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CASE REPORT

DOCK8 deficiency in six Iranian patients

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Key Clinical Message

DOCK8 deficiency is a rare autosomal recessive combined immunodeficiency with high IgE level, eosinophilia, severe eczema, extensive cutaneous viral, and respiratory bacterial infections, mostly in populations with higher prevalence of consanguinity. Molecular diagnosis of this gene is a useful approach for early diagnosis and timely HSCT due to deleterious consequences.

Keywords

Allergy, autosomal recessive, *DOCK8*, hyper IgE syndrome.

Introduction

Homozygous mutations in the *Dedicator of Cytokinesis 8* (*DOCK8*) gene account for most cases of autosomal recessive hyper IgE syndrome (AR-HIES). The genetic alterations in DOCK8 identified so far encompass large deletions, point mutations that alter splicing to cause nonsense mutations, in-frame nonsense mutations, and small insertions and deletions that cause out of- frame nonsense mutations [1]. AR-HIES is considerably rare and has severe outcomes [2]. Along with severe atopic dermatitis, eczema, and recurrent skin infections, these patients suffer from extensive cutaneous viral infections with molluscum contagiosum, herpes simplex virus, varicella zoster, and human papillomavirus (HPV) which are difficult to control, mutilating and often occur concurrently. Vascular disorders and the involvement of the central nervous system appear to be common in AR-HIES and the patients show a high mortality due to sepsis and early onset of malignancies [2, 3]. Patients with DOCK8 deficiency present with multiple abnormalities of the immune system and are usually found to have eosinophilia, lymphopenia as well as

defective T-cell function in addition to the elevated serum IgE levels [1, 4, 5]. DOCK8 is highly expressed in lymphocytes, suggesting crucial functions in these cell types and DOCK8 deficiency appears to impair the CD4+ and CD8+ T-cell proliferative responses [6].

DOCK8 deficiency is a severe disorder and needs to be recognized as it is associated with high mortality and morbidity [3]. Therefore, improving diagnostic methods of DOCK8 deficiency is essential for timely management of these patients for hematopoietic stem cell transplantation (HSCT) [7].

The aim of this study was to describe the phenotypes and to investigate the genetic cause of six patients with persistent viral infections and the clinical phenotype of AR-HIES referred to the Immunology, Asthma and Allergy Research Institute (IAARI) of Tehran University of Medical Sciences in Iran.

Materials and Methods

Patients

The study included six patients referred to IAARI between 2006 and 2013.

All patients and controls or their parental or legal guardians provided written consent for the conducted studies, following local ethics committee requirements.

Lymphocyte markers

Peripheral venous EDTA blood was drawn to determine the CD3, CD4, CD8, CD19, CD16/56 lymphocyte subsets. The preparation of samples was done according to the manufacturer's protocol (Becton Dickinson BD, St. Jose, CA, USA). The percentages of lymphocyte subpopulations were determined by BD FACStar plus flow cytometry. Cell Quest Software was used for analysis.

Serum immunoglobulin and complement levels

IgG, IgA, IgM, and C3 and C4 levels in sera of each patient were quantified using the MININEPH nephelometry kit (Binding Site, Birmingham, UK).

Total serum IgE concentrations were determined by ELISA (EUROIMMUN AG, Lübeck, Deutschland).

Nitro blue Tetrazolium slide test (NBT)

The test was performed on leukocytes activated with PMA (Phorbol-Myristate-Acetate, Sigma Aldrich, St. Louis, MO, USA). Neutrophils ingest the dye, nitro blue tetrazolium (Sigma, Aldrich). The percentage of neutrophils which reduced NBT to blue formazan was determined by counting the positive cells of the Giemsa-stained glass smear. A proportion of more than 95% was considered normal.

T-lymphocyte proliferative responses

Lymphocyte proliferation function was performed on peripheral blood mononuclear cells (PBMCs) according to standard protocols. Proliferation was detected after 3 days using the Cell Proliferation ELISA, BrdU assay kits (Roche Diagnostics GmbH, Mannheim, Germany). In parallel for each patient, an age-matched healthy control was tested.

RIDA allergy screen test

The RIDA Allergy screen kit (R-Biopharm Co., Darmstadt, Germany) was used for the semiquantitative determination of specific IgE in patients' serum against a panel of individual allergens.

