOCK8 in T-cell actin dynamics, which might be important for the formation of the immunologic synapse, leading to T-cell activation, proliferation, and differentiation. *TCR,* T-cell receptor.

Dedicator of cytokinesis 8 is 1 of 11 members of the DOCK protein family.[15] DOCK8 interacts with GTP-bound and GDP-bound forms of Cell division control protein 42 homolog (Cdc42) and ras-related C3 botulinum toxin substrate 1 (Rac1) and the Cdc42 family members TCL and TC10 in a yeast 2-hybrid assay.[14] The protein contains Dock homology region 1 (also known as -zizimin homology 1) and DHR-2 (CZH2) domains, characteristic of DOCK180-related proteins. In some DOCK180-related family members, these domains bind phospholipid and carry out guanine nucleotide exchange factor (GEF) function, respectively.[15,16] Although the GEF activity of DOCK8 remains to be determined, the high DHR-2 domain homology of the DOCK-C subfamily suggests that like orthologs DOCK6 and DOCK7, DOCK8 may be a GEF specific for Cdc42 and/or Rac1[17] (Fig 7).

Dedicator of cytokinesis family proteins play roles in regulation of cell migration, morphology, adhesion, and growth. Immunofluorescence localized transiently transfected and endogenous DOCK8 to the cytoplasm at cell edges forming lamellipodia, which increased when cells were treated with platelet-derived growth factor (PDGF) or FCS. Transient transfection of a C-terminal fragment of DOCK8 containing the DHR-2 domain resulted in formation of vesicular structures, suggesting that DOCK8 may play a role in the organization of filamentous actin[14] (Fig 7).

Patients with AR-HIES have atopic eczema, severe susceptibility to viral infections, and deficient T_H17 cell function.[12] Our present studies demonstrate that DOCK8 deficiency impairs $CD4^+$ and $CD8^+$ T-cell proliferative responses, consistent with a critical role for this protein in T-cell activation and effector functions. It can be speculated, based on the protein domain structure and its conservation across species, that DOCK8 may fulfill an important cytoskeletal function relevant to T-cell activation, such as in the formation of the immunologic synapse (see this article's Table E1 in the Online Repository at www.jacionline.org). Other immune deficiencies, such as the Wiskott-Aldrich syndrome and severe combined immunodeficiency caused by lack of Coronin-1A, also involve deficient function of important cytoskeleton-regulating proteins.[18,19]

The mechanisms by which abnormal T-cell effector functions develop in DOCK8-deficient patients, including impaired T_H17 differentiation, remains to be established. However, a role for DOCK8 in the maintenance of memory T_H17 cells can be inferred, because it was that component of the T_H17 response that appeared most affected in DOCK8-deficient subjects.[12] It is conceivable that T_H17 deficiency in AR-HIES may be symptomatic of a more widespread derangement of T_H-cell differentiation, an eventuality that is currently under investigation. The presence of atopic dermatitis in AR-HIES suggests the action of immune dysregulatory mechanisms, although an additional contribution from a defective barrier function may be involved. Overall, we propose that DOCK8 may play a critical role in cytoskeletal organization, and that its deficiency might result in impaired T-cell activation and effector responses.

Two unrelated patients have been previously described with mental retardation and developmental disability associated with heterozygous disruption of *DOCK8* by deletion and by a translocation breakpoint, respectively. Immunologic abnormalities were not described in these patients. Mapping of the critical region shared by the 2 patients showed truncation of the longest isoform of *DOCK8*. However, these patients had single-copy deletions of *DOCK8* along with disruptions of other genes,[13,20] which may explain why their phenotype differed from those of our patients.

After the initial submission of our work, Zhang et al[11] published homozygous and compound heterozygous mutations in *DOCK8* in a cohort of 12 patients from 8 families. One of their families (#8 in Zhang et al[11]) is family ARH011 in our article. They described the phenotype as a "combined T- B- NK- immunodeficiency" and presented evidence of decreased T, B, and NK–cell numbers. Our assessment of these published cases would suggest that they also have an AR form of HIES, given that the major diagnostic criteria of elevated IgE, eosinophilia, recurrent pneumonia, and skin eczema together with a susceptibility to viral infections were fulfilled in all but 1 patient. However, in contrast with the findings by Zhang et al,[11] studies on the cohort presented herein demonstrate a selective decrease in $CD4^+$ T-cell numbers in many but not all patients. The $CD8^+$ T-cell population is less affected, whereas B and NK cells are usually within the normal range. Furthermore, whereas Zhang et al[11] found a selective defect in $CD8^+$ T-cell activation, our findings are consistent with a more comprehensive T-cell activation defect involving both the $CD4^+$ and $CD8^+$ T-cell subsets. Such a global defect in T-cell activation may explain the severe clinical phenotype of AR-HIES.

