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Brief Communication

Hypomorphic function and somatic reversion of DOCK8 cause combined immunodeficiency without hyper-IgE

Anne-Kathrin Kienzler [a,b,*], Pauline A. van Schouwenburg [a,b], John Taylor [c], Ishita Marwah [a,b], Richa U. Sharma [a,b], Charlotte Noakes [c], Kate Thomson [c], Ross Sadler [d], Shelley Segal [e], Berne Ferry [d], Jenny C. Taylor [f], Edward Blair [g], Helen Chapel [a,b], Smita Y. Patel [a,b]

[a] Nuffield Department of Medicine, Experimental Medicine Division, University of Oxford, UK
[b] Oxford NIHR Biomedical Research Centre, John Radcliffe Hospital, Oxford, UK
[c] Oxford NHS Regional Molecular Genetics Laboratory, Oxford University Hospitals NHS Trust, Oxford, UK
[d] Department of Clinical Laboratory Immunology, Churchill Hospital, Oxford University Hospitals NHS Trust, Oxford, UK
[e] Department of Paediatrics, Children's Hospital, Oxford University NHS Hospitals Trust, Oxford, UK
[f] Oxford Biomedical Research Centre, Wellcome Trust Centre for Human Genetics, Oxford, UK
[g] Department of Clinical Genetics, Churchill Hospital, Oxford University NHS Hospitals Trust, Oxford, UK

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ABSTRACT

Loss-of-function mutations in DOCK8 are linked to hyper-IgE syndrome. Patients typically present with recurrent sinopulmonary infections, severe cutaneous viral infections, food allergies and elevated serum IgE. Although patients may present with a spectrum of disease-related symptoms, molecular mechanisms explaining phenotypic variability in patients are poorly defined. Here we characterized a novel compound heterozygous mutation in *DOCK8* in a patient diagnosed with primary combined immunodeficiency which was not typical of classical DOCK8 deficiency. In contrast to previously identified mutations in *DOCK8* which result in complete loss of function, the newly identified single nucleotide insertion results in expression of a truncated DOCK8 protein. Functional evaluation of the truncated DOCK8 protein revealed its hypomorphic function. In addition we found somatic reversion of *DOCK8* predominantly in T cells. The combination of somatic reversion and hypomorphic DOCK8 function explains the milder and atypical phenotype of the patient and further broadens the spectrum of DOCK8-associated disease.

1. Introduction

Bi-allelic loss-of-function mutations in the guanine-nucleotide exchange factor dedicator of cytokinesis 8 (*DOCK8*) cause autosomal recessive hyper-IgE syndrome. The vast majority of DOCK8-deficient patients present with combined immunodeficiency characterized by recurrent sino-pulmonary and/or gastrointestinal infections, severe cutaneous viral infections, severe atopy, eosinophilia and massively elevated serum IgE levels. Patients also have a predisposition to cancer [1,2].

Recent studies have highlighted the phenotypic variability of patients suffering from DOCK8-deficiency [3,4]. Patients with susceptibility to infection but less severe allergic disease were identified to carry a functional wild-type *DOCK8* allele in lymphocyte subpopulations due to somatic reversion of the mutated *DOCK8* alleles [3].

Here we report for the first time a patient with a hypomorphic mutation in *DOCK8* presenting with recurrent bacterial infections, low serum IgM and IgG, CD4 lymphopenia and severely impaired vaccination responses, but without severe viral infections and severe atopy.

2. Methods

Detailed information can be found in the Supplementary data.

We submitted the variants identified in DOCK8 to be made publically available by ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/). The accession numbers are SCV000257461 (deletion chr9:204193-343954), SCV000257462 (c.65C>T), SCV000257463 (c.289C>A), SCV000257464 (c4107C>G), SCV000257465 (c.5433G>A), and SCV000257466 (c.6019dupT).

Abbreviations: CFSE, Carboxyfluorescein diacetate, succinimidyl ester; DHR1/2, DOCK homology region; DOCK8, Dedicator of cytokinesis 8; EBV, Epstein–Barr-Virus; HC, Healthy control; Mut, Mutated DOCK8 transcript (referring to c.6019dupT); PBMC, Peripheral blood mononuclear cell; PHA, Phytohemagglutinin; Pt, Patient; Trunc, Truncated DOCK8 protein.

