ORIGINAL ARTICLE

Systemic Inflammation and Normocytic Anemia in DOCK11 Deficiency

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ABSTRACT

BACKGROUND

Increasing evidence links genetic defects affecting actin-regulatory proteins to diseases with severe autoimmunity and autoinflammation, yet the underlying molecular mechanisms are poorly understood. Dedicator of cytokinesis 11 (DOCK11) activates the small Rho guanosine triphosphatase (GTPase) cell division cycle 42 (CDC42), a central regulator of actin cytoskeleton dynamics. The role of DOCK11 in human immune-cell function and disease remains unknown.

METHODS

We conducted genetic, immunologic, and molecular assays in four patients from four unrelated families who presented with infections, early-onset severe immune dysregulation, normocytic anemia of variable severity associated with anisopoikilocytosis, and developmental delay. Functional assays were performed in patient-derived cells, as well as in mouse and zebrafish models.

RESULTS

We identified rare, X-linked germline mutations in *DOCK11* in the patients, leading to a loss of protein expression in two patients and impaired CDC42 activation in all four patients. Patient-derived T cells did not form filopodia and showed abnormal migration. In addition, the patient-derived T cells, as well as the T cells from *Dock11*-knockout mice, showed overt activation and production of proinflammatory cytokines that were associated with an increased degree of nuclear translocation of nuclear factor of activated T cell 1 (NFATc1). Anemia and aberrant erythrocyte morphologic features were recapitulated in a newly generated *dock11*-knockout zebrafish model, and anemia was amenable to rescue on ectopic expression of constitutively active CDC42.

CONCLUSIONS

Germline hemizygous loss-of-function mutations affecting the actin regulator DOCK11 were shown to cause a previously unknown inborn error of hematopoiesis and immunity characterized by severe immune dysregulation and systemic inflammation, recurrent infections, and anemia. (Funded by the European Research Council and others.)

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A plethora of human neurologic, vascular, and immune diseases and cancer are associated with aberrant homeostasis of the actin cytoskeleton.[1,2] Actin remodeling is pivotal for numerous cellular processes, including morphologic changes, migration, cell–cell interaction, and signal transduction, all of which are critical for immune-cell development and function.[1]

Dedicator of cytokinesis (DOCK) family members participate in actin cytoskeleton dynamics through their guanine nucleotide exchange factor (GEF) activity, resulting in activation of the small Rho guanosine triphosphatases (GTPases) RAC and cell division cycle 42 (CDC42).[3] Their importance for human immunity has been exemplified by germline mutations in *DOCK2* and *DOCK8* underlying combined immunodeficiencies with severe and recurrent infections, as well as autoimmune manifestations including thrombocytopenia, hemolytic anemia, and vasculitis.[4-6] As is the case with DOCK2 and DOCK8, DOCK11 is predominantly expressed in hematopoietic cells, and in mice it has a role in early B-cell development and function.[7] However, its role in human immune-cell biology and disease remains unknown. Here, we implicate DOCK11 deficiency in an immune dysregulation disorder in patients with susceptibility to infection, systemic inflammation, and normocytic anemia.

Methods

Study Oversight

Written informed consent was provided by the legal representatives of the patients at the respective institutions. All experiments in animals were performed with the approval of the institutional review board at the National Center for Geriatrics and Gerontology in Obu, Japan.

Genetic, Biochemical, and Functional Analyses

Details of genetic analyses and the generation of CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and associated Cas9 homing endonucleases)–edited cells and zebrafish lines are provided in the Supplementary Appendix, available with the full text of this article at NEJM .org. Biochemical and immunochemical assays in which various hematopoietic cell types were used and statistical analysis of the performed experiments are also described in the Supplementary Appendix.

Results

Clinical Histories

We studied four male patients who presented with early-onset immune dysregulation and hematopoietic defects of unknown origin; the patients were from four unrelated families with healthy parents (Fig. 1A, Table 1, and Tables S1 through S4 in the Supplementary Appendix). The patients showed transient (Patients 2 and 4) or chronic (Patients 1 and 3) normocytic (rarely microcytic) anemia of unknown origin (Table 1, and Fig. S1A and S1B). In Patient 1, who received regular erythrocyte transfusions, a bone marrow smear showed moderate erythroid hypoplasia (Fig. 1B). Coombs' tests that were performed in Patient 1 were negative. A morphologic study of erythrocytes showed anisocytosis and poikilocytosis, suggesting a defect in red-cell shape integrity. Known erythrocyte membranopathies involve altered osmotic fragility, hemolysis, and increased levels of bilirubin and lactate dehydrogenase,[8] but these measures were normal or only slightly increased in tested patients (Patients 1 through 3). In addition, Patient 1 had thrombocytopenia (platelet count, 15,000 to 56,000 per cubic millimeter); Patient 3 had thrombocytosis (platelet count, 500,000 to 800,000 per cubic millimeter), which was most likely caused by hyperinflammation; and Patients 2 and 4 had mostly normal platelet counts.

The patients showed a range of early-onset systemic or organ-specific inflammatory manifestations, including recurrent fever and leukocytosis (Patients 1 and 3), skin inflammation and amyloid A amyloidosis (Patient 3), splenomegaly (Patients 1 and 3), systemic inflammatory response syndrome (Patients 2 and 3), and early-onset Crohn's disease (Patient 4) (Fig. 1C through 1F, Table 1, and Fig. S1C). Patients had recurrent respiratory tract infections, bacille Calmette–Guérin vaccine–related lymphadenitis and skin ulcer at injection site (BCGitis) (Patient 1), and one episode of sepsis (Patient 3) (Table 1). Patient 2 had pectus excavatum (Fig. 1G), and pyloric stenosis and recurrent vomiting had developed. Inguinal hernias were present in Patients 1 and 2. Failure to thrive was observed in Patients 1 through 3. Neurologic symptoms, in-

cluding muscular hypotonia and delayed developmental milestones, were observed in Patient 2. The patients received immunosuppressive agents, including glucocorticoids (Patients 1 through 4), tocilizumab and anakinra (Patient 3), and azathioprine (Patient 4). In addition, Patients 1 through 3 received antibiotic prophylaxis and underwent immunoglobulin substitution. No autoantibodies were detected in any of the patients who were evaluated (Table 1; clinical histories are provided in the Supplementary Appendix).