Dedicator of cytokinesis 8 deficiency is characterized by recurrent infections of the upper and lower respiratory tract; susceptibility to severe, recurrent, and mutilating viral infections (especially by molluscum contagiosum and herpes viruses); a severe dermatitis that resembles atopic dermatitis and may often be superinfected; elevated IgE levels; and eosinophilia. Other clinical features such as asthma, allergies, central nervous system symptoms, or autoimmune phenomena are variably associated. Whether there is a genotype-phenotype correlation with regard to the size of the deletion or whether other deleted genes next to *DOCK8* contribute to the phenotype needs to be determined in future studies. It is remarkable that the typical feature of cyst-forming pneumonia (pneumatoceles), as frequently seen in *STAT3*-mutated HIES, is not typical for DOCK8 deficiency and can be used as a discriminating feature.

Subtelomeric deletions often arise at sites of interspersed repetitive genomic sequences such as Alu, long interspersed elements, long terminal repeats, and simple tandem repeats.[21,22] The region surrounding *DOCK8* is particularly rich in these sequences, with 264 such sequences in the region from the p terminus to the end of *DOCK8* (National Center for Biotechnology Information Alu database). In particular, there are *Alu*-Jb sequences near the breakpoints of our patients as mapped by microarray analysis. Abnormal recombination or transposable element activity could contribute to the high frequency of deletions seen. Careful analysis of the breakpoints of these deletions will be helpful in delineating which functions of DOCK8 are affected by the deletions, potentially shedding light on the role of DOCK8 in immune and integument function.

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Clinical implications: Homozygous mutations in *DOCK8* were identified in most patients with AR-HIES. Hence patients with a phenotype of elevated IgE, eosinophilia, and recurrent skin boils, pneumonia, and viral infections (especially molluscum contagiosum and herpes) should be suspected of having mutations in *DOCK8*.

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METHODS

Homozygosity mapping and copy number analysis

Genomic DNA samples from affected individuals and parents were amplified and hybridized to Affymetrix 250 k Nsp or 6.0 SNP Mapping Arrays (Affymetrix, San Jose, Calif) according to the manufacturer's standard protocol. Call rates ranged from 88% to 93%. This resulted in 20 affected individuals available for analysis. The arrays were analyzed with the *crlmm* package v. 1.0 using R v. 2.9.2.[E1] For the purpose of homozygosity mapping, single nucleotide polymorphisms in which there was a low confidence call (<0.99 by crlmm) were excluded from analysis. Replication analysis showed that this reduced the genotyping error rate to <1% (data not shown). After preprocessing, 65,600 SNPs were available for homozygosity mapping, which was performed by using PLINK v.1.06.[E2] Extended stretches of homozygosity were presumed to be identical by descent in consanguineous matings.

Copy number analysis was performed by comparing signal intensities from family members to a single standard reference individual as previously reported.[E3] Briefly, the subject and reference arrays were normalized, and the sums of signal intensities from both alleles were compared after adjusting for effects of PCR efficiency. The log base 2 ratio of the subject to the reference was plotted against physical position on the chromosome, and CNV regions of reduced or expanded copy number were identified by circular binary segmentation.[E4,E5] All CNV analysis was performed by using the *DNAcopy* package v. 1.16 and R v. 2.9.2. Regions of apparent low copy number were confirmed by quantitative PCR of coding exons from genes in the CNV regions.

PCR and sequence analysis

Genomic DNA was extracted from whole blood by using a Gentra Puregene purification kit (Qiagen, Crawley, United Kingdom). For some patients and family members, total RNA was prepared from blood, and cDNA was synthesized as previously described. Exons and flanking intron/exon boundaries from *DOCK8* were amplified from genomic DNA by PCR according to standard protocols with Taq polymerase from PeqLab (Fareham, United Kingdom). Primer sequences are available on request. PCR products were purified by using shrimp alkaline phosphatase (Promega, Madison, Mich) and Exonuclease I (Thermo Scientific, Waltham, Mass); DNA was sequenced with the ABI PRISM BigDye Terminator kit V3.1 (Applied Biosystems, Foster City, Calif), the 3130xl Applied Biosystems Genetic Analyzer, DNA Sequencing Analysis software, version 5.2 (Applied Biosystems), and Sequencher, version 4.8 (Gene Codes Corp, Ann Arbor, Mich).

