Clinical, immunological features and follow up of 20 patients with dedicator of cytokinesis 8 (DOCK8) deficiency

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Abstract
Biallelic mutations in the dedicator of cytokinesis 8 gene (DOCK8) cause a progressive combined immunodeficiency (CID) characterized by susceptibility to severe viral skin infections, atopic diseases, recurrent respiratory infections, and malignancy. Hematopoietic stem cell transplantation (HSCT) is only curative treatment for the disease. However, there is limited information about long-term outcome of HSCT and its effect to protect against cancer development in DOCK8-deficient patients. In this study, we retrospectively evaluated clinical and immunologic characteristics of 20 DOCK8-deficient patients and outcome of 11 patients who underwent HSCT. We aimed to report the experience of our center and the result of the largest transplantation series of DOCK8 deficiency in our country. Median follow-up time is 71 months (min-max: 16-172) in all patients and 48 months (min-max: 5-84) in transplanted patients. Atopic dermatitis (18/20), recurrent respiratory tract infections (17/20), and food allergy (14/20) were the most frequent clinical manifestations. Failure to thrive (13/20), liver problems (12/20), bronchiectasis (11/20), chronic diarrhea (10/21), and autism spectrum disorders (3/20) were remarkable findings in our series. Elevated IgE level (20/20) and eosinophilia (17/20), low IgM level (15/20), and decreased CD3+ T (10/20) and CD4+ T (11/20) cell count were prominent laboratory findings. HSCT was performed in 11 patients. All patients achieved adequate engraftment and showed improvement in their clinical and immunologic findings. Atopic dermatitis and food allergies improved in all patients, and their dietary restriction was stopped except one patient who was transplanted recently. The frequency of infections was decreased. The overall survival is 91% in HSCT-received patients and 80% in all. HSCT at the earliest possible period with most suitable donor- and
INTRODUCTION

DOCK8 deficiency is an autosomal recessive CID syndrome characterized by atopy (dermatitis, food allergies, and asthma), recurrent sinopulmonary and severe/persistent cutaneous viral infections, mucocutaneous candidiasis, early-onset malignancy, and elevated IgE levels and eosinophilia. DOCK8 protein is a member of the DOCK180-related family, which is involved in the cytoskeletal rearrangements. DOCK8 is required for T lymphocyte, natural killer (NK) cell function and survival, dendritic cell migration, B-cell proliferation, immunoglobulin production, and antiviral cytokine production.

Atopic dermatitis (AD), food allergy (FA), and wheezing are usually the initial symptoms of DOCK8 deficiency during the early infancy period. However, chronic or severe infections, autoimmunity, and cancer development may affect the course of the disease and survival. HSCT is the lifesaving therapy in DOCK8-deficient patients; it should be performed as early as possible, before the occurrence of malignancies and fatal infections. HSCT has been shown to cure nearly all clinical and laboratory manifestations of DOCK8 deficiency by reconstituting the normal function of the immune system.

Key Message

In our 20 patients with DOCK8 deficiency, we obtained some different findings with classical findings. In addition, we present the results of the largest transplantation series of DOCK8 deficiency in our country.
Since the parental consanguinity rate is high (23.2%) in Turkey, DOCK8 deficiency has an important place among CIDs. Here, we retrospectively evaluated the clinical and immunologic features and treatment modalities of 20 patients with DOCK8 deficiency and follow-up results of hematopoietic stem cell transplant (HSCT) in 11 patients among them as a single-center experience.

2 | MATERIALS AND METHODS

2.1 | Patients

Twenty patients (11 girls and 9 boys) from 14 families diagnosed and followed up with DOCK8 deficiency from 2004 to 2018 in Ankara University School of Medicine, Department of Pediatric Immunology-Allergy of Children's Hospital, were evaluated retrospectively. DOCK8 protein expression was evaluated by flow cytometry as previously described. Next-generation sequencing (NGS) and then Sanger’s sequencing methods were used to detect and validate the DOCK8 mutations, respectively. The study was approved by the local ethics committee of Ankara University Faculty of Medicine and performed in accordance with the principles of the Declaration of Helsinki. The written informed consent was obtained from all the patients and/or their parents.