Immunophenotyping revealed normal numbers and subset distributions of T cells, natural killer cells, and monocytes (Figs. S2 and S3 and Table S5). Given the immune dysregulation in the patients, we specifically investigated regulatory T cells, the numbers of which were also within the normal range in all the patients who were evaluated (Patients 1, 3, and 4). Patient 4 had an increase in the absolute number of natural killer T cells and double-negative T cells at one time point. B-cell abnormalities included reduced proportions of marginal zone–like and switched-memory B cells (Patient 1) and intermittently increased proportions of $CD21^{low}CD38^{low}$ B cells (Patients 1 through 4) (Fig. S4). Assessment of B-cell differentiation and proliferation in vitro in Patients 1 and 4 showed intact immunoglobulin class-switch recombination in response to both T-cell–dependent and independent stimuli (Fig. S5), a finding that suggests that the reduction of switched-memory B cells in Patient 1 may be due to a B-cell extrinsic defect, possibly resulting from aberrant interactions between T and B cells.

GERMLINE *DOCK11* LOSS-OF-FUNCTION MUTATIONS

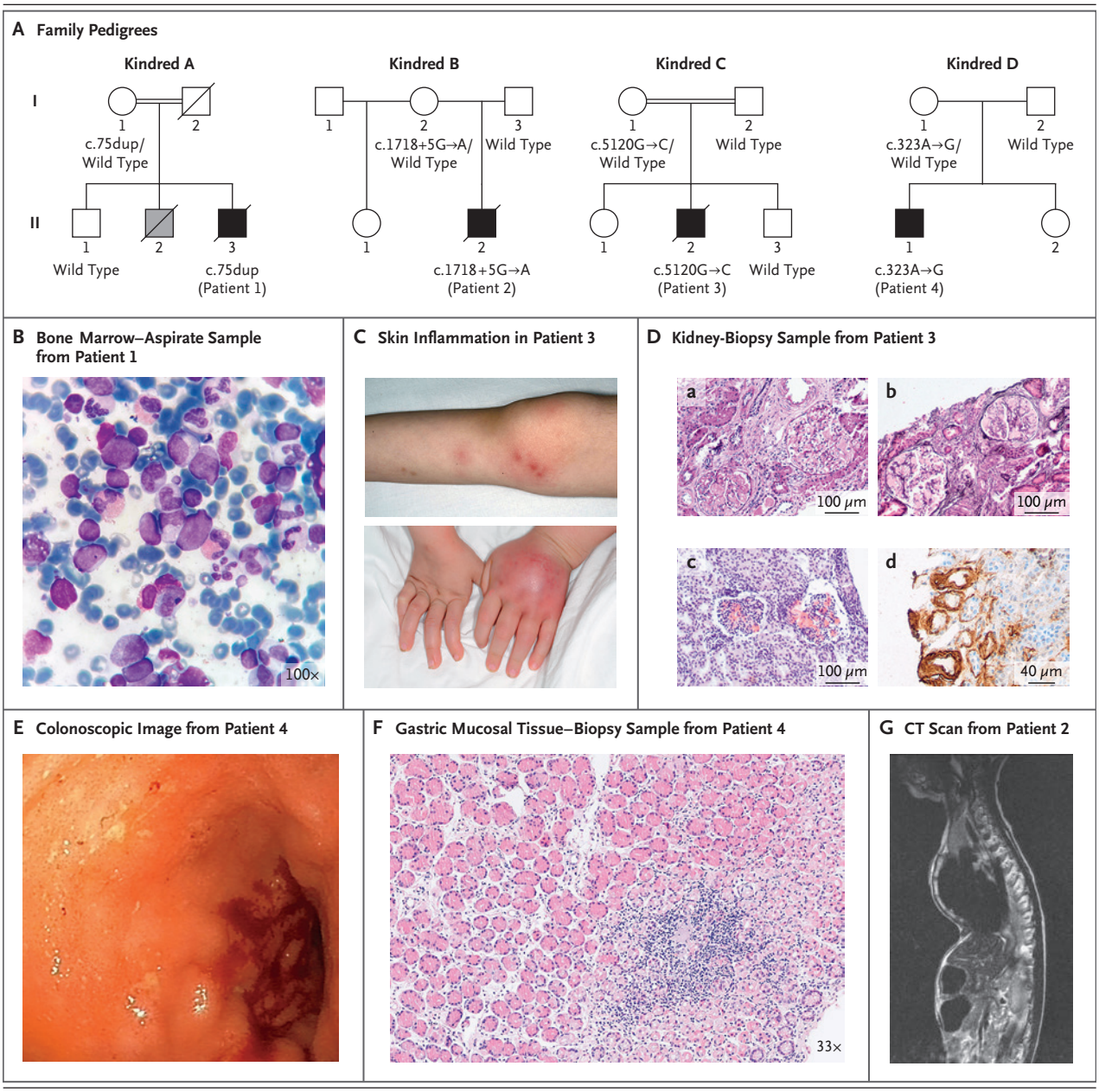
We identified, through exome sequencing, four hemizygous variants in *DOCK11* that cosegregated with disease in an X-linked inheritance pattern (Fig. 1A, and Fig. S6 and Tables S6 through S15). These four variants (Fig. S7A) — an early stop-gain mutation in Patient 1 (c.75dup [p.Glu26Ter]), a splice-site variant in Patient 2 (c.1718+5G→A [p.Pro533_Lys573del]) leading to skipping of exon 15 (Fig. S7B), and two missense mutations in Patients 3 and 4 (c.5120G→C [p.Trp1707Ser] and c.323A→G [p.Tyr108Cys]) — are predicted to be damaging according to the associated Combined Annotation-Dependent Depletion scores.[9] Each of the four healthy male relatives from the maternal side of the families who were tested did not carry the relevant *DOCK11* variants, so there is support for complete penetrance. We did not find evidence of skewed X-chromosome inactivation in the mothers of the patients (Fig. S8).

