Somatic reversion in dedicator of cytokinesis 8 immunodeficiency modulates disease phenotype

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Background: Autosomal recessive loss-of-function mutations in dedicator of cytokinesis 8 *(DOCK8)* cause a combined immunodeficiency characterized by atopy, recurrent infections, and cancer susceptibility. A genotype-phenotype explanation for the variable disease expression is lacking.
Objective: We investigated whether reversions contributed to the variable disease expression.
Methods: Patients followed at the National Institutes of Health's Clinical Center were studied. We performed detailed genetic analyses and intracellular flow cytometry to detect DOCK8 protein expression within lymphocyte subsets.
Results: We identified 17 of 34 DOCK8-deficient patients who had germline mutations with variable degrees of reversion caused by somatic repair. Somatic repair of the *DOCK8* mutations resulted from second-site mutation, original-site mutation, gene conversion, and intragenic crossover. Higher degrees of reversion were associated with recombination-mediated repair. DOCK8 expression was restored primarily within antigen-experienced T cells or natural killer cells but less so in naive T or B cells. Several patients exhibited multiple different repair events. Patients who had reversions were older and had less severe allergic disease, although infection susceptibility persisted. No patients were cured without hematopoietic cell transplantation.
Conclusions: In patients with DOCK8 deficiency, only certain combinations of germline mutations supported secondary somatic repair. Those patients had an ameliorated disease course with longer survival but still had fatal complications or required hematopoietic cell transplantation. These observations support the concept that some DOCK8-immunodeficient patients have mutable mosaic genomes that can modulate disease phenotype over time. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Dedicator of cytokinesis 8, reversion, somatic repair, recombination, gene conversion, intragenic single crossover, T cell, natural killer cell, allergy, immunodeficiency*

Dedicator of cytokinesis 8 (DOCK8) immunodeficiency is caused by autosomal recessive mutations in the *DOCK8* gene, which encodes an atypical guanine-nucleotide exchange factor for cell division control 42 homolog (CDC42) and Ras-related C3 botulinum toxin substrate activation (RAC).[1,2] Initially described as a hyper-immunoglobulinemia E syndrome, this combined immunodeficiency features atopy, recurrent cutaneous and sinopulmonary infections, and cancer susceptibility.[3] Typically, patients have diffuse eczematous dermatitis with bacterial skin infections early in life, along with respiratory tract infections and severe food allergies accompanied by anaphylaxis, asthma, increased serum IgE levels, and eosinophilia. Intractable viral infections of the skin are caused by herpes simplex virus, molluscum contagiosum virus, varicella-zoster virus, and/or human papillomavirus.[4] Mucocutaneous candidiasis can also occur. Death from infections or cancers usually occurs by late adolescence or early adulthood. However, in some patients the disease course is more aggressive, with severe skin disease and life-threatening infections developing at an earlier age.[5,6] Furthermore, patients have been identified who lack atopic dermatitis, food allergies, increased serum IgE levels, and/or eosinophilia. Because known pathogenic mutations in DOCK8 cause loss of protein expression, a molecular explanation for the phenotypic variability remains lacking.

Loss of DOCK8 expression within T cells, B cells, natural killer (NK) cells, and NKT cells can cause abnormal cytokine production, including T_H2 skewing, as well as defects in

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Abbreviations used
DOCK8: Dedicator of cytokinesis 8
HCT: Hematopoietic cell transplantation
NK: Natural killer
SNP: Single nucleotide polymorphism
TCR: T-cell receptor

activation, proliferation, survival, affinity maturation, and cytotoxicity.[1-3,7-12] T cells play a major role in disease pathogenesis because the infection susceptibility is cured by hematopoietic cell transplantation (HCT) when nearly complete donor T-cell chimerism is achieved, even when other leukocyte subsets are of partial donor origin.[13,14] HCT also cures or significantly ameliorates atopic dermatitis, food allergies, increased serum IgE levels, and hypereosinophilia.[13,15-17] However, the minimal level and type of T-cell reconstitution required for cure, as well as the relative contributions of other lymphocytes, are unknown.

Naturally arising somatic reversions of germline mutations have been observed in several primary immunodeficiency disorders, including Wiskott-Aldrich syndrome, severe combined immunodeficiencies, and X-linked lymphoproliferative disease.[18-20] Such cases have provided insights into the relative contributions of loss-of-function mutations in different cell types. Here we sought to determine the circumstances by which reversions occurred in DOCK8 immunodeficiency and whether they could explain phenotypic differences among patients.

METHODS

Patients and their relatives provided written informed consent and were investigated under National Institute of Allergy and Infectious Diseases (NIAID) Institutional Review Board–approved research protocols. Patients 2, 3, 4, 5, 13, 18, and 21 were previously reported as 8-2, 4-1, 4-2, 5-2, 6-1, 2-1, and 1-1, respectively.[1] Patient 1 was reported as ARH011.3.[2] Patients 9, 10, 11, 19, 22, 23, 24, and 27 were also reported elsewhere.[4,11,21] The median ages of patients were calculated from the age of living patients at the most recent evaluation at the National Institutes of Health or when undergoing transplantation or age at the time of death for deceased patients. Disease severity was scored according to the criteria listed in Table E1 in this article's Online Repository at www.jacionline.org. Primers used in this study are listed in Table E2 in this article's Online Repository at www.jacionline.org.

Detailed procedures regarding cell preparation, array comparative hybridization, immunoblotting, flow cytometry, sequencing, and statistical analyses are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Identification of patients who had somatically repaired their germline *DOCK8* mutations

DOCK8 immunodeficiency is caused by autosomal recessive loss-of-function mutations in the *DOCK8* gene.[1,2] We have followed 34 DOCK8-deficient patients from 23 families at the Clinical Center of the National Institutes of Health. Seventeen patients from 11 families formed the core of this study. Clinical diagnoses of DOCK8 immunodeficiency were confirmed by means of mutational analyses showing germline loss-of-function mutations in both *DOCK8* alleles (Table I, columns 3 and 5; Fig 1; and see Figs E1-E8 in this article's Online Repository at www.jacionline.org).

