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ORIGINAL ARTICLE

# Compound Heterozygous DOCK8 Mutations in a Patient with B Lymphoblastic Leukemia and EBV-Associated Diffuse Large B Cell Lymphoma

David Buchbinder[1,2] · Ivan Kirov[2,3] · Jeffrey Danielson[4] · Nirali N. Shah[5] · Alexandra F. Freeman[4] · Rishikesh S. Chavan[2,3] · Helen C. Su[4]

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## Abstract

Mutations in Dedicator of cytokinesis 8 (*DOCK8*) are a rare cause of combined immunodeficiency associated with atopy, infectious susceptibility, and risk for malignancy. We describe a 22-year-old male with a diagnosis of B cell lymphoblastic leukemia followed by Epstein-Barr virus (EBV)-associated diffuse large B cell lymphoma (DLBCL) with compound heterozygous mutations in *DOCK8* and normal intracellular DOCK8 protein expression. Here, B cell lymphoblastic leukemia followed by EBV-associated DLBCL led to the discovery of DOCK8 deficiency. For instances of high clinical suspicion despite normal DOCK8 protein expression, additional functional testing is critical to make a diagnosis. Understanding the spectrum of DOCK8 mutants and their phenotypes will improve our understanding of DOCK8 deficiency.

**Keywords** B cell lymphoblastic leukemia · diffuse large B cell lymphoma · combined immunodeficiency · dedicator of cytokinesis 8

## Abbreviations

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| COG | Children's Oncology Group |
| DLBCL | diffuse large B cell lymphoma |
| *DOCK8* | Dedicator of cytokinesis 8 |
| EBV | Epstein-Barr virus |
| GVHD | graft-versus-host disease |
| HPV | Human Papilloma Virus |
| HSCT | hematopoietic stem cell transplantation |
| MCV | Molluscum |
| VZV | Varicella Zoster Virus |
| WES | whole exome sequencing |

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✉ David Buchbinder
dbuchbinder@choc.org

[1] Department of Hematology, Children's Hospital of Orange County, 1201 W. La Veta Avenue, Orange, CA 92868, USA

[2] Department of Pediatrics, University of California at Irvine, Orange, CA, USA

[3] Department of Oncology, Children's Hospital of Orange County, Orange, CA, USA

[4] Laboratory of Clinical Immunology and Microbiology, NIAID, NIH, Bethesda, MD, USA

[5] Pediatric Oncology Branch, NCI, NIH, Bethesda, MD, USA

## Introduction

Dedicator of cytokinesis 8 (*DOCK8)* bi-allelic mutations are associated with a combined immunodeficiency disorder marked by atopy, infections susceptibility with a preponderance of cutaneous viral disease, and risk for malignancy [1]. DOCK8 protein plays a vital role in cytoskeletal rearrangement, maintenance of cell shape integrity, and immune cell trafficking and survival [2–4]. Atopic features including eczematous dermatitis and food allergies are frequent [5–8]. Chronic viral infections (e.g., Herpes Simplex Virus, Varicella Zoster Virus (VZV), Human Papilloma Virus (HPV), Molluscum (MCV), and Epstein-Barr virus (EBV)) are also frequent [5–8]. Many of these chronic viral infections predispose affected patients to the development of cancer such as HPV-associated carcinoma and EBV-associated lymphoma as well as VZV-associated vasculitis [5–8].

Accurate and timely diagnosis of DOCK8 deficiency is necessary for patient management. The germline mutations in *DOCK8* are homozygous or compound heterozygous deletions that are associated with the loss of DOCK8 protein expression [5–8]. This loss of DOCK8 protein expression in nearly all reported cases justifies screening for the presence of intracellular DOCK8 protein by flow cytometry [9]. Supportive care including antimicrobials and immunoglobulin supplementation are used in suspected or confirmed cases; however, hematopoietic stem cell transplantation (HSCT) is recommended [10, 11]. Here, we describe a 22-year-old male with a history of B cell lymphoblastic leukemia who later developed EBV-associated diffuse large B cell lymphoma (DLBCL). Compound heterozygous mutations in *DOCK8* were identified by whole exome sequencing (WES). The pathogenicity of the variants was assessed.

