

Hematopoietic stem cell transplantation complicated with EBV associated hemophagocytic lymphohistiocytosis in a patient with DOCK2 deficiency

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ABSTRACT

Background. Deducator of cytokinesis 2 (DOCK2) deficiency is a rare autosomal recessive combined immunodeficiency presenting with very early onset, severe bacterial and viral infections. In DOCK2 deficiency; T, B and NK cell numbers are decreased and functions are impaired resulting in severe atrophy of secondary lymphoid tissues. The aim of this report is to provide information on clinical and laboratory features and hematopoietic stem cell transplantation (HSCT) outcomes of a DOCK2 deficient patient. The patient was diagnosed by using a targeted next generation sequencing primary immunodeficiency (PID) panel. Lymphocyte subsets were measured by flow-cytometry.

Case. Here, we describe a patient with DOCK2 deficiency presented with severe combined immunodeficiency. He underwent HSCT without conditioning regimen before the genetic diagnosis and developed hemophagocytic lymphohistiocytosis (HLH) due to Epstein-Barr virus (EBV) infection.

Conclusions. Genetic testing is necessary for early diagnosis of DOCK2 deficiency. The curative treatment should be HSCT soon after diagnosis.

Key words: DOCK2 deficiency, hemophagocytic lymphohistiocytosis, hematopoietic stem cell transplantation, severe combined immune deficiency, EBV associated hemaphagocytic lymphohistiocytosis.

Deducator of cytokinesis 2 (DOCK2) deficiency is a rare autosomal recessive combined immunodeficiency presenting with very early onset, severe bacterial and viral infections. It is caused by homozygous or compound heterozygous mutations in the DOCK2 gene. The curative treatment is hematopoietic stem cell transplantation (HSCT) soon after diagnosis.

DOCK2 protein is predominantly expressed in hematopoietic cells and regulates leukocyte activation/migration by stimulating and activating Rac. Rac is a small signaling G

protein, which is a subfamily of the Rho family of GTPases. T and B cell migration to peripheral lymph node is mediated by CCL21, CXCL12 and CXCL13, homeostatic chemokines expressed in high endothelial venules and stromal cells. It is shown that in vitro stimulation of DOCK2-deficient (DOCK2(-/-)) T and B cells with CCL21, CXCL12 and CXCL13 do not stimulate chemotactic response leading to severe atrophy of secondary lymphoid tissues.^{1,2}

B and NK cell functions are impaired in addition to T cell number/function defects in DOCK2 deficiency. Sanui et al.³ showed that TCR-mediated Rac activation was almost totally abolished in (DOCK2(-/-)) cells. DOCK2 regulates T cell activation through Rac protein activation to provide immunological synapse formation.³ Rac activation is impaired in plasmacytoid

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Received 17th December 2020, revised 14th March 2021,
accepted 9th May 2021.

dendritic cells similar to lymphocytes in DOCK2 deficiency, resulting in the reduction of motility and the loss of polarity during chemotaxis. Recently Moens et al.⁴ showed that reactive oxygen species production are also partially impaired in DOCK2-deficient neutrophils.

As far as we know only 11 patients were reported.^{4,6} Here, we present a DOCK2 deficient patient who underwent HSCT without conditioning regimen and died because of Candida pneumonia after Epstein-Barr virus (EBV) associated hemophagocytic lymphohistiocytosis (HLH).

Case Report

A one-year-old boy was admitted to Hacettepe University Ihsan Dogramacı Children's Hospital with recurrent diarrhea. He had a normal birth-weight, received vaccination according to the routine immunization schedule. During the follow-up period, he had been hospitalized once due to perianal abscess and three times for pneumonia, including Cytomegalovirus (CMV) pneumonia at 4.5 months of age. His parents were consanguineous (first-degree cousins) and had a four-year-old healthy sister.

On physical examination, his body weight was 12 kg (50-75 percentiles (p)), height, 74 cm (<3p), head circumference, 46,5 cm (25p). He had hepatosplenomegaly (both 4 cm below the costal margin).