Immunoblotting revealed absent DOCK11 expression in lymphocytes from Patients 1 and 2 and retained expression of $DOCK11^{W1707S}$ and $DOCK11^{Y108C}$ in cells from Patients 3 and 4, respectively (Fig. 2A, and Figs. S9 and S10). The $DOCK11^{W1707S}$ substitution was in the DOCK homology region 2 (DHR-2) domain harboring the GEF activity, and the $DOCK11^{Y108C}$ variant amino acid was in the N-terminal CDC42-binding region (Fig. S11)[10]; these findings support our hypothesis that the variants impair DOCK11-dependent activation of CDC42. C-C motif chemokine ligand 19 (CCL19)-dependent CDC42 activation was nearly abrogated in B-lymphoblastoid cells from all four patients (Fig. 2B, and Figs. S12 and S13).

LEUKOCYTE MORPHOLOGIC FEATURES, MIGRATION, AND CYTOKINE PRODUCTION

We next tested whether the *DOCK11* mutations might alter CDC42-dependent cell polarization and filopodia formation in lymphocytes.[11] Whereas the majority of control T cells were elongated in a polarized manner and formed actin-rich filopodia on interaction with fibronectin-coated surfaces, the patient-derived T cells were devoid of such protrusions and were more circular (Fig. 3A and 3B, and Fig. S14A). The morphologic defects were phenocopied by knocking in the implicated pathogenic variants into healthy donor T cells or knocking down *DOCK11* in Jurkat T cells (Fig. 3C, Fig. S14B through S14D, and Fig. S15A through S15C), a result consistent with the previous demonstration of induction of filopodia through DOCK11 overexpression in murine bone marrow–derived dendritic cells.[12] Expression of wild-type DOCK11, but not mutant $DOCK11^{W1707S}$, partially restored the number of filopodia per cell, corresponding to the transgene expression level (Fig. S15D through S15F). Taken together, these results show that the implicated *DOCK11* variants lead to deficits in actin remodeling and morphologic features in the patient-derived T cells.

We then investigated whether such defects might affect T-cell migration. Although the patient-derived T cells showed reduced migration across a monolayer of activated endothelial cells under physiological flow conditions (Fig. 3D), their migration speed was enhanced in a confined in



vitro environment (Fig. 3E). Aberrant T-cell migration was confirmed in vivo in a newly generated F0 *dock11*-knockout zebrafish model (Figs. S16 through S18 and Videos S1 and S2 [links to the videos are provided in the Supplementary Appendix]).[13] Collectively, these data indicate that DOCK11-mediated actin remodeling may differentially govern T-cell motility depending on the environmental context.

CDC42 and some of its effectors, such as Wiskott–Aldrich syndrome protein (WASP), are critical to T-cell activation.[5,14-18] Given the hyperinflammatory phenotype of our patients, we hypothesized that DOCK11 regulates T-cell activation and cytokine production. We observed an increase in the proinflammatory cytokines interleukin-2, interferon-γ, and tumor necrosis factor α (TNF-α) in CD8+ T cells from Patient 1 (Fig. 3F), although cytokine levels were normal in CD8+ T cells from Patients 2 and 3 (Fig. S19A). Slightly increased levels of interferon-γ and slightly reduced levels of interleukin-4 were observed in CD4+ T cells from Patients 1 and 3 (Fig. S19B).

Figure 1 (facing page). Identification of Germline *DOCK11* Loss-of-Function Variants.

Panel A shows the pedigrees of four unrelated families. Double lines indicate consanguinity, black solid symbols affected persons with a hemizygous dedicator of cytokinesis 11 (*DOCK11*) variant, and gray solid symbols affected persons with an unknown genotype. Genotypes are indicated below the symbols. Squares indicate male family members, and circles female members. A slash indicates that the person had died. Roman numerals indicate generations, and Arabic numbers persons within a generation. Panel B shows erythroid hypoplasia and hypersegmented neutrophils in a bone marrow–aspirate sample (Wright–Giemsa stain) obtained from Patient 1. Panel C shows erythematous, painful, swollen lesions on the right knee (upper picture) and left hand (lower picture) of Patient 3; the lesions were diagnosed as cellulitis. Panel D shows nodular glomerulosclerosis with extension to interstitial vessels caused by amyloid A deposits in a kidney-biopsy sample obtained from Patient 3. Renal amyloid A amyloidosis was confirmed by a weak periodic acid–Schiff reaction (a), negative methenamine silver staining (b), intense staining of both glomerular and tubular basal membranes for Congo red (c), and immunohistochemical staining of the renal interstitial vessel walls for amyloid A (d). No marked tubular atrophy, interstitial fibrosis, or relevant interstitial inflammation was observed. Panel E shows mucosal erythema with small ulcerations in a macroscopic colonoscopy image in Patient 4. Panel F shows focal, chronic inflammation in a hematoxylin and eosin–stained section of a gastric mucosal tissue–biopsy sample obtained from Patient 4. Panel G shows pronounced pectus excavatum in a computed tomographic (CT) scan in Patient 2.

To overcome the limited availability of primary patient material and the potential effect of drug treatment on cellular functions, we further explored the role of DOCK11 in T cells from a *Dock11*-knockout mouse model[7] and observed no overt abnormalities in T-cell development (Fig. S20A through S20C). However, *Dock11*-knockout mice showed increased rates of proliferation of CD4+ and CD8+ T cells (Fig. S20D and S20E). Lack of DOCK11 in mouse CD8+ T cells led to increased levels of interleukin-2, interferon-γ, and TNF-α (Fig. 3G), findings that were similar to our observations in T cells from Patient 1. Moreover, levels of interleukin-2 were increased in CD4+ T cells, whereas TNF-α and interleukin-4 levels were decreased (Fig. 3H), which was also consistent with DOCK11 regulating cytokine production in T cells. Such regulation is probably cell type–dependent; we observed no changes in cytokine production in a monocyte-like cell line that had been rendered deficient in *DOCK11* (Fig. S21).