The test was performed on panel 3 or 4 according to the manufacturer's instruction. Results were scanned with CCD camera (RIDA X-Screen Reader) and interpreted as class 0–6 corresponding EAST classes. Class ≥ 2 was interpreted as positive (based on reader-specific instructions).

Homozygosity mapping and DOCK8 PCR

Genomic DNA of patients was isolated either from whole blood or PBMCs by using a Gentra Puregene purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For one patient, RNA was extracted from whole blood using the RNeasy Kit (Qiagen) and subsequently reverse-transcribed using Omniscript reverse transcriptase (Qiagen).

To evaluate homozygosity on chromosome 9, the two microsatellite markers D9S917 and D9S1858 were genotyped on the six samples. Primers and other reagents for homozygosity mapping were purchased from Life Technologies GmbH (Darmstadt, Germany), and Qiagen. The polymerase chain reactions (PCR) for homozygosity mapping were performed according to the protocols accompanying the reagents. The PCR products were separated on an ABI3130xl Genetic Analyzer (Applied Biosystems-Life Technologies), GeneMapper® Software v4.1 (Applied Biosystems-Life Technologies, Foster City, California, United State) was used for analysis.

After homozygosity mapping, the coding genomic sequences and flanking intron/exon boundaries of *DOCK8* were amplified from genomic DNA by PCR according to standard protocols using Taq polymerase from PeqLab (Erlangen, Germany). Primer sequences are available on request.

Results

Clinical presentation and patient characteristics

We describe six Iranian patients with the clinical phenotype of AR-HIES. Of the six patients, four were males and two females. The age of the patients at the time of clinical evaluation ranged between 1.5 and 23 years. All patients were originally from Iran, born to consanguineous parents (first degree cousins) and are not related.

HIES scores and the main clinical information of the patients are summarized in Table 1.

Patient 1

The patient's first symptoms began at the age of two years when she presented with cutaneous warts on her hands and arms, and later as well on her knees, shoulders, and back. Further, the patient had generalized eczema, disseminated molluscum contagiosum infection, especially affecting the face and oral thrush. Since the age of 3 years, the patient suffered from purulent otitis along with recurrent respiratory tract infections which led to the formation of bronchiectasis. Due to chronic respiratory illness, the patient developed clubbing of her fingers.

At age 16 years, she was referred to IAARI because of chronic treatment-refractory, recurrent herpetic conjunctivitis (Fig. 1). Despite the use of cidofovir in maintenance dose and surgical procedures, the conjunctival flap did not respond to treatment; hence the patient required hospitalization. She died due to a progressive encephalopathy, with no signs of progressive multifocal leukoencephalopathy (PML) in MRI, at the age of 23.

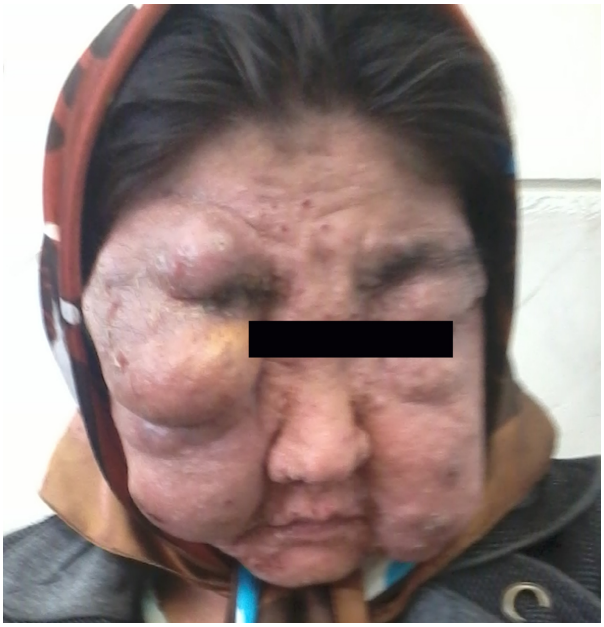

Figure 1. Patient 1 with chronic recurrent herpetic conjunctivitis. Patient 1 suffering from chronic treatment-refractory, recurrent herpetic conjunctivitis. Furthermore, the patient presents with eczema and diffused *molluscum contagiosum* on the face and eyelid and dysmorphic facial features.