2.2 | HSCT characteristics

Eleven of /20 patients (six girls and five boys) are transplanted so far. The median time from diagnosis to HSCT was 8 months (0-48 months).

Myeloablative conditioning (MAC) regimen consisting of IV fludarabine 40 mg/m²/day and IV busulfan 4 mg/kg/day on days -5, -4, -3, and -2 was administered to two patients (P5 and P7), while all others received treosulfan-based reduced-intensity conditioning (RIC). Treosulfan was given at a dose of 42 or 36 g/m² (<1 year of age) in three divided doses on −7, −6, and −5 days. CsA was given alone in six patients transplanted from MRD and in combination with mycophenolate mofetil (MMF) or methotrexate (MTX) in two patients where peripheral blood-derived stem cells (PBSCs) were used. One patient received tacrolimus and MTX. Antithymocyte globulin (ATG) was received weekly prophylactic, antimicrobial, and IVIG therapies.

2.3 | Engraftment

Myeloid engraftment was defined as the absolute neutrophil count being higher than 0.5 × 10⁹/L and platelet counts exceeding 50 × 10⁹/L without transfusion for at least three consecutive days. Immune reconstitution was evaluated by measuring the levels of CD3+, CD4+, CD8+, CD19+, CD20+, CD16+ 56+, CD4+ CD45RA+, and CD45RA+ CD31 (recent thymic emigrant, RTE) cells with flow cytometry (Beckman Coulter, Navios Software, Kaluza, Miami, USA). DOCK8 protein expression was analyzed with flow cytometry before and after 6 months following HSCT as described previously (Figure 1).

2.4 | Chimerism analysis

Myeloid and T-cell donor chimerisms were measured by using PCR-based amplification of short tandem repeat sequences in DNA of the cells following the separation of peripheral blood samples at +1st, 2nd, 3rd, 6th, 9th, 12th, and 18th months following HSCT by Automated Magnetic Cell Sorting (Miltenyi Biotec). Full donor chimerism was defined when >95% of the cells originated from the donor. All patients were nursed in an isolated room, and they all received weekly prophylactic, antimicrobial, and IVIG therapies.

3 | RESULTS

3.1 | Patient characteristics

The clinical characteristics, genetic features, and treatment modalities of the patients are presented in Table 1. The immunologic data are given in Figure 2. All patients were born to consanguineous parents. The median age at diagnosis and at HSCT was 3 years (range: 2 months-14 years) and 6 years (range: 2 months-15 years), respectively. Atopic dermatitis (18/20), recurrent respiratory tract infections (18/20), food allergies (14/20), oral candidiasis (12/20), bronchiectasis (11/20), asthma (8/20), autoimmunity (4/20), and non-Hodgkin lymphoma (NHL) (2/20) were the most prominent clinical manifestations. Failure to thrive was present in 13 of 20 patients. Viral skin infections were less common in our series compared with the literature. Molluscum contagiosum and herpes zoster were not detected in any patient, whereas recurrent oral or labial herpes simplex infections were present in seven patients (P1, P3, P7, P11, P13, P15, and P17) and HPV in only one patient (P2).

Eighteen (90%) patients had been vaccinated with BCG prior to diagnosis. BCG-itis occurred in four patients (P3, P11, P12, and P15), while disseminated BCG disease was detected in one (P6).