Aberrant cytokine production has been linked to defective immune synapse formation in several actin-related defects with immune dysregulation.[16,18,19] However, CD8+ T cells from patients with DOCK11 deficiency showed no overt changes in immune synapse formation, polarization of the microtubule organization center, degranulation, or perforin levels in either T or natural killer cells (Fig. S22). B-cell synapse formation was normal in the primary B cells of Patient 4 and in the B-lymphoblastoid cells of all four patients (Fig. S23); these findings suggest that DOCK11 regulates cytokine production independent of synapse formation.

To explore alternative regulatory mechanisms, we investigated T-cell–receptor signaling and transcriptional regulation, which were previously associated with small Rho GTPase–mediated regulation of cytokine production.[14,15,20-22] T cells from the patients and the *Dock11*-knockout mice showed higher nuclear translocation of the protein nuclear factor of activated T cell 1 (NFATc1), which regulates the transcription of messenger RNAs encoding interleukin-2, interferon-γ, and interleukin-4 (Fig. 3I, and Fig. S24A and S24B).[23] Translocation of NFATc1 to the nucleus is negatively regulated by phosphorylated c-Jun N-terminal kinase (JNK), a CDC42 effector.[24] We observed that JNK phosphorylation was reduced in T cells from *Dock11*-knockout mice (Fig. S24C), a finding that suggests that DOCK11 controls T-cell cytokine levels through the CDC42–JNK–NFATc1 axis.

ANEMIA AND ABERRANT MORPHOLOGIC FEATURES OF RED CELLS

Given the characterized bias in lymphocyte activation, we reasoned that red-cell abnormalities observed in patients with DOCK11 deficiency might be autoimmune-driven. However, the finding of an absence of erythrocyte-directed antibodies supported an erythrocyte-intrinsic role of DOCK11 in erythroid homeostasis.

Zebrafish have been used to model vertebrate hematopoiesis — in particular, erythropoiesis.[25] It is notable that *dock11*-knockout transgenic *fli1:GFP;gata1a:dsRed* embryos showed a striking defect in blood circulation that was characterized by the accumulation of blood cells in the posterior part of the body (Fig. 4A, Fig. S25A, and Videos S3 through S6). The *dock11*-knockout

Table 1. Clinical and Genetic Characteristics of Patients with DOCK11 Deficiency.*

Characteristic	Patient 1†	Patient 2†	Patient 3†	Patient 4
DOCK11 genomic change (HGVS, NC_000023.10)	g.117630009dup	g.117718825G→A	g.117805029G→C	g.117677487A→G
DOCK11 cDNA change (HGVS, NM_144658.3)	c.75dup	c.1718+5G→A	c.5120G→C	c.323A→G
DOCK11 protein change (HGVS, NP_653259.3)	p.Glu26Ter	p.Pro533_Lys573del	p.Trp1707Ser	p.Tyr108Cys
ClinVar accession number	SCV003841184	SCV003841185	SCV003841186	SCV003841187
Age at onset	40 days	Birth	4 mo	2 yr
Red-cell manifestations	Transfusion-dependent anemia Anisocytosis	Transient anemia Anisocytosis Fragmentocytes	Anemia Anisocytosis	Anisocytosis
Inflammatory manifestations	Leukocytosis Hepatosplenomegaly ARDS	SIRS	Leukocytosis SIRS Amyloid A amyloidosis Hepatosplenomegaly	Early-onset Crohn's disease
Infections	Otitis media Mastoiditis BCGitis	Pneumonia Bronchitis Gastroenteritis	Pneumonia Gastroenteritis Sepsis	Gastroenteritis (rotavirus)
Immunoglobulin levels	Low-normal IgG Normal IgM and IgE	Normal IgG, IgA, and IgM	Low IgG, IgA, and IgM Normal IgE	Normal IgG, IgA, and IgM Increased IgE
Gastrointestinal manifestations	None	Pyloric stenosis Recurrent vomiting	None	Constipation Diarrhea Rectal bleeding Colitis Antrum gastritis
Skin manifestations	Skin ulcer	Wrinkled skin	Cellulitis Myositis Arthritis	None
Neurologic manifestations	Facial-nerve palsy	Delayed developmental milestones Floppy infant Muscular hypotonia	None	None
Miscellaneous	FTT BCG vaccine–related lymphadenitis Inguinal hernia	FTT Pectus excavatum Inguinal hernia	FTT Nephrotic syndrome Hypothyroidism	None
Therapy	Blood transfusions IVIg Prednisolone	Antibiotic prophylaxis IVIg Gastric-tube feeding Budesonide	Steroids Cyclosporine Colchicine Anakinra Tocilizumab, IV or SC Sirolimus Secukinumab Antibiotic prophylaxis IVIg or SCIg	5-aminosalicylic acid mesalamine Budesonide Azathioprine

* ARDS denotes acute respiratory distress syndrome, BCG bacille Calmette–Guérin, BCGitis BCG vaccine–related lymphadenitis and skin ulcer at injection site, cDNA complementary DNA, FTT failure to thrive, HGVS Human Genome Variation Society, IV intravenous, IVIg intravenous immune globulin, SC subcutaneous, SCIg subcutaneous immune globulin, and SIRS systemic inflammatory response syndrome.
† Patient 1 died at 6 years of age after undergoing splenectomy. Patient 2 died at 2 years 10 months of age and Patient 3 at 6 years 1 month of age, both due to multiorgan failure related to sepsis or SIRS.