The patient was the last child of seven pregnancies. Two of her older siblings died at birth, one at age five due to respiratory difficulties; the other three siblings and the parents are phenotypically healthy.

Patient 2

This female patient suffered from *Aspergillus fumigatus* sinusitis and pneumonia starting at the age of 2 years.

Table 1. Demographic and clinical hallmarks of six patients with DOCK8 deficiency.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Consanguinity	+	+	+	+	+	+
Age (y)	23*	19*	8*	8*	4	1.5*
Gender	Female	Female	Male	Male	Male	Male
HIES score	41	42	42	51	41	35
Molluscum contagiosum infection	Positive	Positive	Negative	Negative	Negative	Negative
Main clinical manifestation	Persistent herpetic keratitis, Encephalopathy	Fungal sinusitis (Aspergillus), Pneumonia, Hemolytic anemia	Encephalopathy	Osteomyelitis, Septicemia, Aneurysm	Eczema, Severe food allergy, Failure to thrive	Severe food allergy, Failure to thrive
DOCK8 mutation	Homozygous deletion Exon 1–48	Homozygous deletion Exon 1–44	Homozygous deletion Exon 11–14	Homozygous deletion Exon 11–13	Homozygous deletion Exon 25–26	Homozygous deletion Exon 25–26

*Age at death.

Recurrent respiratory tract infections led to parenchymal abnormalities and the formation of tubular bronchiectasis in right middle lobe. However, allergic bronchopulmonary aspergillosis (ABPA) can be considered for this patient though the clinical symptoms and laboratory findings of this patient do not fulfill the criteria for ABPA.

The patient, aged 17 years, was referred to IAARI because of chronic recurrent respiratory tract infections, resistant to a broad spectrum of antibiotics. She developed hemolytic anemia resulting in hemoglobin levels as low as 4 g/dL, after the administration of cotrimoxazol. Bone marrow aspiration was not diagnostic.

The patient suffered from a generalized *molluscum contagiosum* infection involving especially the eyelids, ears, uvula, and anus, but had no history of eczema or skin abscesses. Failure to thrive and short stature led to growth hormone infusions; further, decreased bone density with coarse trabeculation was reported. Due to recurrent and persistent upper and lower respiratory tract infections (sinusitis and pneumonia) along with severe cough and severe hemolytic anemia, the patient underwent several hospitalizations. She died at the age of 19 years because of respiratory failure.

The patient had two brothers and one sister, all alive and phenotypically healthy.

Patient 3

This male patient suffered from asthma and severe atopic dermatitis since early infancy. Furthermore, the patient developed chronic sinusitis as well as otitis and had several episodes of pneumonia which required hospitalization and antibiotic therapies. Generalized lymphadenopathy was seen from 2 years of age.

At the age of seven, the patient had trauma-induced insomnia for 48 hours which was followed by lethargy and loss of appetite. Within 3 months after trauma, the patient developed urine and fecal incontinence. He was hospitalized in the pediatric intensive care unit because of an altered mental status, fever, chills, and sweating; also presenting hepatomegaly and abdominal distention. Brain MRI showed signs of PML; however, CSF was negative for JC polyoma virus in PCR. The patient died at the age of 8 years due to neurological deterioration.

Patient 4

The male patient suffered from itchy skin and diarrhea since the first year of life. Eczema and asthma due to food allergies were also seen. At the age of 4 years, left leg arterial thrombosis led to the amputation of the left first toe. The patient was hospitalized three times because of septicemia, dysentery, and osteomyelitis. Subsequently, aortic and abdominal aneurysms were diagnosed by Doppler sonography. At the age of 8 years, the patient died due to the rupture of the aortic aneurysm.

Patient 5

This male patient had severe eczematous skin starting 1 month after birth and suffered from severe food allergies. He has a history of two to three common colds per year and two episodes of impetigo. Severe reactions to insect bites led to the formation of blistering rashes on his fingers.

Extremely limited nutrition due to his multiple food allergies, especially reactions to milk, wheat, and egg white, led to failure to thrive. This patient is the only patient still alive from our DOCK8 cohort; currently he is 4 years old. However, the preliminary HSCT workup was done for him, but the parents did not accept undergoing HSCT. The first child of the family died due to lymphoma at the age of 8 years. She had history of severe eczema, extreme eosinophilia, and recurrent infections of lungs and ears.