## Case Description

A 22-year-old Caucasian male with nasal polyps and history of B lymphoblastic leukemia was diagnosed with EBV-associated DLBCL. His past medical history was remarkable for recurrent otitis media (12 months of age) and transient warts (4 years of age), without atopic features or peripheral blood eosinophilia. At 15 years of age, a B lymphoblastic leukemia was diagnosed. He was treated according to Children's Oncology Group (COG) Protocol AALL0232, achieved remission at the end of induction and completed 3 years of therapy. Infectious complications included osteomyelitis, sepsis with *Clostridium septicum*, nodular lung infiltrates suggestive of fungal disease, intestinal perforation complicated by enterocutaneous fistula formation, and *Prevotella loescheii* bacteremia.

At 22 years of age, still in remission from his B lymphoblastic leukemia, abdominal pain in association with cholelithiasis led to an incidental finding of a lung mass positive for EBV-associated DLBCL. He was treated according to Children's Oncology Group protocol ANHL1131, Group B was switched to Group C1 arm after residual disease was noted following the first cycle of consolidation. Complete remission was achieved. Infectious complications included a soft tissue infection, sepsis with *Streptococcus mitis*, and bacteremia with *Pseudomonas aeruginosa*. Given the second malignancy, the patient underwent testing for a primary immunodeficiency disorder. WES (Fulgent Genetics, Temple City, California) identified compound heterozygous mutations in *DOCK8* [NM_203447.3, c.1128_1132del (p.Ser376Argfs*4) and c.4474-1G>C]. The former variant is predicted to result in a frameshift in exon 11 and premature truncation of the gene product and the latter variant alters a highly conserved canonical splice acceptor site and is predicted to result in aberrant splicing. These variants are observed at a frequency of < 0.01% and the highest allele frequency of these variants is < 0.01% based on the Broad Institute database.

While in remission from his DLBCL, he was evaluated for haploidentical HSCT. Laboratory evaluation prior to HSCT included a complete blood cell count which demonstrated leukopenia (2600/μL), neutropenia (2100/μL), and panlymphocytopenia (0/μL) (Supplemental Table 1). Quantitative immunoglobulin levels documented an IgG level of 618 mg/dL (normal 613-1295), IgM level of < 6 mg/dL (normal 40–230), IgA level of 209 mg/dL (normal 82-453), and IgE level of 4.8 kU/L (normal < 214). Antibody responses to tetanus (0.04 IU/mL), *Haemophilus influenzae* Type b (0.11 mg/L), and pneumococcal 13-valent conjugate vaccine were suboptimal (< 1.3 μg/mL for 11 out of 13 serotypes assessed).

The patient is day +135 following a haploidentical peripheral blood stem cell transplant. Conditioning included fludarabine, cyclophosphamide, busulfan, and total body irradiation as previously published [10]. He received 5.9 × 10^6 CD34/kg from his father (EBV seropositive). Graft-versus-host disease (GVHD) prophylaxis included cyclophosphamide, tacrolimus, and mycophenolate mofetil. He achieved neutrophil engraftment on day +22. Whole blood chimerism demonstrated 100% donor DNA (day +26). His course was complicated by fevers and *Clostridium difficile* colitis on day +0. Fevers recurred on day +15 in association with *Stenotrophomonas maltophilia* bacteremia. The patient received defibrotide for a mild sinusoidal obstruction syndrome (day +27) with a peak total bilirubin of 6.5 mg/dL. He developed acute GVHD of the skin (grade 1, stage 2) treated with topical hydrocortisone and tacrolimus. VZV infection (day +57) was documented and treated with valacyclovir. Fevers also occurred on day +100 in association with *Corynebacterium jeikeium* bacteremia.