DOCK8-deficient patients normally express no DOCK8 protein in lysates from purified primary T cells (Fig 2, *A*, left panel). As expected, patients also expressed no DOCK8 in B cells (Fig 2, *A*, middle panel) or monocytes (Fig 2, *A*, right panel). However, in some patients normal or near-normal levels of DOCK8 were detected in primary T cells (Fig 2, *A*, middle and right panels). The discrepancy between germline mutations and actual protein expression suggested somatic mosaicism occurring within T cells. The germline mutations had been identified by sequencing genomic DNA from neutrophils. When we compared these against mutational analyses performed on primary T cells and, in some cases, NK cells, we discovered somatic repair in 17 patients (Table I, column 4).

Somatic repair could be categorized into one of 3 groups. In the first group somatic repair resulted from point mutations, which corrected for germline-encoded deleterious single-base substitutions. Patients 1 and 3 had second-site mutations (Fig 1, *A*, left panel, and see Fig E1), whereas patient 4 had an original-site mutation (Fig 1, *A*, right panel). These abolished use of the germline-encoded cryptic splice site or premature stop codon. Patients 2, 5, 6, 7, and 8 were obligate for either a second-site mutation or original-site mutation.

In the second group somatic repair resulted from recombination-mediated gene conversion. For example, in patients 10 and 11, genotyping of single nucleotide polymorphisms (SNPs) throughout the *DOCK8* gene indicated which portions of the *DOCK8* alleles were derived from each parent (Fig 1, *B*, and see Fig E4). In DNA from primary T cells, the paternally inherited large deletion was present, but the maternally inherited indel was absent. Furthermore, maternal SNPs upstream of the deletion were also absent. Thus we inferred that gene conversion repaired the indel on the maternally inherited allele by using the intact undeleted portion of the paternally inherited allele. Gene conversion was likely responsible for somatic repair in T cells from patients 14 and 17 (see Figs E7 and E8).

In the third group somatic repair resulted from recombination-mediated intragenic single crossover. For example, analysis of genomic DNA from primary T cells of patient 9 showed that both maternally and (presumed) paternally inherited mutations and SNPs were present throughout the entire *DOCK8* gene (Fig 1, *C*). However, when sequencing was performed after cloning PCR-amplified regions of cDNA prepared from primary T cells, neither the indel nor the missense mutation was detected. A single wild-type transcript was present with the 5′ portion containing nonmaternal SNPs and the 3′ portion containing maternal SNPs. Thus we inferred that an intragenic single crossover event generated a new allele that lacked both mutations while simultaneously generating a second new allele that contained both mutations and underwent nonsense-mediated decay. Intragenic single crossover was also responsible for somatic repair in T cells from patients 12 (Fig E5) and 16 (Fig E8) and probably patient 13 (Fig E6).

To summarize, 17 DOCK8-immunodeficient patients had somatic mosaicism, which resulted from repair of germline *DOCK8* mutations through compensatory point mutations or recombination. Recombination-mediated gene conversion or intragenic crossover occurred in all patients from our cohort who had a germline mutation on 1 allele plus an intact region corresponding to this mutation on the other allele (Table I, column 2). By contrast, in patients with overlapping deletions on both alleles, repair was not possible and was not observed (see Table E3 in this article's Online Repository at www.jacionline.org and data not shown).

TABLE I. *DOCK8* mutational analyses in the National Institutes of Health patients who have somatic repair

Patient no.	Germline mutations	Nomenclature	Mechanism of somatic repair	Supporting evidence
1	Homozygous splicing mutations (exon 11)	c.1214A>G, p.K405RfsX15	Second-site mutations differing in T and NK cells	Engelhardt et al[2]; Fig E1
2			Not determined: second-site mutation or original-site reversion	Zhang et al[1] and Engelhardt et al[2]
3	Large deletion (entire gene) + nonsense mutation (exon 11)	Chr9:g.(163,190_204,193)_(538,588_544,450)del, plus c.1153G>T, p.E385X	Second-site mutation	Zhang et al[1]; Fig 1, *A*
4			Original-site reversion	
5	Large deletion (promoter to exon 17) + nonsense mutation (exon 8)	Chr9:g.(163,190_204,193)_(361,777_370,184)del, plus c.745C>T, p. R249X	Not determined: second-site mutation or original-site reversion	Zhang et al[1]
6	Homozygous nonsense mutations (exon 19)	c.2044G>T, p.E682X	Not determined: second-site mutation or original-site reversion	Fig E2
7	Homozygous nonsense mutations (exon 41)	c.5182C>T, p.R1728X	Not determined: second-site mutation or original-site reversion	Fig E2
8			Not determined: second-site mutation or original-site reversion	
9	Small indel (exon 19) + missense mutation (exon 44)	c.2174_2175delinsAC>T, p.H725LfsX45, plus c.5627C>T, p.P1876L	Intragenic single crossover	Fig 1, *C*; Fig E3
10	Large deletion (exon 21 to end of gene) + small indel with frameshift mutation (exon 12)	Chr9:g.(383,073_383,756)_(474,634_474,667)del, plus c.1266delC, p.W423TfsX18	Gene conversion (exon 12)	Fig 1, *B*; Fig E4
11				
12	Large deletion (promoter to exon 13) + small indel with frameshift mutation (exon 32)	Chr9:g.(1_163,131)_(368,288_ 368,361)del, plus c.4031_4032insT, p.D1344RfsX2	Intragenic single crossover; additional intragenic double crossover	Fig E5
13	Large deletion (exons 13 to 26) + splicing mutation (intron 5)	Chr9:g.(340,142_356,076)_(405,056_416,292)del, plus c.538-18C>G, p.E180VfsX4	Intragenic single crossover, or gene conversion (exons 13 to 26)	Zhang et al[1]; Fig E6
14	Nonsense mutation (exon 17) + small indel with frameshift mutation (exon 36)	c.1895G>A, p.W602X, plus c.4540delG, p.E1514KfsX8	Gene conversion differing in T cells (exon 17) and NK cells (exon 36)	Fig E7
15			Not determined: all possible	
16	Large deletion (exons 5 to 9) + splicing point mutation (intron 23)	Chr9:g.(300,972_301,582)_(323,232_323,291)del, c.(325_921del), p.A109_K307del, plus c.2767-1G>A, p.K924TfsX15	Intragenic single crossover	Fig E8
17			Gene conversion (intron 23), or original-site reversion (intron 23)	