Patient 6

The male patient had a history of severe atopic dermatitis and generalized eczema especially to milk and egg white which was improved by elimination diet. Starting from infancy, he suffered from recurrent pneumonia, cough, vomiting, and perforated otitis media. Recurrent urinary tract infections and chronic diarrhea were also seen. The patient was hospitalized twice; once due to sepsis at the age of 14 months and the second time, because of food intolerance due to severe food allergies with reported eosinophilic esophagitis and eczematoid skin lesions at the age of 15 months. The patient suffered from developmental delay, failed to thrive, and passed away at the age of 18 months.

Immunological assessment

The details of the immunologic evaluations are listed in Table 2. All six patients had elevated IgE levels except one patient who had eosinophilia. In one patient (Patient 2) elevated IgG and IgM levels were detected, whereas, Patient 3 and 6 had reduced IgM levels and Patient 5 reduced IgG levels, respectively. IgA levels were within normal range in all six patients.

Four of the six patients presented elevated white blood cell counts at the time of blood drawing, whereas, CD3+ T-cell counts were reduced in five patients. Four of the patients had decreased percentages of CD4+ T cells; CD8+ T cells were found reduced in four but elevated in Patient 2 and 6, leading to an inversion of the CD4+/

Table 2. Immunologic parameters of six patients with DOCK8 deficiency.

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age of evaluation (y)		23	19	8	8	4	1.5
WBC (cells/mL)		7,580 (4,500–11,000)	**15,550** ↑ (4,500–11,000)	**14,440** ↑ (4,500–13,500)	**29,900** ↑ (4,500–13,500)	12,320 (5,000–15,500)	**20,940** ↑ (6,000–17,000)
Eosinophils/μL		**1,743** ↑ <700	**5,390** ↑ <700	245 <700	**8,319** ↑ <700	**2,210** ↑ <700	**3,874** ↑ <700
IgE (IU/mL)		**>10,000** ↑ <100	**3,615** ↑ <100	**2,890** ↑ <90	**2,500** ↑ <90	**3,468** ↑ <60	**2,900** ↑ <60
IgG (mg/dL)		1,229 (639–1,349)	**2,161** ↑ (639–1,349)	982 (764–2,314)	1,048 (764–2,314)	**378** ↓ (500–1,300)	591 (424–1,051)
IgA (mg/dL)		163 (70–312)	146 (70–312)	285 (70–303)	96 (70–303)	64 (40–180)	35 (14–123)
IgM (mg/dL)		85 (56–352)	**498** ↑ (56–352)	**35** ↓ (69–387)	79 (69–387)	49 (40–180)	**29** ↓ (48–168)
Isohemagglutinin Titer	Anti-A	1:2	Neg	0	ND	1:2	ND
	Anti-B	1:2	Neg	Neg	ND	Neg	ND
	Blood Group	0	AB	B	ND	B	ND
CD3 (%)		**48.87** ↓ (57–86)	75.35 (57–86)	**48.5** ↓ (55–78)	**23.03** ↓ (55–78)	**31.31** ↓ (43–76)	**60.81** ↓ (62–69)
CD4 (%)		**24.09** ↓ (29–57)	**18.44** ↓ (29–57)	32.1 (27–53)	**15** ↓ (27–53)	27.87 (23–48)	**18.52** ↓ (30–40)
CD8 (%)		**17.4** ↓ (25–51)	**51.09** ↑ (25–51)	**14.4** ↓ (19–34)	**4.39** ↓ (19–34)	**5.19** ↓ (14–33)	**40.78** ↑ (25–32)
CD19 (%)		**30.54** ↑ (4–16)	15.73 (4–16)	18.5 (10–31)	**69.09** ↑ (10–31)	25.33 (14–44)	28.12 (14–44)
CD16/CD56 (%)		7.85 (5–30)	**0.78** ↓ (5–30)	11.7 (4–26)	ND	23.08 (4–23)	**2.87** ↓ (4–23)
Lymphocyte Transformation Test (LTT Ratio)	PHA (>3)	**1.02** ↓ (3.37)	**2.15** ↓ (3.4)	3.7 (4.2)	ND	**2.13** ↓ (3.87)	ND
	BCG (>2.5)	**1.03** ↓ (2.5)	ND	2.8 (3.6)	ND	ND	ND
Results of age-matched healthy controls are in parenthesis.	*Candida albicans* Antigen (>2.5)	**1.6** ↓ (2.53)	**2.0** ↓ (3.0)	**2.1** ↓ (2.6)	ND	**1.51** ↓ (4.9)	ND

Values outside reference ranges are highlighted in bold. Arrows indicate values greater than (↑) or less than (↓) the reference ranges. Normal ranges are from the 10th to 90th percentiles (19).