Several hepatic problems due to infections (CMV in 4, C parvum in 1, hepatitis B in 1, and liver abscess in one patient), drug-related side effects, or unknown reasons were detected in 12 of 20 patients. Cholestatic liver disease was diagnosed in two patients (P4 and P11). Agents known to cause hepatitis (hepatitis B and C viruses, CMV, and EBV) were found as negative in these patients. Cryptosporidium parvum (C parvum) was ruled out by PCR in only one patient (P11). The other patient was evaluated by acid-staining test for cryptosporidium. P4 died due to cholestatic liver failure. P11 was successfully treated by liver transplantation followed by HSCT.
| Patient no/ gender | Age at OS/D (y) | Failure to thrive | Infections/mo | Allergy | AI |
|-------------------|----------------|------------------|--------------|---------|----|
| P1 M              | 0.5/12         | +                | Rec. bronchiolitis (Rhinovirus, Coronavirus 229E) Otitis Rec. pneumonia (S aureus, S pneumonia, H influenza) Oral candidiasis and HSV Chronic diarrhea Chronic hepatitis B infection CMV viremia | AD Asthma FA (milk, egg, beef) | ITP AIHA |
| P2 F              | 0.5/7          | -                | Rec. otitis HPV | AD FA (milk, egg) | - |
| P3 F              | 0.5/3          | +                | Rec. bronchiolitis Otitis Local BCG infection Suppurative abscess, frowcles Oral HSV infection Oral candidiasis | AD FA (milk, egg, banana) | - |
| P4 M              | 0.1/8          | -                | Rec. bronchiolitis Hepatic abscess Chronic diarrhea | - | - |
| P5 M              | 0.5/3          | +                | Rec. bronchiolitis Otitis Pneumonia Oral candidiasis | AD Asthma FA (milk, egg) | - |
| P6 F              | 0.5/1.5        | +                | Rec. bronchiolitis (RSV, Rhinovirus) Otitis Septicemia (A xylosioidans) Disseminated BCG infection (an abscess in right popliteal fossa with bilateral inguinal and axillary lymphadenopathies, fever, and weight loss) | AD FA (egg, milk) | - |
| P7 F              | 0.2/4          | -                | Rec. bronchiolitis (RSV, Adenovirus, Rhinovirus) Oral candidiasis Pneumonia (P aeruginosa) Oral HSV infection Chronic diarrhea Renal abscess | Asthma AD | - |
| P8 M              | 0.5/3          | +                | Rec. bronchiolitis (Rhinovirus, bocavirus) Rec. pneumonia (Parainfluenza type 3, RSV) Otitis Oral candidiasis Chronic diarrhea (C parvum) | AD Asthma FA (milk, egg, beef) | - |
| P9 F              | 0.2/3          | +                | Pneumonia (CMV, Parainfluenza type 3, Rhinovirus) Otitis (S aureus) Oral candidiasis Chronic diarrhea | Neonatal rash AD Asthma FA (egg, milk) | AIHA |
| P10 F             | 0.1/0.1        | +                | Chronic diarrhea (Vancomycin-resistant enterococci) Bronchiolitis (Rhinovirus) | Neonatal rash AD | - |
| Malignancy                              | GIS                  | Organ damage and others                                                                 | Mutation                                                      | HSCT | Outcome        |
|----------------------------------------|----------------------|----------------------------------------------------------------------------------------|                                                               |      |                |
| Supraclavicular plasmacytoma           | HSM                  | Esophageal papillomas and eosinophilic esophagitis                                    | BE Splicing mutation exon 5 c528G > C                         | +    | Alive/well    |
|                                        |                      | IBD Hormone deficiency                                                                  |                                                               |      |                |
|                                        |                      | Celiac disease                                                                        |                                                               |      |                |
|                                        |                      | Hepatic fibrosis                                                                       |                                                               |      |                |
| NHL B cell (intracranial)              | -                   | -                                                                                      | Exon39:c.C4902G:p.Y1634X                                       | -    | Died           |
| Plasma cell cheilitis                  |                      | -                                                                                      |                                                               |      |                |
| NHL B cell (intracranial)              | BE                  | -                                                                                      | Proximal deletion in Exon 1                                   | -    | Died           |
|                                        |                      | -                                                                                      |                                                               |      |                |
|                                        |                      | Cholestatic liver disease                                                               | c137G > A (p.G46D) homozgyous                                 | -    | Died           |
|                                        |                      | Osteopenia                                                                            | IVS16-1G > C splice acceptor site mutation before exon 17     | +    | Alive/ cGvHD  |
|                                        |                      | -                                                                                      |                                                               |      |                |
|                                        |                      | Esophageal papillomas and esophagitis                                                  |                                                                |      |                |
|                                        |                      | Hepatic fibrosis                                                                       |                                                               |      |                |
|                                        |                      | Choledochal cysts                                                                      |                                                               |      |                |
|                                        |                      | -                                                                                      | IVS16-1G > C splice acceptor site mutation before exon 17     | -    | Alive          |
|                                        |                      | -                                                                                      |                                                               |      |                |
| Plasma cell cheilitis                  | IBD                  | BE                                                                                     | Exon 28- 48 homozygous deletion                               | +    | Alive/well    |
|                                        |                      | -                                                                                      |                                                               |      |                |
| Intestinal plasmacytoma                | HSM                  | Esophageal papillomas and esophagitis                                                  |                                                                |      |                |
|                                        |                      | Nodules on duodenum and ascending colon                                                 |                                                               |      |                |
|                                        |                      | Hepatic fibrosis                                                                       |                                                               |      |                |
|                                        |                      | IBD Hormone deficiency                                                                  |                                                               |      |                |
|                                        |                      | Celiac disease                                                                        |                                                               |      |                |
|                                        |                      | -                                                                                      | 2bp insertion in exon 26: c.3176-3177insXX                    | +    | Alive/well    |
|                                        |                      | -                                                                                      |                                                               |      |                |
|                                        |                      | HSM Transaminitis                                                                      |                                                              | +    | Died           |
|                                        |                      | IBD Osteopenia                                                                         | homozgyous rs151094543                                        |      |                |
|                                        |                      | PDD Osteopenia                                                                         |                                                               |      |                |
|                                        |                      | -                                                                                      | homozgyous rs151094543                                        | +    | Alive/well    |
|                                        |                      | -                                                                                      |                                                               |      |                |
| (Continues)                            |                      | -                                                                                      |                                                               |      |                |
Ten patients had chronic diarrhea, and inflammatory bowel disease (IBD) was detected in 8 of them by performing colonoscopy and biopsy. These patients were screened for gastrointestinal infectious agents with a PCR panel and with microscopy, culture, and antigen tests. Vancomycin-resistant enterococci were detected in P10. *Cryptosporidium parvum* (*C. parvum*) was only demonstrated in P8 by acid-staining test known to have low sensitivity. *Cryptosporidium* was screened by acid-staining test so it might have