Germline mutational analyses were performed on genomic DNA isolated from neutrophils and in some cases also *Herpesvirus saimiri*–transformed T cells or EBV-transformed B cells. Somatic mutational analyses were performed on genomic DNA and cDNA, as indicated. Parenthetical information indicates where the germline mutation or somatic repair occurred. See Fig 1 and Figs E1 to E8 for supporting genetic data. Patients 1 to 8 had point mutation–mediated repair, whereas patients 9 to 17 had probable recombination-mediated repair.

Reversions are enriched in T cells

To determine in which cells somatic repair occurred, we developed an intracellular flow cytometric method to quantify DOCK8 protein. In PBMCs from healthy control subjects, we detected high levels of DOCK8 in T, B, and NK cells (Fig 2, *B*). As expected, patients with unrepaired germline *DOCK8* mutations expressed minimal DOCK8. Heterozygous carriers expressed intermediate levels. A similar expression pattern occurred in monocytes, despite higher nonspecific background. By contrast, we observed DOCK8 within T cells from patients who had somatic repair, at levels slightly decreased or similar to those in healthy control subjects (Fig 2, *C*). DOCK8-positive cells ranged up to 94% of total T cells (Table II). Proportions of DOCK8-expressing NK cells were generally lower or absent, but reached 84% in 1 patient (Table II). Low proportions of DOCK8-expressing B cells were also observed (Table II). These trends were mirrored at the genetic level in patients 10 and 11, as determined by estimating proportions of repaired lymphocyte subsets after PCR amplification, cloning, and sequencing of transformants (see Table E4 in this article's Online Repository at www.jacionline.org).

To characterize further the revertant T cells, we costained for additional cell-surface markers along with intracellular DOCK8 protein (Fig 2, *C*, and Table II). DOCK8 was expressed in $CD4^+$