embryos were anemic; they harbored a lower percentage of erythroid cells (Gata1a$^+$) than did controls. In contrast, the percentage of endothelial cells (Fli1$^+$) was similar in the *dock11*-knockout embryos and controls (Fig. 4B, and Fig. S25B and S25C). Moreover, *dock11* knockdown recapitulated the anemia observed in *dock11*-knockout embryos (Fig. 4B). Because CDC42 is critical

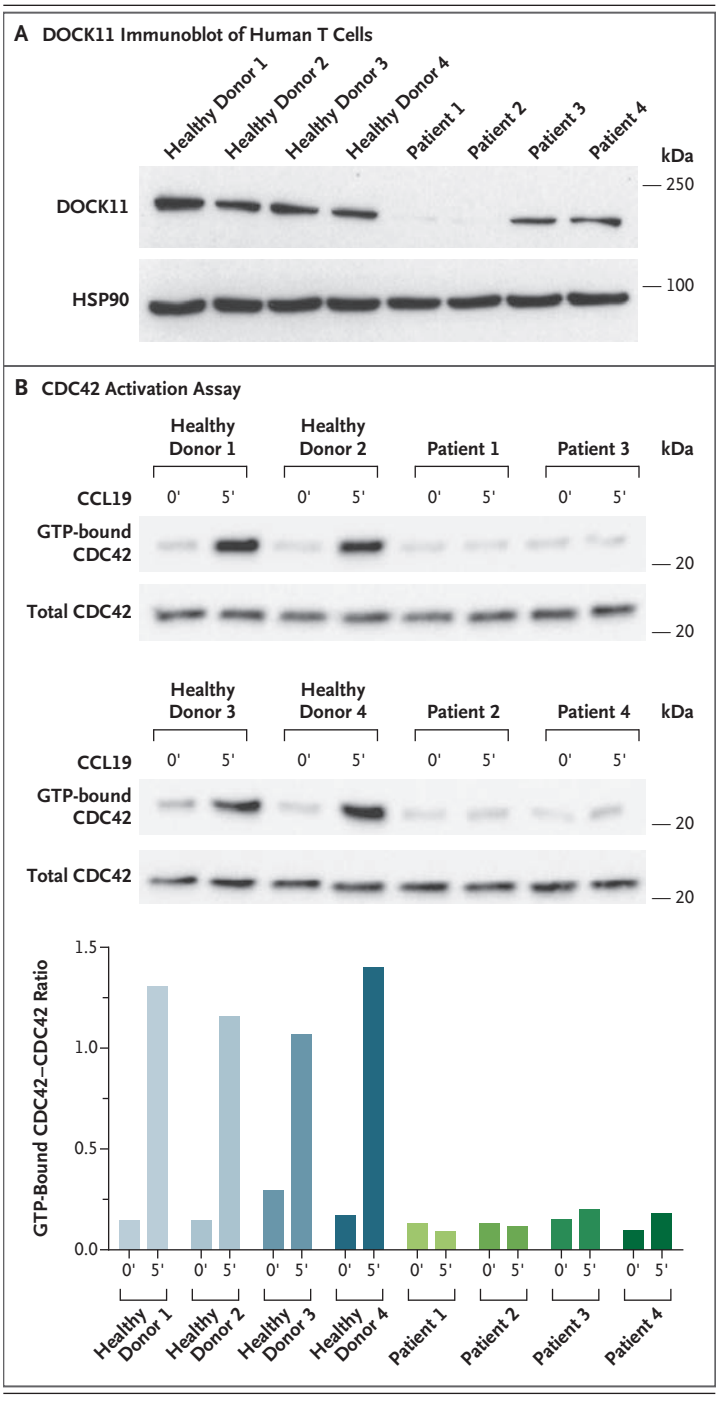


Figure 2. DOCK11 Protein Expression and CDC42 Activation in Lymphocytes.

Panel A shows absent DOCK11 expression in T cells from Patients 1 and 2 and retained DOCK11 expression in T cells from Patients 3 and 4 in representative immunoblots. Antibodies against heat shock protein 90 (HSP90) were used as a loading control. Panel B shows representative immunoblots and quantification of guanosine triphosphate (GTP)–bound (active) cell division cycle 42 (CDC42) and total CDC42. CDC42 activation on C-C motif chemokine ligand 19 (CCL19) stimulation was lower in B-lymphoblastoid cells from the patients than in the cells from healthy donors. The graph shows the ratio of GTP-bound CDC42 to total CDC42. Biologic replicates and quantification of the immunoblots displayed in Panels A and B are shown in Figures S9B and S12, respectively, in the Supplementary Appendix.

for murine erythropoiesis,[26,27] we speculated that a reduction in Gata1a$^+$ cells in *dock11*-knockout zebrafish embryos results from defective Cdc42 activity. The *cdc42*-knockdown embryos showed a reduction in the number of erythrocytes that was similar to the reduction in the *dock11*-knockout embryos (Fig. 4C).

To test our hypothesis that impaired activation of Cdc42 by Dock11 causes the anemia phenotype, we expressed a constitutively active form of human CDC42 (CDC42^{Q61L}) in *dock11*-knockout zebrafish embryos. Expression of CDC42^{Q61L} led to restoration of the numbers of Gata1a$^+$ cells (Fig. 4D). The percentages of apoptotic and mitotic red cells in *dock11*-knockout embryos were unaltered (Fig. S25D and S25E), although the morphologic features of the red cells were abnormal (Fig. S26). Moreover, the altered nuclear–cytoplasmic ratio, a measure for terminal differentiation, suggested hampered erythroid maturation.

To explore whether DOCK11 plays a similar role in human erythropoiesis, we investigated its role in CD34+ cord blood cells. DOCK11 was expressed at different stages during erythroid differentiation of CD34+ cells (Fig. S27A). On *DOCK11* knockdown, cell expansion during differentiation was reduced, concomitant with increased cell death, but no difference in the percentage of apoptotic cells was detected (Fig. 4E and Fig. S27B). A relative paucity of CD117+CD36+ and CD71+CD235+ erythroid cells was identified, a finding that is consistent with early developmental delay (Fig. 4F and Fig. S27C). Collectively, these experiments highlight the pivotal function of the DOCK11–CDC42 axis in regulating erythropoiesis across species.