| Patient no/ gender | Age at OS/D (y) | Failure to thrive | Infections/mo | Allergy | AI |
|-------------------|----------------|-----------------|---------------|---------|----|
| P11 F | 0.5/5 | + | Rec. bronchiolitis  
*Adenovirus, Parainfluenza type 4*  
Otitis  
CMV and EBV viremia  
Local BCG infection  
Oral candidiasis  
Oral HSV infection  
Chronic diarrhea  
Suppurative skin abscess | AD  
Asthma  
FA (milk, walnut) | AIHA |
| P12 M | 0.8/14 | + | Chronic diarrhea  
Otitis  
Rec. bronchiolitis (*Rhinovirus*)  
Pneumonia  
Suppurative skin abscess (*S. aureus*)  
Local BCG infection | AD  
Asthma  
FA (egg, milk) | AIT |
| P13 F | 0.3/3 | - | Severe HSV infection  
Otitis  
Rec. bronchiolitis (*Coronavirus OC43*)  
Pneumonia (*S. pneumonia*) | AD  
FA (egg, milk) | - |
| P14 F | 0.3/7 | + | Pneumonia (*P. aeruginosa*)  
Rec. bronchiolitis (*Influenza B, Rhinovirus*)  
Otitis  
Suppurative skin abscess  
Local BCG infection  
Chronic diarrhea | AD  
FA (egg, milk, peanut, hazelnut)  
Asthma  
Chronic urticaria and angioedema | - |
| P15 M | 0.3/2 | + | Rec. bronchiolitis  
Otitis  
Sepsis  
Oral HSV infection  
Local BCG infection  
Oral candidiasis | AD | - |
| P16 F | 0.7/0.7 | - | Upper respiratory viral infection  
CMV viremia | AD  
FA (milk, egg) | - |
| P17 M | 0.3/1.5 | - | Oral candidiasis  
Oral HSV infection | AD  
FA (milk, egg) | - |
| P18 M | 0.1/1.5 | - | Oral candidiasis  
Rec. bronchiolitis | AD  
FA (milk, egg) | - |
| P19 M | 2.5/5 | - | Rec. bronchiolitis  
Rec. lymphadenopathy  
Papulopustular wounds | AD  
Urticaria  
Angioedema | - |
| P20 F | 0.6/1 | + | Rec. bronchiolitis  
Rec. pneumonia and otitis  
Chronic diarrhea | - | - |

(Continues)
After 2015, acid-staining and PCR techniques were used together to screen the patients for *C. parvum*. Esophageal papilloma and eosinophilic esophagitis were detected in 5 of 20 patients (P1, P6, P8, P11, and P12). All transplanted patients (P1, P8, P11, and P12) recovered.