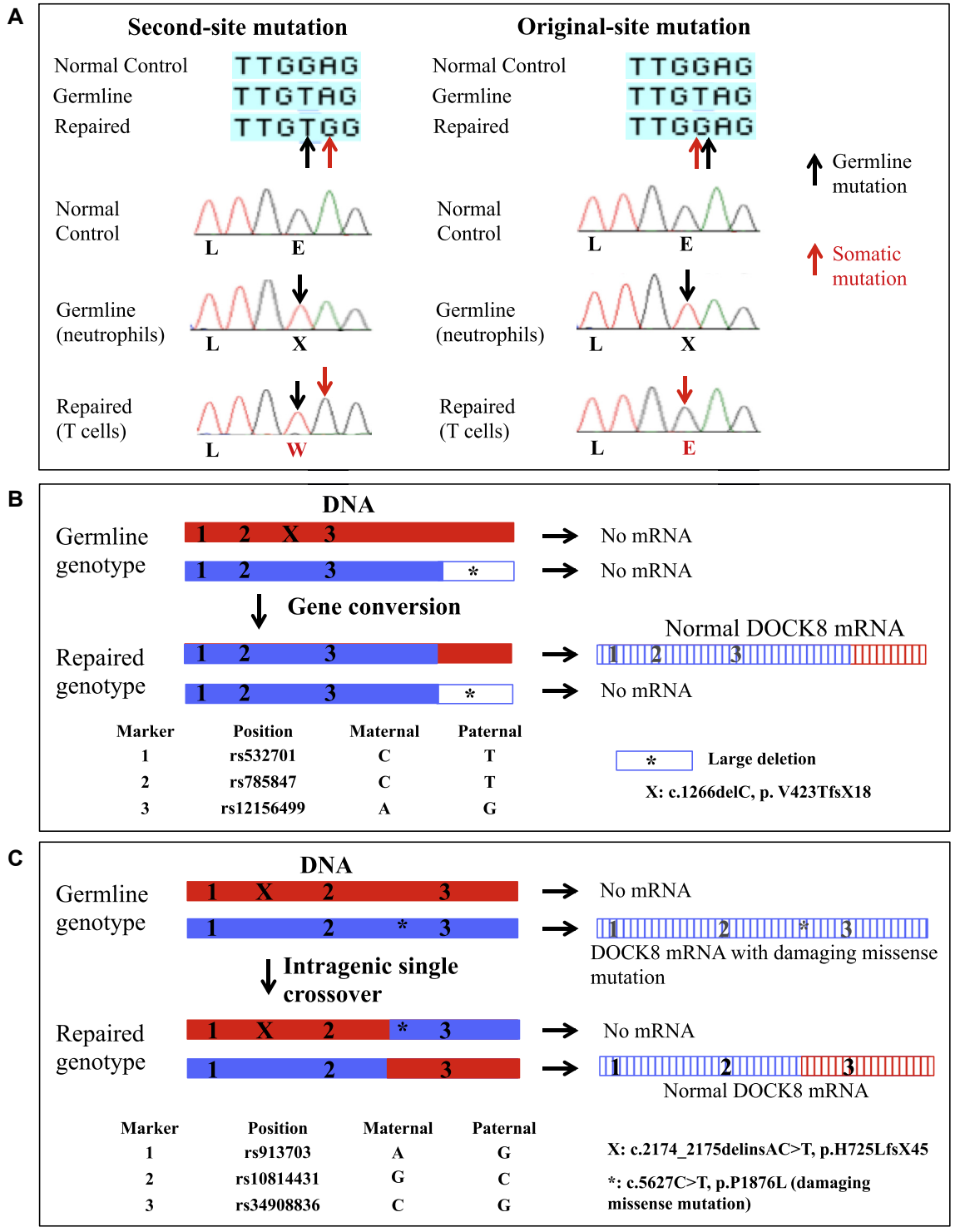

FIG 1. Mechanisms underlying somatic repair of *DOCK8* mutations: representative examples. **A,** *Left,* second-site mutation in patient 3. *Right,* Original-site reversion in patient 4. *Black* and *red arrows* designate germline and somatic mutations identified from DNA of neutrophils and primary T cells, respectively. **B,** Gene conversion in patients 10 or 11. **C,** Intragenic single crossover in patient 9. *Red* and *blue* designate maternally and paternally derived alleles, respectively, as inferred by the genotyped SNPs and mutations. Hatching indicates mRNA. Additional details are provided in Figs E3 and E4.

and especially $CD8^+$ T cells. Expression was also enriched in T cells bearing the effector/memory phenotypic marker CD45RO but less frequently in $CD45RA^+$ T cells. Up to 28% of $CD4^+CD45RA^+$ T cells expressed DOCK8. Several patients showed DOCK8 in greater than 90% of their $CD8^+CD45RA^+$ T cells (Table II), which includes highly differentiated effector

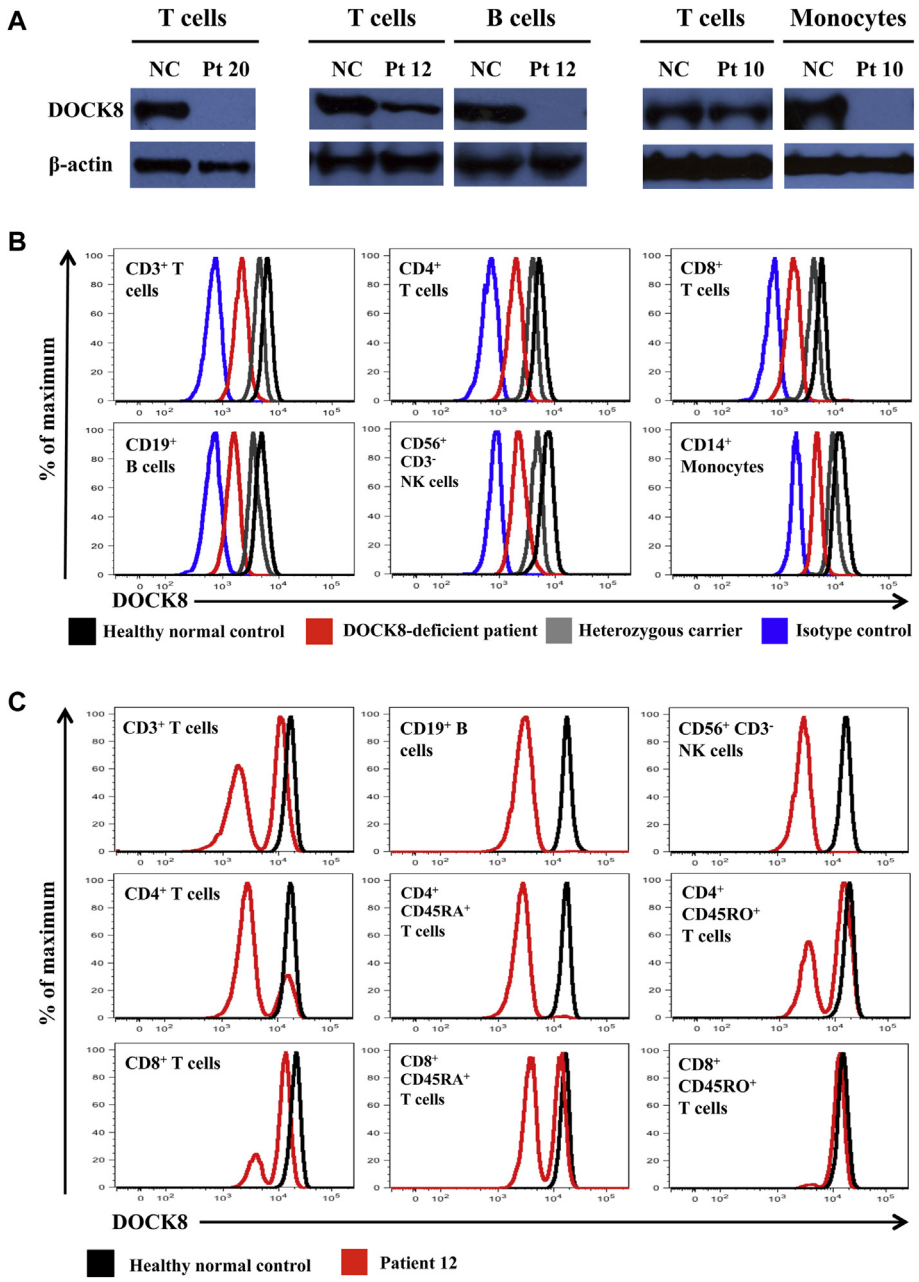

FIG 2. DOCK8-immunodeficient patients having T-cell reversions. **A,** Immunoblotting for DOCK8 or β-actin proteins in primary T cells, B cells, or monocytes. Patient 20 has a homozygous large deletion. Patients 12 and 10 have somatic repair. *NC*, Healthy control subject. **B,** Representative flow cytometric histograms showing intracellular DOCK8 expression in gated subsets from a healthy control subject *(black)*, a DOCK8 heterozygous carrier *(gray)*, patient 19 with a large homozygous deletion *(red)*, or isotype control staining *(blue)*. **C,** Histograms are from patient 12, who has somatic repair.