* Corresponding author at: University of Oxford, NDM Experimental Medicine and BRC Translational Immunology Laboratory, John Radcliffe Hospital, Level 7, Room 7400, Headley Way, Oxford OX3 9DU, UK.
E-mail address: anne-kathrin.kienzler@ndm.ox.ac.uk (A.-K. Kienzler).

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3. Case presentation

The female patient is the only child of non-consanguineous, healthy parents. She presented aged eight with a two-year history of recurrent bacterial chest infections and radiological signs of early bronchiectasis. The patient also had a long-standing history of mild eczema and asthma requiring treatment with inhaled corticosteroids and beta-agonists. All routine childhood immunizations were received uneventfully. Immunological evaluation (Table 1) revealed low serum IgM, normal IgA and IgE, and borderline-low IgG levels which dropped significantly over 12 months. Measurement of responses to previous immunizations demonstrated protective levels of IgG to tetanus toxoid but absent IgG to *Haemophilus influenzae type b*, pneumococcal polysaccharides and measles. Also, despite a history of a normal course of chicken-pox, varicella zoster virus IgG was undetectable. Lymphocyte subset analysis demonstrated low $CD4^+$ T cell numbers and low frequencies of $CD27^+$ effector B cells (Table 1). Following the failure of antibiotic prophylaxis alone to reduce the infection burden, immunoglobulin replacement therapy was commenced with a good clinical response. Sequence analysis of recombination-activating gene (*RAG*) *1*, *RAG2*, and DNA cross-link repair 1C (*DCLRE1C* encoding Artemis) did not reveal any mutations. Therefore the patient was given a diagnosis of undefined primary combined immunodeficiency.

Table 1
Immunological characteristics of the patient.

Parameter	Patient		Normal range
Serum immunoglobulin			
IgM (g/L)	↓	0.23	0.4–2.5
IgG (g/L)	N/(↓)[a]	7.04	6.0–13.0
IgA (g/L)	N	2.75	0.8–3.0
IgE[b] (kU/L)	N	190	<380
Leukocyte count (no./μL) and phenotype (%)			
Lymphocytes (/μL)	N	1170	1000–5300
$CD19^+$ B cells			
$CD19^+$ (/μL)	N	530	200–600
$CD38^{++}IgM^{++}$ transitional (%)	↑	14.2	4.6–8.3[c]
$CD27^-IgD^+$ naive (%)	↑	91	47.3–77.0[c]
$CD27^+IgD^+$ natural effector (%)	↓	3.44	5.2–20.4[c]
$CD27^+IgD^-$ switched memory (%)	↓	1.12	10.9–30.4[c]
$CD3^+$ T cells			
$CD3^+$ (/μL)	↓	570	800–3500
$CD4^+$ (/μL)	↓	180	400–2100
$CD4^+CD45RA^+$ naïve (%)	↓	39	46–77[d]
$CD4^+CD45RO^+$ memory (%)	↑	61.1	13–30[d]
$CD4^+CD45RA^+CD31^+$ RTE (%)	↓	32.5	42–74[d]
$CD8^+$ (/μL)	N	280	200–1200
$CD8^+CD45RA^+$ naïve (%)	↓	41.4	63–92[d]
$CD8^+$ $CD45RO^+$ memory (%)	↑	58.6	4–21[d]
$CD16^+CD56^+$ NK cells (/μL)	N	80	70–1200
Eosinophils (/μL)	↑	1150	<350
TRECs (/10^6 MNC)	↓	1197	>10,000
Specific IgG responses			
Tetanus toxoid (IU/ml)	N	0.03	>0.01
Haemophilus influenzae type b (μg/ml)	↓	<0.15	0.15–1.0
Pneumococcal polysaccharides (U/ml)	↓	1	>14
Measles	Absent		
Varicella zoster	Absent		
T cell proliferation			
PHA	↓↓ absent at 10 years of age ↓ decreased at 15 years of age		

N, value within normal range; ↑, value above normal range; ↓, value below normal range; RTE, recent thymic emigrants; TREC, T cell receptor rearrangement excision circle; PHA, phytohemagglutinin.

[a] Serum IgG dropped within a year after initial presentation.

[b] Serum IgE was measured after identification of the DOCK8 mutation on serum samples frozen before start of immunoglobulin replacement therapy.