Laboratory tests showed normal total lymphocyte count (8400/mm³ (3200-12300)), normal immunoglobulin levels (IgA:79,7mg/dl (17-69)), IgG:2880mg/dl (463-1006), IgM:51,9mg/dl (46-159)) with low CD3 (31% (53-75)), CD4 (8% (32-51)), normal CD16/56 (4% (3-15)) and high CD19 (65% (16-35)) ratio and very low naive CD4+ T cells (0.2% (57.1-84.9)), naive CD8+ T cells (0.4% (28.4-80.6)) and recent thymic emigrants (1% (31-81)), suggestive of severe combined immunodeficiency. Monthly intravenous immunoglobulin (IVIG) treatment (400 mg/kg/dose), trimethoprim

sulfamethoxazole and fluconazol prophylaxis were started. At 14 months of age, HSCT (6,6X10⁸nucleated cells/kg) was performed from his HLA-identical father without any conditioning regimen. He was discharged three weeks after HSCT as he was well and an increase in CD3 after HSCT compatible with engraftment was recorded.

After receiving the informed consent from the parents, next-generation sequencing (NGS) primary immunodeficiency (PID) panel of 266 genes (ThermoFisher-Ion Torrent Platform) was performed on the patient and a homozygous DOCK2 mutation (NM_004946.2 c.1773_1774insG) (p.L592Afs*76) was found. The mutation was novel, not found in the healthy population (Gnomed, 1000G). Mutation taster predicts that the variant is disease-causing (CADD_Score:35). This is a null variant that causes a frameshift defect. The probability of being loss-of-function intolerant (pLI) score of the DOCK2 gene indicates that the gene function is not tolerant for loss-of-function variants. The c.1773_1774insG variant is classified as a pathogenic causative variant for the disease according to the American College of Medical Genetics and Genomics 2015 guidelines.⁷ The variant was detected as a heterozygous state in the parents by another NGS platform (Miseq-Illumina San Diego, CA) using PCR primers designed in-house and was visualized with ALAMUT® VISUAL (Interactive Biosoftware: France) software. We gave genetic counseling to the parents. Although there were other consanguineous marriages in the family, our patient was the only affected person.

Three months after HSCT, the patient was admitted to another center with a swelling on the neck. Physical examination revealed cervical lymphadenopathy, massive hepatosplenomegaly (6 cm and 9 cm below the costal margins respectively), and maculopapular rash on arms and trunk. As the skin biopsy was compatible with grade 1 graft versus host disease, cyclosporine was started. We did an extensive infection workup. It included blood

cultures, polymerase chain reaction (PCR) testing for mycobacteria and Herpes family, respiratory viruses, and parvovirus. Epstein-Barr Virus viremia (EBV DNA, 991 copies/mL) was present, and acyclovir was started. Despite the broad-spectrum antibiotic therapy, the fever continued, and the patient was evaluated for hemophagocytic lymphohistiocytosis (HLH). Anemia (hemoglobin:7,8g/dl) thrombocytopenia (59000/mm³), neutropenia (500/mm³), hyperferritinemia (6932µg/L) and hypertriglyceridemia (743mg/dl) were observed. Microscopic evaluation of bone marrow aspiration biopsy showed megaloblastic changes. However, hemophagocytosis was not present. Based on laboratory and clinical findings, the patient met the criteria for HLH. Corticosteroids and cyclosporine treatment were given according to the HLH-2004 protocol. Etoposide was not given as EBV DNA was high (20634 copy/ml). The chimerism analysis was low (6%), and the patient was given lymphocyte infusion from his EBV IgG positive donor for EBV reactivation. A second HSCT with conditioning regimen and interferon alpha-2b treatment was planned, but it could not be performed as the patient developed pneumonia. *Candida inconspicua* was isolated from bronchoalveolar lavage culture. Despite antifungal treatment, the pneumonia did not improve. Unfortunately, the patient died of respiratory insufficiency and multiorgan failure.

Discussion

DOCK2 deficiency is caused by compound heterozygous or homozygous mutations of the DOCK2 gene (Fig. 1). It is one of the rare CIDs reported in 11 patients.⁴⁻⁶ Six out of 11 underwent HSCT (Table I). Three patients received myeloablative while one received reduced-intensity conditioning regimens. One patient underwent HSCT from the match related sibling without conditioning, and she was well. In case of severe viral infections in CID, it is difficult to decide to give a conditioning regimen to the patient. As he had a very low T-Cell Receptor Excision Circles (TREC) (1% (31-81)) and T cell activation and had a history of severe cytomegalovirus (CMV) infection, we performed HSCT from his HLA-identical father without giving any conditioning regimen. After the diagnosis of DOCK2 deficiency, and due to the low level of chimerism, we planned a second HSCT. However, the patient died from EBV-associated secondary HLH and fungal pneumonia.