T cells that share CD45RA with naive T cells but lack CCR7 expression. Such effector memory $CD45RA^+CD8^+$ T cells, which are seen in states of chronic viral infections, are expanded in DOCK8-immunodeficient patients.[8] These data were corroborated by calculated frequencies of repaired sequences in $CD4^+$ or $CD8^+$ T-cell subsets that had been further sorted based on CD45RA expression (see Table E4).

Thus, among our 17 patients, reversions accumulated to the highest extent in T cells, particularly antigen-experienced T cells. Because somatic repair occurred in less than approximately one third of naive $CD4^+$ T cells, this process inefficiently corrected the defect throughout the full T-cell repertoire. Repair also occurred to variable degrees in other lymphocyte subsets and in some patients reached high levels in NK cells. Interestingly, recombination-mediated repair was associated with higher levels of reversion compared with levels seen with somatic repair caused by compensatory point mutations (Table II). Recombination often targets repetitive sequences within a gene, but exact breakpoints

TABLE II. Proportions of lymphocyte subsets expressing DOCK8

Patient no.	$CD3^+$ T cells	$CD4^+$ T cells	$CD4^+CD45RA^+$ T cells	$CD4^+CD45RO^+$ T cells	$CD8^+$ T cells	$CD8^+CD45RA^+$ T cells	$CD8^+CD45RO^+$ T cells	$CD19^+$ B cells	$CD56^+CD3^-$ NK cells
1	57	43	4	56	49	10	82	3	43
2	7	6	0	9	10	7	14	1	1
3	22	28	1	48	23	8	30	10	4
4	26	26	15	38	53	59	34	5	3
5	10	6	2	15	24	2	59	1	0
6	5	4	0	9	4	0	5	4	0
7	1	1	0	6	0	ND	ND	1	1
8	1	1	0	4	1	ND	ND	1	2
10	90	83	23	87	96	91	97	1	1
11	83	80	14	86	95	94	95	1	1
12	60	23	3	62	76	46	96	0	0
13	69	56	3	82	68	60	61	4	0
14	86	57	3	72	88	79	89	8	44
15	94	85	15	88	96	97	95	ND	84
16	94	96	28	99	96	96	97	1	3
17	75	67	11	96	93	91	97	2	1
Healthy control subjects	98 ± 2	99 ± 1	100 ± 1	99 ± 1	99 ± 2	99 ± 2	99 ± 2	99 ± 2	98 ± 2

Flow cytometric analyses for intracellular DOCK8 protein and the indicated cell-surface markers were performed on PBMCs. Percentages of different lymphocyte subsets that were positive for DOCK8 are shown. Patient 15 had previously received rituximab. Patients 1 to 8 had point mutation–mediated repair, whereas patients 10 to 17 had probable recombination-mediated repair. The means ± SDs for 14 healthy control subjects are shown based on gates established from patients' cells.
ND, Not determined.

cannot be resolved at the nucleotide level. Because of this limitation, the actual numbers of different recombination events we observed might be underestimations.

Reversions occurring in multiple lymphocyte lineages

We next analyzed the repertoire of revertant T cells. In patient 12, cells were stained for intracellular DOCK8 and costained with a panel of antibodies to identify rearranged T-cell receptor (TCR; Fig 3, *A*). Of the DOCK8-expressing T cells, approximately 40% were TCRγδ and approximately 60% were TCRαβ. Among the latter, Vβ1 (TRBV9) and Vβ18 (TRBV30) subsets were preferentially expanded in contrast to T cells lacking DOCK8 expression or T cells from healthy control subjects (see Fig E9 in this article's Online Repository at www.jacionline.org). These results suggested that reversion conferred a survival advantage for DOCK8-expressing T cells, as had been seen in adoptive transfer studies in mice.[7,8] However, the markedly increased frequency of revertants in antigen-experienced cells, along with effector memory $CD45RA^+CD8^+$ T-cell expansion, suggested that chronic antigenic stimulation also contributed to the expansion of such repaired cells. This was supported by studies in patient 10, who had Vβ8 (TRBV12) or Vβ13.1 (TRBV6-5) subsets expanded to more than 20% each of total $CD8^+CD45RA^+$ cells (Fig 3, *B*). After sorting these 2 expanded subsets, the clonotype or clonotypes they contained were identified by using DNA sequencing of CDR3. The Vβ8-expressing T-cell subset contained 3 clonotypes, and the Vβ13-expressing T-cell subset contained 1 clonotype. DOCK8 mutational analysis showed somatic repair in all 3 Vβ8-expressing clonotypes but not in the single Vβ13-expressing clonotype.

DOCK8 reversions occurring in different T cells could be explained by a single recombination event that occurred early in a hematopoietic progenitor, followed by selective outgrowth in certain clones. However, additional analyses supported the possibility that multiple recombination events had occurred in separate lymphocyte lineages. Patient 14 had 2 different gene conversion events, with the nonsense mutation repaired in T cells and the indel repaired in NK cells (see Fig E7). T cells from patient 12 also had intragenic single crossover and at least 1 other repair event (see Fig E5). Finally, patient 1 had different second-site mutations in DOCK8-expressing T cells and NK cells (see Fig E1), indicating that non–recombination-mediated somatic repair had occurred independently in different cell lineages.

Disease course in patients having reversions

Spontaneously arising somatic reversions have been likened to "natural gene therapy" and have been associated with improved disease in patients with some primary immunodeficiencies and inherited skin diseases.[18,19,22,23] To investigate whether patients having reversions had less severe disease, we devised a scoring system that gauged the severity of accumulated disease features among patients who had somatic repair compared with those who did not (see Table E1). Patients with reversions had a median age that was 9.5 years older at last evaluation, suggesting an improved overall survival (Fig 4, *A*; $P = .02$, Mann-Whitney test). Although total disease scores were similar (Fig 4, *B*; $P = .20$, Mann-Whitney test), when scores were stratified by age, they decreased with age for patients who had somatic repair but increased with age for patients without repair (Fig 4, *C*). However, improvement remained insufficient for disease elimination because 6 patients underwent HCT for uncontrolled viral infections and a seventh patient died; these outcomes were comparable with those seen in patients without reversions (see Table E1).