Three of DOCK8-deficient patients (P5, P7, and P8) have previously been reported in the literature. Six novel DOCK8 mutations were detected in 17 patients and are reported for the first time (P11, P12, P14, P15/P16, P18, and P19/P20) (Table 1).

| Malignancy                                      | GIS                                      | Organ damage and others | Mutation                                                                 | HSCT | Outcome  |
|------------------------------------------------|------------------------------------------|-------------------------|-------------------------------------------------------------------------|------|----------|
| -                                              | Esophageal papillomas and esophagitis    | BE                      | c.250DelG (p.D85Tfs*46) (p.Asp85Thr*46) frameshift mutation             | +    | Alive/well |
| -                                              | IBD                                      | BE                      | IVS 19 3C > G (c.2206-2C > G homozygous)                                  | +    | Alive/well |
| -                                              | Hepatic fibrosis                         | BE                      | Exon 1-7 homozygous deletion                                             | +    | Alive/well |
| Cervical lymph node plasmacytoma               | Hepatic fibrosis IBD                     | BE                      | c3067_3068insTA (p.V1024Lfs*13) homozygous frameshift                   | +    | Alive/well |
| -                                              | Transaminitis                            | -                       | c.1492C > T(p.Q498*) (p.Gln498*) causes premature stop codon             | -    | Alive     |
| -                                              | Transaminitis                            | -                       | c.1492C > T(p.Q498*) (p.Gln498*) causes premature stop codon             | -    | Alive     |
| -                                              | -                                        | -                       | exon39:c.C4902G:p.Y1634X                                                 | +    | Alive     |
| -                                              | -                                        | -                       | c3067_3068insTA (p.V1024Lfs*13) homozygous frameshift                   | -    | Alive     |
| -                                              | Transaminitis                            | BE                      | c137G > A (p.G46D) (pGly46Asp) homozygous                               | -    | Alive     |
| -                                              | -                                        | -                       | c137G > A (p.G46D) (pGly46Asp) homozygous                               | -    | Alive     |

Abbreviations: AD, atopic dermatitis; AI, autoimmunity; AIHA, autoimmune hemolytic anemia; AIT, autoimmune thyroiditis; BE, bronchiectasis; D, diagnosis; EE, eosinophilic esophagitis; FA, food allergy; HSM, hepatosplenomegaly; IBD, inflammatory bowel disease; ITP, immune thrombocytopenia; mo, microorganism; OS, onset of symptoms; PDD, pervasive developmental disorder.

TABLE 1 (Continued)
to steroid and IVIG. Splenectomy was performed prior to HSCT. Autoimmune thyroiditis was detected in P12.

In addition to previously reported data about a patient (P7), we detected plasmacytoma in four patients (P1, P2, P8, and P14).

Three patients (P9, P10, and P12) without any history of problems at birth had pervasive developmental disorder (PDD) and speech problems. Mild cerebral atrophy was detected in P9 by cranial MRI, but MRI results were normal in other two patients.

All patients had elevated serum IgE levels with a median of 2740 kU/mL (range: 358-36,000 kU/mL). Three patients diagnosed before the first year of life had normal peripheral blood eosinophil counts, while the other 17 patients (85%) had eosinophilia (median: 1200/mm³, range: 600-39300/mm³). Low serum IgM levels were detected in 15 patients (75%), CD3+ T-cell lymphopenia in 10 (50%), CD4+ T-cell lymphopenia in 11 (55%), and CD8+ T-cell lymphopenia in 6 (30%). Lymphocyte activation response to PHA was found as being low in 10 patients (50%).

All patients received Ig replacement therapy, trimethoprim-sulfamethoxazole, acyclovir, and fluconazole prophylaxis. Severe oral herpes infection of P7 was treated successfully with interferon α2b, while in P6, interferon α2b treatment was ceased due to side effects (fever and myalgia) early after its initiation.

3.2 | Outcome in patients who did not receive HSCT

A total of three patients died: two (P2 and P3) because of intracranial NHL and one (P4) because of cholestatic liver failure before the definitive DOCK8 diagnosis. Donor screening is still being processed in six patients.