Patients with DOCK2 deficiency are highly prone to bacterial, viral, and fungal infections. Four out of the eleven patients previously reported died from infections (Table I). Two of the patients who died had multiple organ failures after varicella infection. The others died from recurrent CMV and *Klebsiella* infection and sepsis, respectively. The present patient had

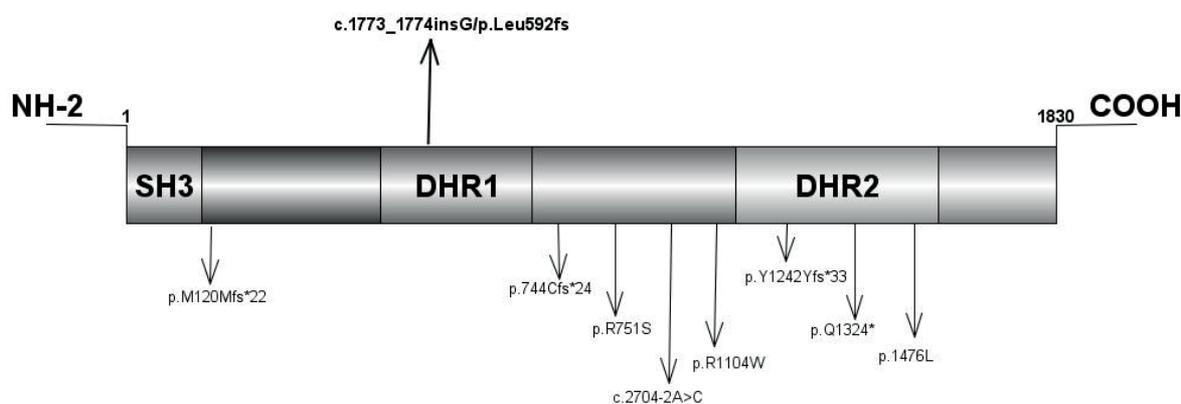


Fig. 1. Structure of DOCK2 protein showing the different domains and defined mutations.

Table I. DOCK2 defective patients, review of the literature.

Patients	Dobbs et all. P1	Dobbs et all. P2	Dobbs et all. P3	Dobbs et all. P4	Dobbs et all. P5	Moens et all. P6
DOCK2 mutation	p.Y1242Yfs*33	p.R1104W p.Q1324*	p.R751S	p.F744Cfs*27	p.M120Mfs*22 p.P1476L	c.2704-2 A>C
Ethnic origin	Lebanese	Finnish	Turkish	Turkish	Hispanic	Moroccan
Age at evaluation	5 months	2.5 years	6.3 years	1 years	4 months	One months
Sex	Male	Female	Male	Male	Male	Male
Symptoms at onset	bronchiolitis caused by RSV	granulomatous inflammation in lungs caused by M. avium intracellulare	meningoencephalitis caused by mumps	neonatal-onset chronic diarrhea, oral moniliasis	Interstitial pneumonia	Ulcerative perianal dermatitis, E. coli pyelonephritis
Other symptoms and findings	recurrent pneumoniae	recurrent otitis media, pneumonia and diarrhea, recurrent thrombocytopenia and thrombosis	recurrent upper and respiratory tract infection	growth failure, hepatomegaly	Rectal fistula	nephrotic syndrome, Omenn Syndrome
Viral infections	RSV	M. avium intracellulare	Mumps, Varicella	parainfluenza virus, type 3 adenovirus, CMV, K. pneumoniae		-
HSCT	+	+	-	-	+	-
Donor	MMRD	MUD			MRD	
Conditioning regimen	myeloablative conditioning with busulfan and fludarabine	reduced-intensity conditioning with treosulfan, fludarabine, and alemtuzumab			Myeloablative conditioning with busulfan and cyclophosphamide	
Cause of death	Alive	Alive	Varicella pneumonia	Sepsis (K.pneumoniae)	Alive	ARDS and capillary leak syndrome

ATG: anti-thymocyte globulin, GVHD: graft versus host disease, HSCT: hematopoietic stem cell transplantation, MMRD: mismatched related donor, MRD; matched related donor, MUD: matched unrelated donor, NA: not available

early-onset severe CMV infection. After HSCT, he suffered from EBV-associated secondary HLH and died from Candida pneumonia. In this case, IVIG was started even though immunoglobulin levels were normal. Because the patient's clinical and immunological features were compatible with severe combined immunodeficiency.