Total infectious disease burden, including viral disease burden, respiratory tract infections, other invasive or serious bacterial infections, or fungal or opportunistic infections increased with age. These measures were similar between both groups, although

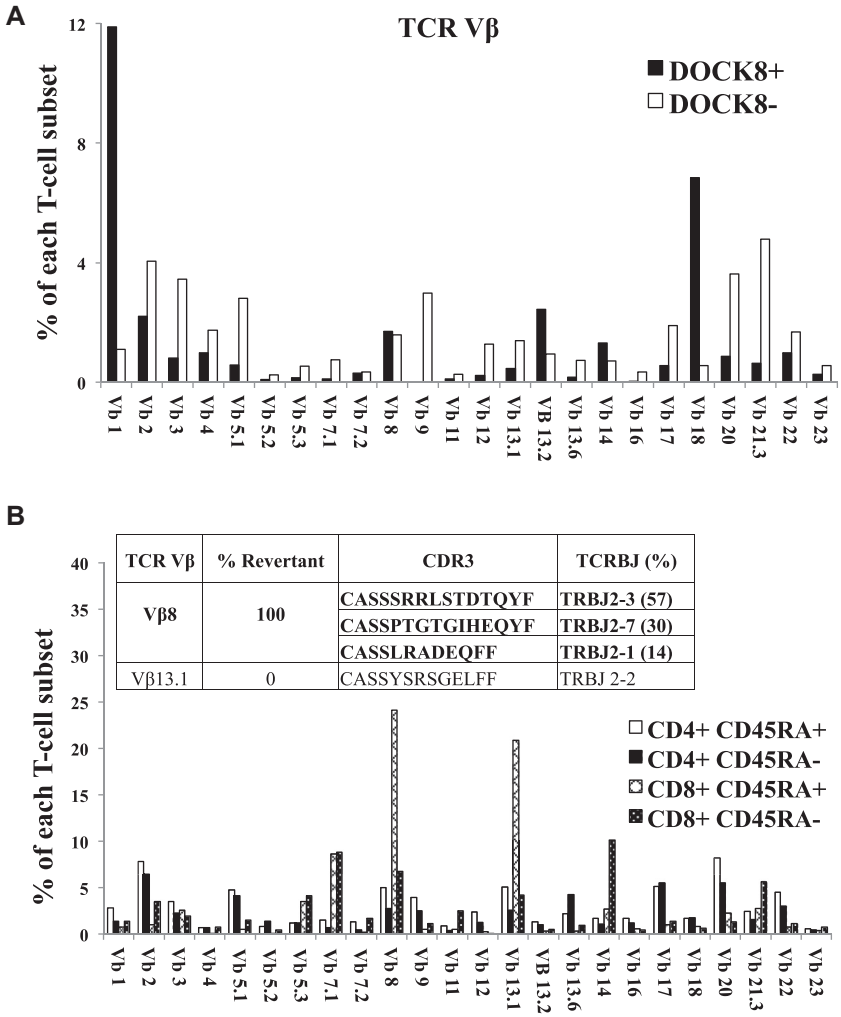

FIG 3. Reversions in multiple T-cell clonotypes. TCRVβ repertoire analyses performed on purified T cells. **A,** Patient 12's cells also stained for intracellular DOCK8. Healthy control cells are shown in Fig E9. **B,** Patient 10's cells also stained for the indicated cell-surface markers. Vβ subsets are expressed as a percentage of each T-cell subset, as indicated in the key. *Insets* indicate the CDR3-sequenced clonotypes contained within the sorted cell subsets, with DOCK8-repaired clonotypes shown in boldface.

fewer patients with reversions had staphylococcal skin and soft tissue infections (50% vs 92%; $P = .002$, Fisher exact test). The severity of functional antibody impairment was decreased (impaired specific antibodies to protein and polysaccharide antigens, 62% vs 92%; $P = .037$, χ^2 test), whereas the extent of lymphopenia did not differ. Failure to observe significant differences in overall infections might be partially due to the effectiveness of overall prophylactic management, including immune globulin and antibiotics.

Interestingly, atopic disease burden was decreased in patients who had reversions (Fig 4, *D*; $P = .007$, Mann-Whitney test). Although eczema, asthma, and eosinophilic gastrointestinal disease were similar (see Table E1), the frequency and severity of food allergies were decreased (food allergies without anaphylaxis, 6% vs 40%; food allergies with anaphylaxis, 38% vs 50%; $P = .03$, χ^2 test), and growth was also improved (poor growth, 19% vs 77%; $P = .003$, Fisher exact test). Furthermore, the severity of peripheral eosinophilia was decreased (absent, 37% vs 0%; mild-to-moderate eosinophilia, 44% vs 8%; severe eosinophilia, 19% vs 92%; $P < .001$, χ^2 test). Several patients also had normal serum IgE levels, although this did not reach statistical significance (33% vs 0%; $P = .14$, Fisher exact test). Finally, other disease features, including vascular abnormalities, autoimmunity, or malignancy, were similar in both patient groups.