3.3 | Outcome in patients receiving HSCT

Engraftment was achieved in all patients following HSCT. Median duration for engraftment of neutrophils, thrombocytes, and lymphocytes was 13 days (range: 11-19 days), 15 days (range: 13-19 days), and 15 days (range: 11-41 days), respectively. Full T-cell chimerism (95% or more) was achieved in all patients except one (P13) who was transplanted from a haploidentical donor. P9 died due to pneumonia during the 14th month of transplantation at a local hospital although she had full donor engraftment. In transplanted patients, the median follow-up time is 48 months (min-max: 5-84 months) and overall survival rate is 91% (10/11). The data related to HSCT characteristics and outcomes are presented in Table 2.

AD and food allergies were resolved in 10 patients, and diet restrictions were ended at a median of 3 months (range: 2-12 months) following HSCT. Allergen-specific IgE levels were also found negative in these 10 patients. No allergic reaction was detected in patients with free diet. Both the frequency and severity of infections significantly reduced in all patients. Their pulmonary function tests were improved by 45% (5/11) after transplantation. Chronic diarrhea resolved in all patients. However, growth failure did not improve after HSCT in 6 patients (P1, P5, P11, P12, P13, and P14).

Five patients (P3, P5, P7, P8, and P10) had active infections at the time of the transplant. Acute GvHD (Grade I-III) developed in five
patients (45%), and chronic GvHD developed in four (36%). Four patients who developed sinusoidal obstruction syndrome (SOS) (36%) (P1, P9, P11, and P14) had liver failure at various stages before transplantation. They all received prophylactic defibrotide treatment. All patients having SOS responded to supportive care and defibrotide treatment.

IVIG replacement was stopped in seven patients at a median of months (range: 6-14 months). One patient who was recently transplanted and three patients with cGvHD still continue to receive immunosuppressive treatment and IVIG replacement.

As for laboratory results, eosinophilia disappeared in all patients during the post-HSCT period (median: 120/mm³, min-max: 0-330/mm³). Serum total IgE levels returned to normal or to near-normal levels in 10 patients [post-HSCT median: 40 kU/mL, min-max: 2-428]. Serum IgM levels were low in 8 of 11 and were normalized following transplantation in six patients. CD3+ T-cell counts were low in 6 of 11 patients, and CD4+ T-cell counts were low in 8 of 11 patients. After HSCT, all patients had normal CD3+ T, and CD4+ T-cell counts normalized in six patients. In two siblings with Omenn phenotype (P8 and P9), CD19+ B-cell levels reached normal levels after transplantation. In four patients, DOCK8 expression was examined by flow cytometry both pre- and post-HSCT. All patients had a significant increase in DOCK8 expression on T cells after HSCT.

CD4+ CD45RA+ CD31+ T-cell (RTE) counts were low in eight patients based on age-specific normals at diagnosis (median: 71/mm³, min-max: 42-1900/mm³). RTE counts did not reach age-specific normal values but increased significantly (median: 179/mm³, min-max: 76-1200/mm³). The clinical and laboratory features of the patients and outcomes related to HSCT are summarized in Table 3.

4 | DISCUSSION

Here, we report clinical and immunologic findings of 20 DOCK8-deficient patients while reviewing the outcomes of 11 transplanted cases among them. Our study is the largest single-center cohort reported from Turkey. Furthermore, six novel mutations were reported in this cohort. Our patients had unique features that Turkish patients participating in previous studies did not have. In addition to classical clinical features, gastrointestinal problems (liver and inflammatory bowel disease) were more common, while viral skin infections were less common in our series compared with the literature. BCG vaccine-related complications were seen in five patients for the first time. The median age of our patients (3 years, 2 month-14 years) was lower than the previous studies, and mild clinical findings were found in patients diagnosed during infancy period. Molluscum contagiosum or herpes zoster infections were not detected in our series. HPV was detected in only one patient. Mucosal herpes simplex virus (HSV) infections were observed at similar rates with the literature (35%). Gastrointestinal problems (liver and inflammatory bowel disease) in this study were more common than in previous studies. These abovementioned differences might be due to patients’ young age, the characteristics of the genetic mutations, and epigenetic factors influencing the genotype.