Immunoblotting

Cells were lysed in 1% NP-40 Nonidet P-40, 150 mmol/L NaCl, 25 mM/L HEPES, pH 7.4, and cell lysis buffer supplemented with protease inhibitors (Roche, Indianapolis, IN). Whole cell lysates (50 μg/lane) were resolved by SDS-PAGE and transferred to nitrocellulose filters. The latter were blocked with 5% nonfat milk, then immunoblotted with polyclonal rabbit anti-DOCK8 (Sigma-Aldrich, St. Louis, MO). The blots were developed by using horseradish peroxidase–conjugated secondary antibodies and enzyme-linked chemiluminescence, and exposed to film.

Proliferation studies

PBMCs were resuspended at 1×10^6 cells/mL in RPMI medium supplemented with 10% FCS. The cells were seeded in 96-well plates at 2×10^5 cells/well and were left otherwise untreated or were stimulated with 1 μg/mL anti-CD3 (OKT3, Ortho Biotech). After 3 days, the cell cultures were pulsed with ^{3}H-thymidine at 0.4 μCi and analyzed for radioisotype incorporation by using a β-counter.

CFSE dilution studies

PBMCs were suspended at 10^7/mL in PBS and loaded with 5 μmol/L CFSE (Invitrogen) for 15 min in the dark, washed, and resuspended at 2×10^6/mL in RPMI/10% FCS. CFSE-labeled cells were placed in culture and treated either with 50 U/mL recombinant human IL-2 (Peprotech) (control cells) or stimulated with anti-CD3 plus anti-CD28 mAb coated beads (Miltenyi Biotech) for 3 days. The cells were then harvested and analyzed for CFSE fluorescence by flow cytometry. T-cell subsets were concurrently identified using phycoerythrin–anti-CD4 and APC–anti-CD8 antibodies (BD Biosciences, San Jose, California).

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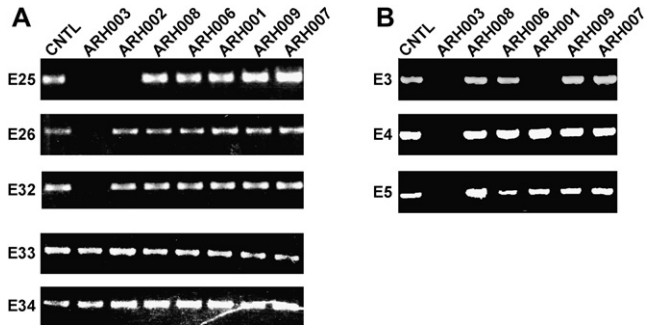

FIG E1. PCR identification of homozygous deletions affecting *DOCK8* in patients with AR-HIES with CNV abnormalities at 9p. **A,** The deletion in ARH002 ends between E25 and E26, whereas that of ARH003 ends between E32 and E33. **B,** The deletion in ARH001 ends between E3 and E4. All 3 deletions were found to extend proximally beyond exon 1, but their precise 5'' edges have not been established. Note that ARH006, who has a heterozygous deletion at 9p, successfully amplifies the respective exons, as would be expected given his possession of an undeleted copy of *DOCK8*. *CNTL,* Control.