DISCUSSION

DOCK8 deficiency usually leads to death by late adolescence or early adulthood unless curative HCT is performed.[13,14] Nevertheless, the HCT risk/benefit ratio might not be obvious for some patients who have less severe disease. We now identify one important source for the phenotypic variation among patients: revertant mosaicism. Reversions have been observed in cases of several primary immunodeficiencies, including Wiskott-Aldrich syndrome, where it occurs in approximately 11% of patients.[18-20] Here we found reversions in half of the DOCK8-immunodeficient patients who we follow, most of whom have a nonconsanguineous background. This high prevalence probably reflects *DOCK8*'s location within a recombination hotspot that is characterized by many subtelomeric repetitive sequences.[2] Such locations are

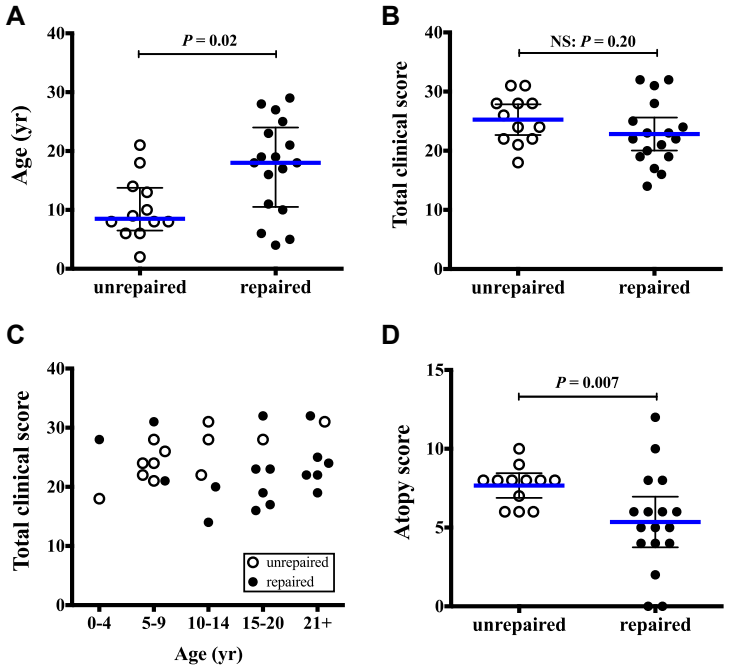

FIG 4. Clinical scoring in patients with reversions. Patients with reversions *(solid circles)* were compared with those without *(open circles)*. **A,** Age (median ± quartiles). **B,** Total clinical score. **C,** Clinical score stratified by age group. **D,** Atopic score. Scoring criteria are provided in Table E1. Fig 4, *B* and *D*, show means ± 95% CIs. *P* values were calculated by using the Mann-Whitney test. *NS*, Not significant.

known to contribute to large intragenic germline deletions found in patients with other human diseases[24] and could also contribute to the recombination-mediated somatic repair seen here.

Among our patients, reversions occurred most frequently within T cells. In patients with other diseases, reversion is often associated with a survival or growth advantage.[18,22,23,25] Thus our observations suggest that DOCK8 confers a selective advantage, especially in T cells. This is consistent with published findings in mice showing preferential outgrowth of Dock8-expressing T cells after bone marrow adoptive transfers.[7-9] DOCK8 might also confer a selective advantage in NK cells because we observed that the proportion of NK cells that were revertant increased in patient 1 from approximately 15% to 43% over a period of 2.5 years (data not shown). Revertant T and NK cells could theoretically correct the T_H2 skewing, defective NK cell cytotoxicity, and other T-cell abnormalities seen in patients with this disease, thereby modulating phenotype over time. That reversion predominates in these cell types probably also reflects their higher proliferative rates in the periphery.[26-28]

Although cure might also require correction in multiple lineages, including dendritic cells, a key role for T cells was previously suggested by a report of a DOCK8-deficient patient in whom HCT established donor engraftment of 98% of T cells but only 35% of B cells, 53% of mononuclear cells, and 6% of granulocytes.[13] Those levels completely cured infectious complications and markedly improved atopic disease. By contrast, despite high reversion frequencies in T cells, our patients still had pronounced infections, even though atopy was improved and median age increased. The different outcomes could be explained by differences in the repertoire of DOCK8-expressing T cells after transplantation compared with spontaneous repair (ie, complete vs partial correction). Given the broad infection susceptibility of DOCK8-deficient patients, cure would require that reversions occur in a diverse repertoire of T cells. Somatic repair failed to achieve this in our patients, as demonstrated by low numbers of corrected naive phenotype $CD4^+$ T cells.

Currently, a diagnosis of DOCK8 deficiency can often be made by using commercially available deletion analysis, which detected approximately 60% of the families of patients in our cohort who had deletions in 1 or both alleles (data not shown). Full sequencing of the *DOCK8* gene and confirmation of loss of protein expression by means of immunoblotting are available only through a few research laboratories. Our results now demonstrate that intracellular flow cytometric detection of DOCK8 protein could serve as a simple and rapid method for diagnosis. Because B cells show minimal reversion, their analysis is highly sensitive for detecting DOCK8 deficiency and, in fact, identified all patients tested by using this screening methodology. Monitoring the proportions of DOCK8-expressing lymphocytes over time with disease activity might be useful in selected patients. However, our data suggest that in most cases reversions at best delay the progression of disease but do not abrogate the need for HCT. Thus patients with homozygous large deletions or compound heterozygous overlapping large deletions, who are incapable of generating revertants, can be predicted to have more severe disease and earlier severe complications. In this patient subgroup especially, we advocate early HCT to minimize the development of infection-related disease pathology that might otherwise complicate delayed HCT.