There is limited information about the speech and developmental problems in DOCK8 deficiency. Three of our patients have speech problems and pervasive developmental problems. Because all these patients’ parents are consanguineous and two of the patients are siblings, it suggests that other genetic etiologies may be responsible for the PDD.

More than 60% of the non-transplanted patients are reported to die due to malignancy or severe infections before the age of 30 years. In our series, three patients died before the detection of DOCK8 mutations due to central nervous NHL (P2 and P3) and liver failure (P4). Thus, HSCT is suggested to all patients with DOCK8 deficiency; even patients with significant comorbidities should undergo HSCT as early as possible after aggressive treatments of these conditions to minimize complications and to achieve engraftment.

Recently, HSCT outcomes of 81 transplanted patients with DOCK8 deficiency from 22 centers were published. In this cohort, the median follow-up time after HSCT was 26 months (3-135), and 84% of the patients were alive. Survival rates were higher in the patients receiving transplants before 8 years of age and receiving RIC (treosulfan based or reduced-dose busulfan) than those undergoing HSCT later than 8 years of age and receiving MAC. Of 73 patients with available chimerism data, 65 (89%) had >90% donor T-cell chimerism at the last follow-up. The outcome of HSCT even with different conditioning regimens, different GvHD prophylaxis, and different donors has been quite successful in all reported cases.

In our study, survival rate was 91% despite the fact that most of our patients had organ damage including severe inflammatory states and half of them had active infections at the time of HSCT. MAC consisting of busulfan 4 mg/kg/day and fludarabine 40 mg/m²/day IV on days −5, −4, −3, and −2 was given to the first two patients transplanted with MRD as treosulfan could not be obtained at that time. Then, we preferred treosulfan-based RIC in patients who had a high risk of developing transplant-related toxicity, particularly SOS and infections. Complete chimerism was achieved in all patients except one (P13) who received RIC regimen and was transplanted from a haploidentical donor. OS was 91% in transplanted patients similar to previous studies. In our study, cGvHD rate is higher than the previous studies. It could be related to severe organ damage and inflammatory morbidities due to delayed diagnosis and HSCT in our cohort. All patients who developed cGvHD were older than 4 years of age. Severe cGvHD was the most important complication we have seen after HSCT in DOCK8-deficient patients.

Previous series report persistence of food allergies after HSCT. However, in our series food allergies were improved in all patients except for one patient who was transplanted recently. We did not observe allergic reactions in the patients after stopping dietary restrictions.

Although the underlying cause of development of autoimmunity is not clear in DOCK8 deficiency, autoimmune diseases—even celiac disease—disappeared in our patients after transplantation.
Eight of 11 patients undergoing HSCT had failure to thrive. Only two patients were able to achieve normal growth after transplantation (P8 and P10). Two of the patients with growth retardation (P1 and P12) were transplanted after the age of 14. Four patients (P5, P11, P13, and P14) received long-term steroid therapy for chronic GvHD treatment. P13 and P14 who were transplanted in the last two years should be monitored for their growth in the long run.

Clinical and laboratory findings improved in all patients after HSCT. Our results suggest that HSCT in the earliest period with MRD, MUD, or haploidentical donors, together with patient-specific appropriate conditioning regimens, and GvHD prophylaxis