DISCUSSION

We identified rare hemizygous loss-of-function mutations in *DOCK11* that led to impaired CDC42 activation as the cause of a novel immune dys-

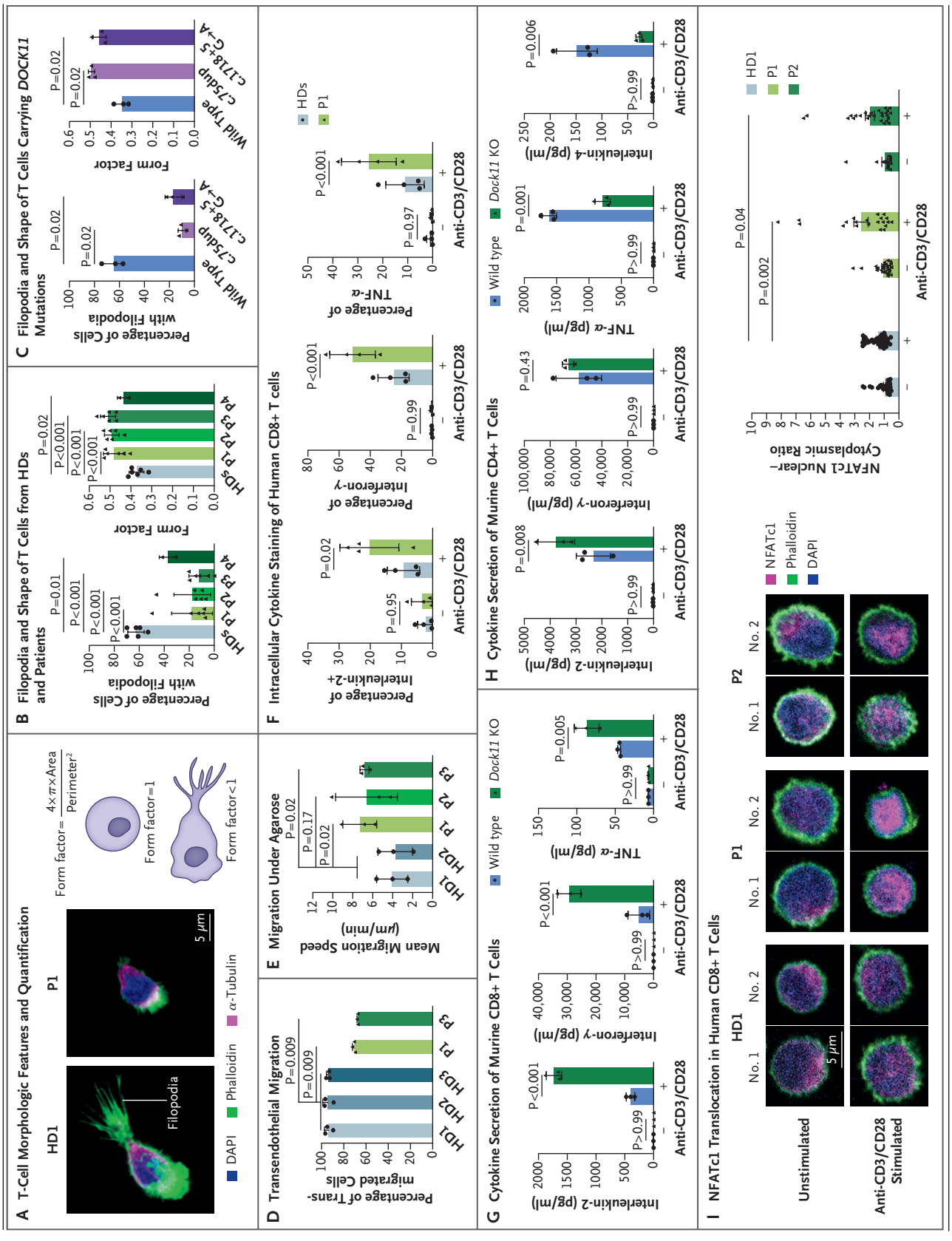


Figure 3 (facing page). DOCK11 Deficiency and Morphologic and Functional Defects in Lymphocytes.