## Methods

Patient blood samples were obtained after the provision of written informed consent, using an Institutional Review Board approved consent. Intracellular flow cytometric detection of DOCK8 protein as described in [12] was utilized to assess for the presence of DOCK8 protein. We assessed the function of DOCK8 protein by measuring the elongation of lymphocytes as they migrated through a collagen matrix as described in [3] with the following modifications: collagen mixture was made same-day, then $2 \times 10^6$ cells were suspended in 450 μl of collagen mixture and allowed to polymerize at 37 °C for at least 90 min before being visualized using an AF6000 LX microscope (Leica) on a motorized stage, using a × 20 dry objective lens. Images were acquired at 60-s intervals.

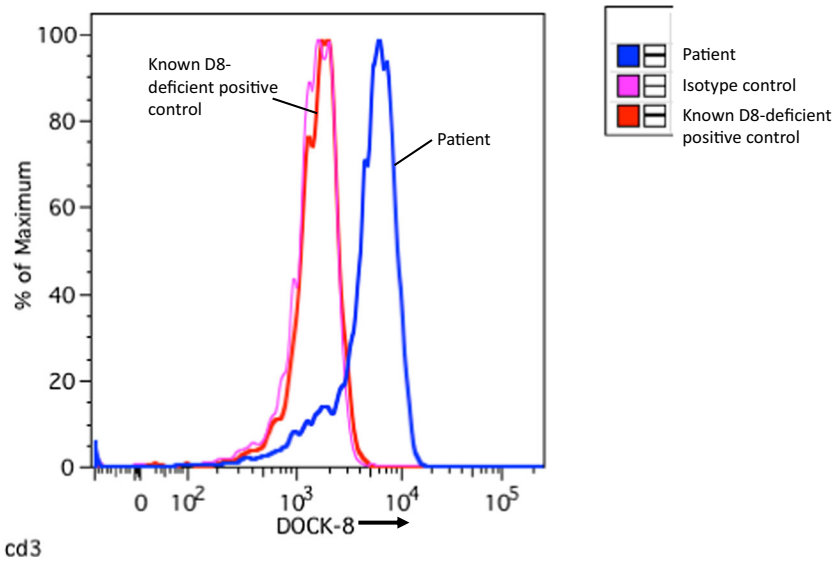


**Fig. 1** Representative flow cytometry histogram showing intracellular DOCK8 expression from our DOCK8-deficient patient, a known DOCK8-deficient positive control patient (red), and an isotype control staining

## Results and Discussion

Flow cytometric quantification of intracellular DOCK8 protein was normal when compared to normal control and a known DOCK8-deficient control [Chr9: g.328113C>T, NM_001193536.1:c.782C>T, p.(Ala261Val)] as depicted in Fig. 1. Additional functional assessment of DOCK8 protein was completed. When migrating through a collagen matrix, live cell imaging demonstrated abnormal elongation of patient lymphocytes as depicted in Fig. 2a when compared to normal