### TABLE 2  HSCT-related features, follow-up, and outcome

| Patient no/Age at HSCT (year) | Conditioning regimen | GvHD prophylaxis | GvHD treat. | Donor type | Stem cell source | CD34 + cell dose (/kg x10^6) |
|-------------------------------|----------------------|------------------|-------------|------------|-----------------|-----------------------------|
| P1/15                         | Treo 42 g/m^2 Flu 150 mg/m^2 ATG | CSA + MMF | CS, Tacrolimus and MSC | MMUD | BM | 1.46 |
| P5/4                          | Bu 16 mg/kg Flu 160 mg/m^2 | CsA + MTX | CS, Tacrolimus, MSC, Ruxolitinib | MRD | PBSC | 8 |
| P7/8                          | Bu 16 mg/kg Flu 160 mg/m^2 | CsA | - | MSD | BM | 5.1 |
| P8/6                          | Treo 42 g/m^2 Flu 150 mg/m^2 | CsA | - | MSD | BM | 6.4 |
| P9/5                          | Treo 42 g/m^2 Flu 150 mg/m^2 | CSA | - | MRD | BM | 7 |
| P10/0.2                       | Treo 36 g/m^2 Flu 150 mg/m^2 | CsA | - | MRD | BM | 14.2 |
| P11/6                         | Treo 42 g/m^2 Flu 150 mg/m^2 | CSA | CS, Tacrolimus and MSC | MRD | BM | 9.6 |
| P12/14                        | Treo 42 g/m^2 Flu 150 mg/m^2 | CSA + MMF | - | MRD | PBSC | 5 |
| P13/3                         | Treo 42 g/m^2 Flu 150 mg/m^2 ATG | CSA + MMF PT/ Cy | Tacrolimus, MMF and MSC | Haplo | BM | 5 |
| P14/9                         | Treo 42 g/m^2 Flu 150 mg/m^2 | CSA | Tacrolimus, MMF, MSC, Sirolimus | MSD | BM | 6.4 |
| P17/2                         | Treo 42 g/m^2 Flu 150 mg/m^2 | Tacrolimus + MTX | - | MRD | BM | 4.6 |

Abbreviations: A, alive; aGvHD, acute graft-versus-host disease; ATG, antithymocyte globulin; BM, bone marrow; Bu, busulfan; CS, corticosteroid; CsA, cyclosporine A; Flu, fludarabine; Haplo, haploidentical; MMF, mycophenolate mofetil; MMUD, mismatched unrelated donor; MRD, matched related donor; MSC, mesenchymal stem cell; MV, mechanical ventilation; MSD, matched sibling donor; MTX, methotrexate; MUD, matched unrelated donor; PBSC, peripheral blood stem cell; PMNCs, peripheral blood mononuclear cells; SOS, sinusoidal obstruction syndrome; Treo, treosulfan; W, well.

(Continues)
is lifesaving for DOCK8 deficiency. cGvHD is the most important complication after HSCT. In our opinion, GvHD prophylaxis is very important and GvHD findings should be followed very closely and treated aggressively. Long-term follow-up is necessary to see how immunologic features progress and how effects on the prevention of malignancy and survival in transplanted patients will be.

Despite most of the mutations found in DOCK8-deficient patients are loss-of-function mutations that abolish DOCK8 protein expression, very low but detectable levels of DOCK8 protein can be expressed in some patients. The residual DOCK8 protein contributes to the variable disease phenotype. In our cohort, four patients with mild phenotype (P15, P16, P18, and P20) diagnosed
TABLE 3 Clinical and immunologic improvement in transplanted patients

|                          | Before HSCT (n) | After HSCT (n) |
|--------------------------|----------------|---------------|
| Severe infections        | 11             | 0             |
| Allergy                  |                |               |
| Atopic dermatitis        | 11             | 0             |
| Asthma                   | 4              | 2             |
| Food allergy             | 11             | 1             |
| Gastrointestinal disorders|               |               |
| Chronic diarrhea         | 8              | 0             |
| Liver disease            | 6              | 3             |
| Autoimmunity             | 4              | 0             |
| Immunologic characteristics|              |               |
| Eosinophilia             | 11             | 0             |
| Elevated total IgE level | 11             | 1             |
| Low IgM level            | 8              | 2             |
| CD3+ T-cell lymphopenia  | 6              | 0             |
| CD4+ T-cell lymphopenia  | 8              | 2             |
| CD19+ B-cell lymphopenia | 4              | 2             |
| Decreased lymphocyte activation response | 9 | 0 |

*P17 was recently transplanted.

P1 had chronic hepatitis B infection, and P5 and P14 had chronic liver GvHD.

P13’s immunosuppressive treatment was discontinued 2 mo ago, and P14 was still receiving immunosuppressive treatment.

in early infancy demonstrated some residual DOCK8 protein expression by flow cytometric analysis. Therefore, examination of DOCK8 protein expression by flow cytometry in early infancy in suspected patients is a rapid and reliable diagnostic approach.

We suggest that all patients suspected to have DOCK8 deficiency be followed up closely, and DOCK8 expressions be measured by flow cytometry and confirmed by genetic study and performing HSCT as soon as possible.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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