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Key messages

- **Somatic repair within lymphocytes occurs in some DOCK8-immunodeficient patients, especially when recombination is possible.**
- **A rapid intracellular flow cytometry–based assay can be used to screen for DOCK8 deficiency and identify reversions.**
- **Reversions occur most often in T and NK cells, are associated with improved disease phenotypes, but are inadequate to eliminate disease.**

REFERENCES

1. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, et al. Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med 2009;361:2046-55.
2. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, Lopez-Herrera G, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. J Allergy Clin Immunol 2009;124:1289-302.e4.
3. Su HC, Jing H, Zhang Q. DOCK8 deficiency. Ann N Y Acad Sci 2011;1246:26-33.
4. Chu EY, Freeman AF, Jing H, Cowen EW, Davis J, Su HC, et al. Cutaneous manifestations of DOCK8 deficiency syndrome. Arch Dermatol 2011;148:79-84.
5. Alsum Z, Hawwari A, Alsmadi O, Al-Hissi S, Borrero E, Abu-Staiteh A, et al. Clinical, immunological and molecular characterization of DOCK8 and DOCK8-like deficient patients: single center experience of twenty five patients. J Clin Immunol 2013;33:55-67.
6. Sanal O, Jing H, Ozgur T, Ayvaz D, Strauss-Albee DM, Ersoy-Evans S, et al. Additional diverse findings expand the clinical presentation of DOCK8 deficiency. J Clin Immunol 2012;32:698-708.
7. Lambe T, Crawford G, Johnson AL, Crockford TL, Bouriez-Jones T, Smyth AM, et al. DOCK8 is essential for T-cell survival and the maintenance of CD8(+) T-cell memory. Eur J Immunol 2011;41:3423-35.
8. Randall KL, Chan SS, Ma CS, Fung I, Mei Y, Yabas M, et al. DOCK8 deficiency impairs CD8 T cell survival and function in humans and mice. J Exp Med 2011;208:2305-20.
9. Randall KL, Lambe T, Johnson A, Treanor B, Kucharska E, Domaschenz H, et al. Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. Nat Immunol 2009;10:1283-91.
10. Jabara HH, McDonald DR, Janssen E, Massaad MJ, Ramesh N, Borzutzky A, et al. DOCK8 functions as an adaptor that links TLR-MyD88 signaling to B cell activation. Nat Immunol 2012;13:612-20.
11. Mizesko MC, Banerjee PP, Monaco-Shawver L, Mace EM, Bernal WE, Sawalle-Belohradsky J, et al. Defective actin accumulation impairs human natural killer cell function in patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol 2013;131:840-8.
12. Crawford G, Enders A, Gileadi U, Stankovic S, Zhang Q, Lambe T, et al. DOCK8 is critical for the survival and function of NKT cells. Blood 2013;122:2052-61.
13. Bittner TC, Pannicke U, Renner ED, Notheis G, Hoffmann F, Belohradsky BH, et al. Successful long-term correction of autosomal recessive hyper-IgE syndrome due to DOCK8 deficiency by hematopoietic stem cell transplantation. Klin Padiatr 2010;222:351-5.
14. Gatz SA, Benninghoff U, Schutz C, Schulz A, Honig M, Pannicke U, et al. Curative treatment of autosomal-recessive hyper-IgE syndrome by hematopoietic cell transplantation. Bone Marrow Transplant 2011;46:552-6.
15. Metin A, Tavil B, Azik F, Azkur D, Ok-Bozkaya I, Kocabas C, et al. Successful bone marrow transplantation for DOCK8 deficient hyper IgE syndrome. Pediatr Transplant 2012;16:398-9.
16. Boztug H, Karitnig-Weiss C, Ausserer B, Renner ED, Albert MH, Sawalle-Belohradsky J, et al. Clinical and immunological correction of DOCK8 deficiency by allogeneic hematopoietic stem cell transplantation following a reduced toxicity conditioning regimen. Pediatr Hematol Oncol 2012;29:585-94.
17. Barlogis V, Galambrun C, Chambost H, Lamoureux-Toth S, Petit P, Stephan JL, et al. Successful allogeneic hematopoietic stem cell transplantation for DOCK8 deficiency. J Allergy Clin Immunol 2011;128:420-2.e2.
18. Wada T, Candotti F. Somatic mosaicism in primary immune deficiencies. Curr Opin Allergy Clin Immunol 2008;8:510-4.
19. Palendira U, Low C, Bell AI, Ma CS, Abbott RJ, Phan TG, et al. Expansion of somatically reverted memory CD8+ T cells in patients with X-linked lymphoproliferative disease caused by selective pressure from Epstein-Barr virus. J Exp Med 2012;209:913-24.
20. Jongmans MC, Verwiel ET, Heijdra Y, Vulliamy T, Kamping EJ, Hehir-Kwa JY, et al. Revertant somatic mosaicism by mitotic recombination in dyskeratosis congenita. Am J Hum Genet 2012;90:426-33.
21. Dasouki M, Okonkwo KC, Ray A, Folmsbeel CK, Gozales D, Keles S, et al. Deficient T Cell Receptor Excision Circles (TRECs) in autosomal recessive hyper IgE syndrome caused by DOCK8 mutation: implications for pathogenesis and potential detection by newborn screening. Clin Immunol 2011;141:128-32.
22. Kawai T, Saito M, Nishikomori R, Yasumi T, Izawa K, Murakami T, et al. Multiple reversions of an IL2RG mutation restore T cell function in an X-linked severe combined immunodeficiency patient. J Clin Immunol 2012;32:690-7.
23. Lai-Cheong JE, McGrath JA, Uitto J. Revertant mosaicism in skin: natural gene therapy. Trends Mol Med 2011;17:140-8.
24. Purandare SM, Patel PI. Recombination hot spots and human disease. Genome Res 1997;7:773-86.
25. Trifari S, Scaramuzza S, Catucci M, Ponzoni M, Mollica L, Chiesa R, et al. Revertant T lymphocytes in a patient with Wiskott-Aldrich syndrome: analysis of function and distribution in lymphoid organs. J Allergy Clin Immunol 2010;125:439-48.e8.
26. Macallan DC, Asquith B, Irvine AJ, Wallace DL, Worth A, Ghattas H, et al. Measurement and modeling of human T cell kinetics. Eur J Immunol 2003;33:2316-26.
27. Zhang Y, Wallace DL, de Lara CM, Ghattas H, Asquith B, Worth A, et al. In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection. Immunology 2007;121:258-65.
28. Macallan DC, Wallace DL, Zhang Y, Ghattas H, Asquith B, de Lara C, et al. B-cell kinetics in humans: rapid turnover of peripheral blood memory cells. Blood 2005;105:3633-40.