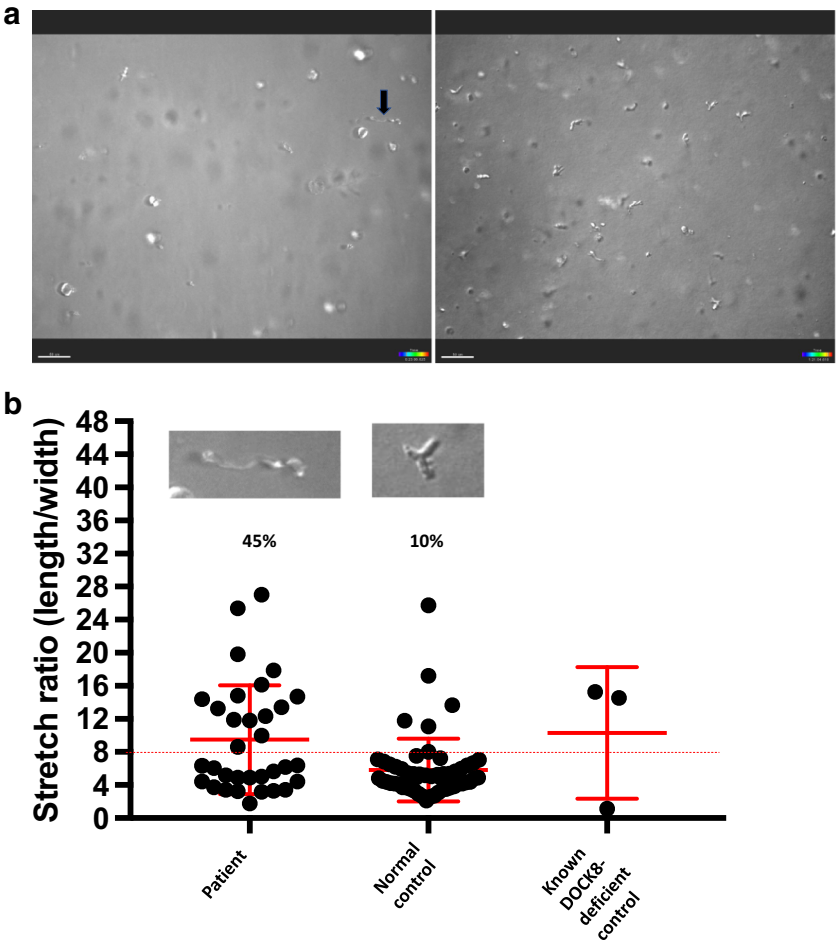


**Fig. 2** **a** Live cell imaging of T cells from our DOCK8-deficient patient (left panel) and a healthy control (right panel). Note the elongation of patient T cells (arrows). **b** Proportions of resting T cells that were abnormally elongated from our DOCK8-deficient patient, a healthy control, and a DOCK8-deficient patient control

control. As depicted in Fig. 2b, when studied, 45% of the patient's lymphocytes demonstrated abnormal elongation (stretch ratio > 8 defined by length/width), as compared with 10% of lymphocytes from a normal control.

To our knowledge, B lymphoblastic leukemia has not been previously reported in association with DOCK8 deficiency. In a survey of 136 patients with DOCK8 deficiency, 17% had either hematological or epithelial cell cancers in childhood [5]. Like our case, the diagnosis of cancer can be the first overt sign of immunodeficiency [5–8]. EBV infections are drivers of lymphoproliferation such as diffuse large B cell lymphoma as occurred in our case [5–8]. Other lymphoid malignancies have also been described such as Burkitt lymphoma, non-Hodgkin lymphoma, cutaneous T cell lymphoma-leukemia, T cell lymphoma, and acute myeloid leukemia [5–8]. Human papilloma virus–associated squamous cell carcinoma involving the skin, cervix, or penis may also occur [5–8].

Our case underscores that for instances of high clinical suspicion despite normal DOCK8 protein expression, functional testing is crucial. Among patients with DOCK8 deficiency, large deletions affecting *DOCK8* are frequent (~2/3rd of patients) [5–8]. With rare exceptions, the majority of *DOCK8* mutations result in the loss of DOCK8 protein expression. This facilitates our ability to rapidly screen for DOCK8 deficiency among suspected patients [9]. Absent or significantly reduced levels of DOCK8 protein can be documented using flow cytometric techniques. In our patient, functional testing supported a diagnosis of DOCK8 deficiency and ultimately HSCT.

In summary, B lymphoblastic leukemia followed by the development of EBV-associated DLBCL led to the discovery of DOCK8 deficiency. Our patient's course was atypical for the lack of high IgE, which is seen in the majority of DOCK8 deficient patients; however, our patient had a history of warts and low serum IgM. Our case underscores, for rare instances of high clinical suspicion despite normal DOCK8 protein expression, functional testing is crucial to make a definitive diagnosis. Understanding the spectrum of *DOCK8* mutants and their phenotypes will improve our recognition and treatment of this disease.

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**Authorship Contributions** All authors helped draft and approved the submitted manuscript. HCS and DB coordinated investigation of the patient's immunological analyses and creation of the manuscript. DB, IK, and RSC diagnosed and treated the patient's immune disorder. JD and HCS helped characterize the immunological disturbance. NNS, AFF, and HCS helped review the literature and illustrate the manuscript. DB, IK, and RSC recognized the patient's enigmatic presentation, initiated diagnostic studies, and made possible his multi-institutional investigation.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declared that have no conflict of interest.

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