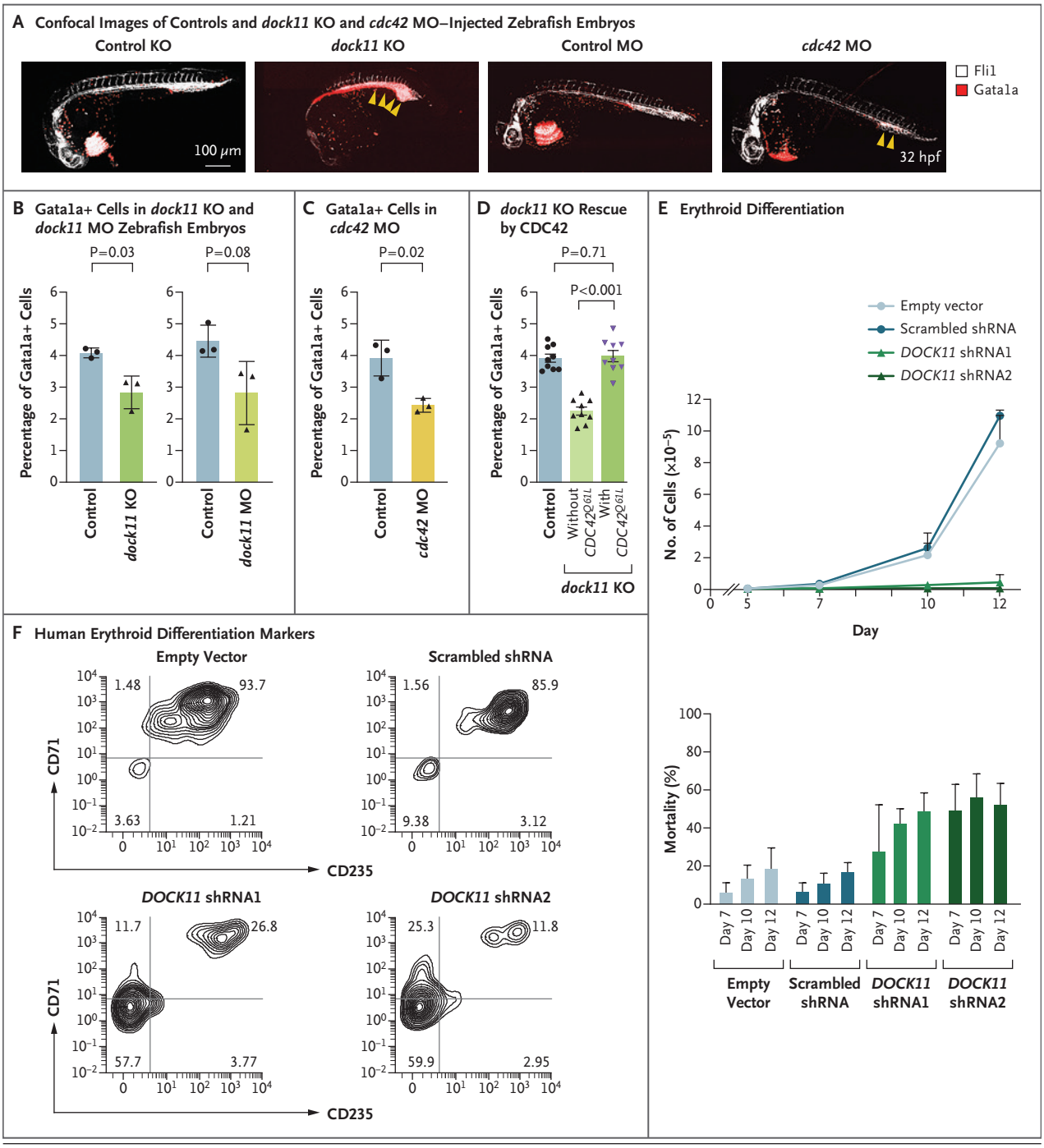
Panel A shows representative images of T-cell morphologic changes on interaction with fibronectin (left side of panel) in Healthy Donor (HD) 1 and Patient (P) 1 and the quantification scheme used (right side of panel). Magnified images correlate with those in Figure S14A. The form factor equals 1 when the object is perfectly circular. DAPI denotes nuclear marker 4′,6-diamidino-2-phenylindole. Panel B shows that the percentage of T cells with filopodia was lower in the patients than in the HDs. Cell circularity was higher in the T cells from the patients than in those from the HDs. Panel C shows the quantification of confocal images. The number of cells with filopodia was lower and cell circularity (form factor) was greater in *DOCK11* knock-in T cells (*DOCK11* c.75dup and c.1718+5G→A) than in wild-type cells. Panel D shows that the transmigration capacity of T cells was lower in the patients than in the HDs. Panel E shows that the mean migration speed of the CD8+ T cells under agarose was higher in the patients than in the HDs. Panel F shows that the intracellular cytokine levels on anti-CD3 and anti-CD28 (anti-CD3/CD28) stimulation (plus sign), as assessed by flow cytometry, was higher in the T cells from P1 than in those from the HDs. The minus sign denotes no stimulation and TNF-α tumor necrosis factor α. Panels G and H show that the cytokine levels in CD8+ (Panel G) and CD4+ (Panel H) T cells from *Dock11*-knockout (KO) mice were altered on anti-CD3/CD28 stimulation, as compared with wild-type cells. Panel I shows two representative images per condition (No. 1 and No. 2, left side of panel) and quantification of nuclear factor of activated T cell 1 (NFATc1) nuclear–cytoplasmic ratio from one representative experiment (right side of panel). The degree of nuclear translocation on anti-CD3/CD28 stimulation was higher in the T cells from the patients than in those from the HDs. In Panels B and C and E through H, the results are given as mean values, with standard deviations (I bars), derived from multiple measurements. In Panel D, the results are given as mean values, with the standard deviations (I bars), derived from five imaging fields in each of three measurements. In Panel I, the results are given as mean values of multiple measurements, with standard errors (I bars). Solid triangles indicate T cells from the patients (Panels B, D through F, and I), *DOCK11* knock-in T cells (*DOCK11* c.75dup and c.1718+5G→A) (Panel C), or T cells from *Dock11*-knockout mice (Panels G and H). Solid circles indicate the respective controls including HDs (Panels B, D through F, and I), unedited HD-derived wild-type T cells (Panel C), and wild-type mice (Panels G and H). In Panels B through E, statistical analysis was performed with the use of a Mann–Whitney test; in Panels F through H, with the use of a two-way analysis of variance with a Šidák correction test for multiple comparison; and in Panel I, with the use of an unpaired Student's t-test. Details are provided in the Supplementary Appendix.

regulation disorder. Clinically, DOCK11 deficiency includes phenotypes associated with *CDC42* mutations, such as recurrent infections, immune dysregulation, anemia, platelet anomalies, and neurodevelopmental abnormalities. Unlike patients carrying *CDC42* mutations,[28-31] patients with DOCK11 deficiency had no detectable facial abnormalities, neutropenia, monocytopenia, or hemophagocytic lymphohistiocytosis.

Despite the systemic inflammatory phenotype in DOCK11 deficiency, we did not detect autoantibodies, alterations in regulatory T-cell numbers, or overt defects in immune synapse assembly, features that are seen in other genetic disorders involving aberrant actin assembly.[17,19,32,33] It is notable that patients with DOCK11 deficiency showed increased frequencies of $CD21^{low}$ B cells and double-negative T cells, which indicate a propensity toward immune dysregulation.[34] Identification of additional patients with *DOCK11* mutations would permit delineation of a broader phenotypic spectrum of the disease.