[c] 5–95 percentile range for age-matched controls adopted from [7].

[d] 10–90 percentile range for age-matched controls adopted from [8].

4. Results and discussion

To identify the underlying disease cause, we undertook whole exome sequencing (WES) on the patient and both parents. A novel heterozygous frameshift variant was detected in *DOCK8* in the patient and her mother. Sanger sequencing confirmed a single-nucleotide duplication [c.6019dupT (p.Tyr2007Leufs*12)] within the conserved DOCK homology region 2 (DHR2) domain of *DOCK8*, leading to a frameshift and premature stop-codon (Fig. 1, A and C, and Supplementary Table 1]. As autosomal recessive mutations in *DOCK8* cause combined immunodeficiency, we screened for further variants in *DOCK8*. Analysis of single nucleotide polymorphisms (SNPs) across *DOCK8* in the trio revealed apparent loss of paternal contribution of two SNPs in a 5′ region of the gene (Supplementary Table 1), indicating the possibility of a paternally inherited deletion. Array comparative genomic hybridization analysis confirmed a large deletion encompassing exons 1–14 of *DOCK8* in the patient and her father (approx. 140 kb deletion of 9p24.3, base pair 204,193 to 343,954) (Fig. 1, B and C). This novel compound heterozygous mutation in *DOCK8* was the only disease-causing variant identified in the patient (Supplementary Tables 2–4).

The deletion in *DOCK8* is predicted to result in the absence of any protein expression since the deletion includes the start codon. The frameshift mutation is predicted to result in the production of a truncated protein lacking 81 amino acids (~11 kDa). Indeed, patient EBV cells expressed low amounts of a truncated DOCK8 protein, but not the full-length protein (Fig. 1D). We hypothesized that this truncated DOCK8 protein has hypomorphic function accounting for the milder clinical presentation of our patient.

Previous studies of DOCK8-deficient patients report impaired T cell proliferation [1,2]. At the age of ten years, both $CD4^+$ and $CD8^+$ patient T cells did not proliferate in response to mitogen (PHA) stimulation (Fig. 2A), consistent with an inability of the truncated DOCK8 protein to transmit proliferative signals. Interestingly, when studying T cell proliferation at the time of WES (four years later), proliferation of both $CD4^+$ and $CD8^+$ patient T cells was present, although reduced compared to a healthy control (Fig. 2B). We hypothesized that this difference in T cell proliferation could be explained by somatic reversion of *DOCK8*. Indeed, Sanger sequencing of *DOCK8* cDNA of T cells and subsequent peak height quantification revealed that two thirds of all *DOCK8* transcripts are wild-type (Fig. 2C), showing somatic reversion of *DOCK8* as previously described [3]. Somatic reversion of *DOCK8* in T cells was confirmed by pyrosequencing of *DOCK8* (Fig. 2D). Therefore improved T cell proliferation over time is likely to be due to somatic reversion of *DOCK8* in patient T cells.

The frequency of somatic reversion in B cells was half compared to the *DOCK8* reversion in T cells (Fig. 2D) indicating a higher proportion of B cells expressing only the truncated DOCK8. Interestingly, patient $CD19^+$ B cells immortalized with EBV expressed only mutated *DOCK8* transcripts (Fig. 2E) suggesting selective outgrowth of cells that did not undergo somatic reversion. As migration of DOCK8-deficient B cells has previously been shown to be impaired [5], we investigated the functionality of the truncated DOCK8 protein in a transwell assay using the patient EBV B cells expressing only the truncated version of DOCK8. Migration of patient EBV B cells was comparable to the EBV B cell lines of healthy controls and significantly better than that of EBV B cells of a patient with complete loss-of-function mutation in DOCK8 ($DOCK8^{null}$) (Fig. 2F). This shows that migration was not significantly affected by the truncation of the DOCK8 protein.

DOCK8 is a large protein with at least two described functional domains, the N-terminal DOCK homology region (DHR) 1 domain and the C-terminal DHR2 domain. There is only little data available on which downstream cellular functions are mediated by each domain. Our data showing normal migration of patient EBV cells expressing a truncated DOCK8 protein in which the C-terminal DHR2 domain is disrupted suggest that this domain is dispensable for lymphocyte migration. In line, Ham et al. [6] showed that the N-terminal region of