HLH is a rare and highly fatal multisystem inflammatory syndrome. HLH is common in patients with primary immunodeficiencies, particularly those with abnormal T cell functions. However, secondary HLH after transplantation in PID is uncommon.^{8,9} Asano et al.¹⁰ reported that secondary HLH developed in 37 out of 5427 HSCT cases, and four out of 37 were PID. Ali et al.¹¹ reported EBV-associated HLH in three out of 408 patients who underwent allogeneic HSCT,

and all three patients died. Epstein-Barr virus is the most frequently associated infectious agent with HLH.¹¹ In these cases, it is challenging to control HLH and EBV.¹⁰ Therefore, EBV titer monitoring is needed in the patients before and after the HSCT. In the present case, the HLH-2004 protocol was given without etoposide, because of high EBV DNA copy numbers (20634 copy/ml). Anti-CD20 antibody (rituximab) improves outcomes in patients with EBV-associated lymphoproliferative disorders by depleting B cells.¹² However, we could not give the patient the rituximab therapy. Because the patient's fever persisted, and we could not rule out sepsis. HLH could have possibly occurred in the patient due to graft failure.

In plasmacytoid dendritic cells, induction of type I interferons (IFN) critically depends on

Table I. Continued.

Patients	Moens et all. P7	Moens et all. P8	Moens et all. P9	Alosaimi et all. P10	Alosaimi et all. P11	Our patient P12
DOCK2 mutation	c.2704-2 A>C	c.2704-2 A>C	c.2704-2 A>C	Del 902-1078	Phe848fs	p.L592Afs*76
Ethnic origin	Moroccan	Moroccan	Moroccan	NA	NA	Turkish
Age at evaluation	NA	NA	NA	2 weeks of age	5 months	16 months
Sex	Female	Male	Female			Male
Symptoms at onset		NA	NA	chronic diarrhea, recurrent sinopulmonary infection, CMV viremia	chronic diarrhea, recurrent pneumonia, oral candidiasis	recurrent diarrhea
Other symptoms and findings		NA	NA	-	NA	perianal abscess, recurrent pneumonia, Growth retardation, EBV associated HLH
Viral infections	Varicella	NA	NA	CMV	-	CMV, EBV, Candida inconspicua
HSCT	-	+	+	+	-	+
Donor	NA	NA	MRD	MUD		MRD
Conditioning regimen		NA	Without conditioning	myeloablative conditioning with busulfan, fludarabine, and ATG		Without conditioning
Cause of death	Varicella infection	Alveolar hemorrhage after HSCT	Alive	Alive	Sepsis	GVHD Pneumoniae

ATG: anti-thymocyte globulin, GVHD: graft versus host disease, HSCT: hematopoietic stem cell transplantation, MMRD: mismatched related donor, MRD; matched related donor, MUD: matched unrelated donor, NA: not available

IFN regulatory factor 7 (IRF-7) and IkappaB Kinase IKK α , which activates IRF-7 after binding.¹³ In DOCK2 $^{-/-}$ plasmacytoid dendritic cells, phosphorylation of IKK- α and nuclear translocation of Interferon Regulatory Factors (IRF-7) were impaired, resulting in a selective loss of IFN- α /IFN- β induction. Recombinant IFN- α 2b replacement protects DOCK2 deficient fibroblasts from virus-induced cell death. During the HLH process, we planned to give interferon-alpha 2b treatment to the patient. However, we could not give as we lost the patient within a few days.

In conclusion, DOCK2 deficiency is a rare form of CID. Its diagnosis is challenging because these patients may have normal lymphocyte count on routine laboratory analysis. Accurate diagnosis usually requires genetic testing, and early diagnosis may increase the chance

of effective treatment. The curative treatment should be HSCT soon after diagnosis. Further studies are needed to define other treatment options for DOCK2 deficient patients.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ESA, DC, IT; data collection: ESA, IB; analysis and interpretation of results: CT, BC, DC; draft manuscript preparation: ESA, DC, IT. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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