DOCK11-deficient T cells showed reduced transendothelial migration in vitro, yet also increased migration speed under confinement in vitro and in vivo, which suggests that DOCK11 is involved in leukocyte diapedesis and interstitial migration. Together with the impaired capacity to form filopodia, this may translate into aberrant target recognition and tissue positioning. Such homing and migration alterations in T-cell subsets could contribute to infection susceptibility and severity.

Autoinflammation, which has been observed in several actin-related deficiencies, has been linked to increased inflammasome-mediated secretion of interleukin-1β and interleukin-18.[28,31,35-37] However, we did not find evidence of this mechanism in DOCK11-deficient monocytes. Instead, we observed altered levels of proinflammatory cytokines and an increased degree of nuclear translocation of NFATc1 in T cells from two patients. Similarly, T cells from the *Dock11*-knockout mice showed aberrant JNK phosphorylation, nuclear translocation of NFATc1, and altered cytokine production. These data, in combination with data from studies involving mice showing that CDC42 negatively regulates effector and memory T-cell activation,[14,15] implicate the DOCK11–CDC42 axis in T-cell proliferation, activation, and cytokine production.



Defects in DOCK8, another CDC42-activating GEF, cause type 2 cytokine skewing and reduced proliferation.[5,16] Given the developmental stage–specific role of CDC42 in regulating T-cell activation[15] and the link between specific spatiotemporal patterns of CDC42 with T-cell functions,[38] we speculate that GEFs, including DOCK8 and DOCK11, regulate CDC42-dependent processes in a manner dependent on cell type, localization, and stimulus. The distinct clinical presentation of patients with DOCK11 deficiency as opposed to DOCK2 and DOCK8 deficiencies further supports the notion that DOCK family proteins promote nonredundant Rho GTPase activation.[4,5]

Figure 4 (facing page). DOCK11 Deficiency and Anemia and Aberrant Red-Cell Morphologic Forms across Species.

Panel A shows representative confocal images of the accumulation of intravascular blood (yellow arrows) in *dock11*- KO and *cdc42* morpholino (MO)–mediated knockdown *fli1:GFP;gata1a:dsRed* transgenic zebrafish embryos as compared with their respective controls. Single color images are shown in Figure S25A. The abbreviation hpf denotes hours postfertilization. Panels B and C show that the Gata1a+ erythroid cell number, assessed by flow cytometry, is lower in *dock11* KO and *dock11* MO-mediated (Panel B) or *cdc42* MO-mediated (Panel C) knockdown zebrafish embryos than in their respective controls. Panel D shows rescue of Gata1a+ erythroid cell numbers in *dock11* KO embryos injected with $CDC42^{Q61L}$ messenger RNA for expression of constitutively active CDC42. Panels E and F show human CD34+ cells during erythroid differentiation, which are transduced with empty, scramble, or *DOCK11* short hairpin RNA (shRNA)–containing pLKO.1-CMV-tGFP vectors at day 2 and sorted for green fluorescent protein (GFP)–positive cells at day 5. Panel E shows that the cell counts were lower (upper graph) and mortality was higher (lower graph) at different stages of differentiation (days 5 through 12) in *DOCK11*-silenced cells than in control cells. Panel F shows a delay in erythroid differentiation in *DOCK11*-silenced cells in representative flow-cytometry plots of GFP-positive cells stained with CD71 and CD235 (glycophorin A) after 10 days in erythroid culture. In Panels B, C, and E, the results are given as the mean values of multiple measurements, with standard deviations (I bars or T bars [lower graph in Panel E]). In Panel D, the results are given as the mean values of multiple measurements, with standard errors (I bars). Solid triangles indicate Gata1a+ cells from *dock11*-knockout, *dock11*-knockdown or *cdc42*-knockdown zebrafish embryos (Panels B through D), or *DOCK11*-silenced CD34+ cells (Panels E and F). Solid circles indicate the respective control zebrafish embryos (Panels B through D) and control CD34+ cells (Panels E and F). In Panels B and C, statistical analysis was performed with the use of a paired Student's t-test, and in Panel D, with the use of a Mann–Whitney test. Details are provided in the Supplementary Appendix.

Data from the patients and the zebrafish model indicate that the anemia was not autoimmune-related. The erythroid hypoplasia in Patient 1 is consistent with a bone marrow defect and the observed erythroid-differentiation defect in *DOCK11*-knockdown CD34+ cord blood cells. The erythrocyte morphologic features in the zebrafish model were reminiscent of the anisopoikilocytosis that was observed in the patients with DOCK11 deficiency. The contribution of aberrant assembly of the red-cell membrane skeleton (in which actin is a component) to the anemia is probably less important; we did not observe increased red-cell hemolysis, which is characteristic of erythrocyte membranopathies.[8]

Clinical management of complex immune dysregulation disorders remains challenging because of the need for immunosuppressive agents, the use of which further increases the risk of severe and life-threatening infections. The high mortality among the patients with DOCK11 deficiency that we observed in this study (Patients 1, 2, and 3 had died during the evaluation [Table 1]) underlines the importance of developing more specific treatment strategies. In a finding that was consistent with the unaltered levels of interleukin-1β in a *DOCK11*-knockout monocyte-like cell line, the interleukin-1–receptor antagonist anakinra, used in other autoinflammatory conditions,[28] had no sustained effect in Patient 3. It was intriguing that targeting interferon-γ by emapalumab was successful in one patient with a missense variant in *CDC42*.[28] Emapalumab or other drugs targeting specific cytokines with increased levels in DOCK11-deficient T cells may be thus beneficial, yet finding the right balance between efficiency and potential side effects is challenging. Allogenic hematopoietic stem-cell transplantation had been considered for the three patients who died during the evaluation. The clinical condition of the remaining patient is currently stable, but in case of deterioration, the risk–benefit for hematopoietic stem-cell transplantation will be reassessed. Future studies may also assess whether gene therapy may offer an alternative curative treatment strategy.

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APPENDIX

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