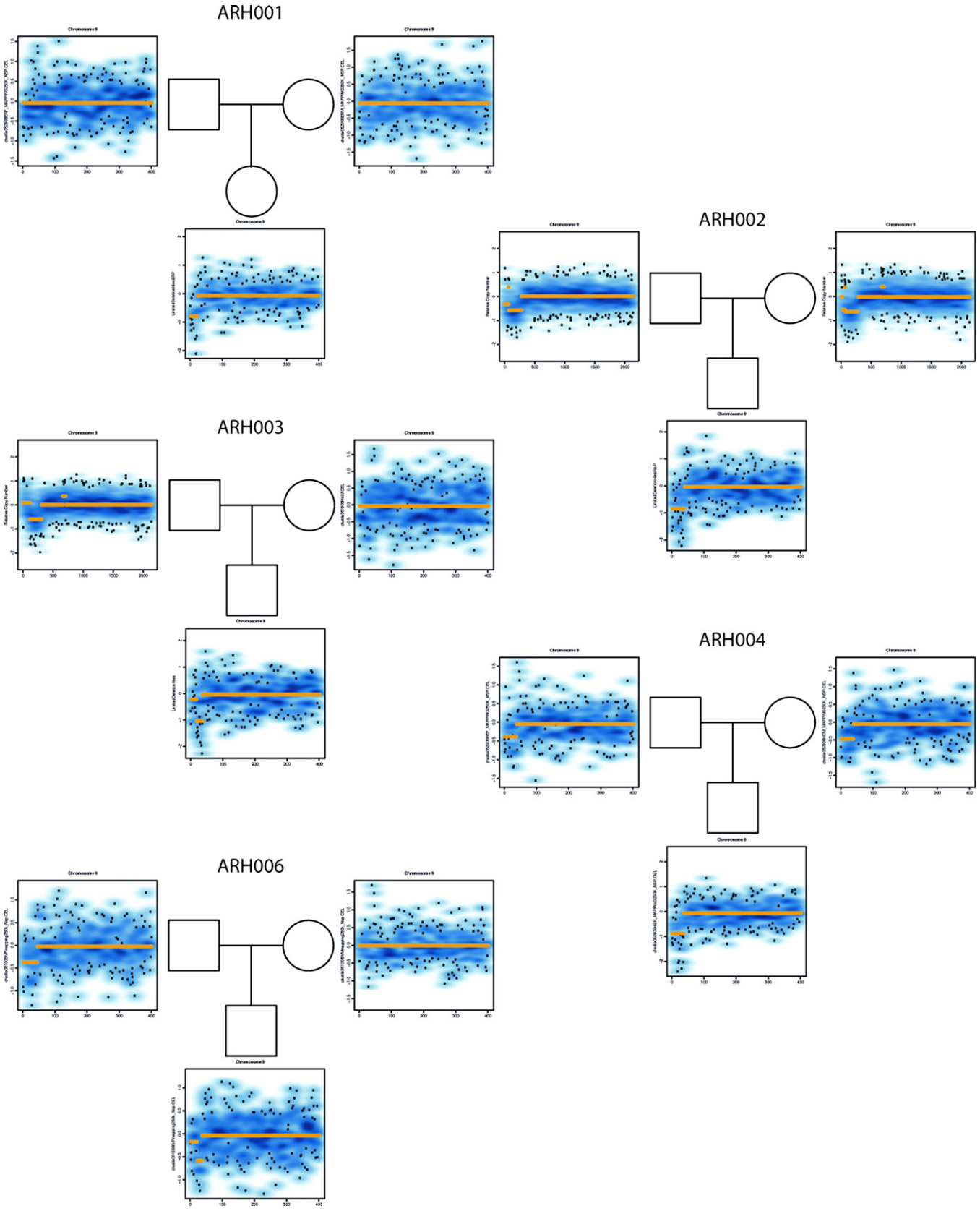

FIG E2. Heritability of copy loss in 4 families with AR-HIES. ARH004 shows typical mendelian inheritance of a deletion. ARH006 demonstrates partial repair of the CNV, presumably by gene conversion.[E6] ARH001 shows a *de novo* homozygous deletion. Parentage for ARH001 was confirmed by SNP inheritance. No mendelian errors were found in 44,645 genotypes surveyed. ARH007 is a patient without a deletion but with extensive homozygosity at this locus.

TABLE E1. Proteins similar to DOCK8 are found in a variety of euteleosts, suggesting evolutionary conservation in greater than 90% of vertebrate organisms

Species	Symbol	Protein	DNA	d	dN/dS	dNR/dNC
Pan troglodytes	DOCK8	99.1	99.1	0.009	0	0
Canis lupus familiaris	DOCK8	94	90.4	0.103	0	0
Mus musculus	DOCK8	91.8	87.1	0.141	0	0
Rattus norvegicus	DOCK8	92	87.4	0.138	0	0
Gallus gallus	DOCK8	82.3	76.3	0.285	0	0
Danio rerio	DKEY-91M11.1	68.3	66.9	0.436	0	0

The table gives the percentage protein or DNA homology between *Homo sapiens* and the listed model organisms, as well as the number of nucleotide substitutions per site *(d)* and the ratio of nonsynonymous to synonymous substitutions (*dN*/*dS* or *dNR*/*dNC*). HomoloGene, http://www.ncbi.nlm.nih.gov/homologene/, accessed 10/15/09.