WBC, white blood cells; ND, not done; Neg, negative; NBT, nitro blue tetrazolium test; PHA, phytohemagglutinin; BCG, Bacille Calmette–Guérin.

CD8+ ratio in these two patients. B-cell counts were elevated in two patients but normal in four; NK cell numbers where reduced in two of five patients.

NBT test was normal in all six patients, likewise complement, C4 and CH50 levels were within normal ranges in the four patients tested. C3 levels were found reduced in Patient 3 but elevated in Patient 4.

In the lymphocyte proliferation test (LTT), three out of the four patients evaluated showed a reduced lymphocyte proliferation; only one patient revealed values within normal ranges after stimulation with phytohemagglutinin (PHA) and Bacillus Calmette–Guérin (BCG) but a reduced stimulation index (SI) following stimulation with Candida.

Five out the six patients suffered from allergies. Details of the RIDA allergy screen test (Panel 3 or 4) are listed in Table 3. Patient 5 and Patient 6, in particular, show multiple severe food allergies mainly to milk, wheat flour, egg white, and peanut but also allergic reactions against egg yolk, hazelnut, fish, potato, carrot and others have been detected. Moreover, all five patients showed mild to intermediate allergic reactions to grass mixtures or birch or both. For Patient 2, the RIDA screening test was not performed as this patient did not have allergies.

Homozygosity mapping and DOCK8 mutations

Homozygosity mapping plays an important role in the detection of recessive mutations in families of consanguineous marriages. The method takes advantage of the

Table 3. RIDA allergy screen test (Panel 3 or 4) in HIES (DOCK8-deficient) cases.

Allergen	Patient 1	Patient 3	Patient 4	Patient 5	Patient 6
Milk	**1+**	Neg	**5+**	**4+**	**4+**
Wheat flour	Neg	**2+**	**4+**	**4+**	**6+**
Rye meal	ND	ND	ND	**4+**	ND
Egg white	Neg	Neg	**5+**	**4+**	**3+**
Egg yolk	Neg	Neg	**4+**	Neg	**3+**
Peanut	**1+**	Neg	**2+**	**2+**	**4+**
Hazelnut	Neg	Neg	Neg	**3+**	**6+**
Fish	ND	ND	**3+**	**2+**	ND
Soya beans	Neg	Neg	Neg	Neg	**3+**
Potato	Neg	Neg	Neg	**2+**	**3+**
Carrot	Neg	Neg	Neg	**2+**	**4+**
Casein	ND	ND	ND	**4+**	ND
Celery	ND	ND	ND	**2+**	ND
Tomato	ND	ND	ND	**2+**	ND
Apple	ND	ND	ND	**3+**	ND
Sesame seed	ND	ND	ND	**2+**	ND
Grass mixture	**2+**	**1+**	Neg	Neg	**3+**
Birch	**2+**	Neg	**3+**	**3+**	**2+**

Bold numbers represent EAST classes based on reader specific instructions; Neg, Negative; ND, Not Done.

fact that inbred affected individuals are likely to inherit two recessive copies of the disease allele from a common ancestor. Since small chromosomal regions tend to be transmitted as a whole, affected individuals will also have identical-by-descent alleles at markers located nearby the disease locus, and thus, will be homozygous at these markers [8]. Homozygosity mapping with microsatellite markers on chromosome 9p was therefore used as diagnostic tool to assess the likelihood of the patients to carry homozygous *DOCK8* mutations.

All six patients were homozygous at the DOCK8 locus; Patients 1 and 2 were homozygous for the D9S1858 marker only, all other patients were homozygous for both markers tested (D9S917 and D9S1858).

Subsequently, all 48 exons encoding DOCK8 were amplified by PCR to detect possible homozygous whole-exon deletions. We identified two large and four smaller deletions in the genomic DNA of our six patients (Fig. 2). For Patient 1, we were unable to amplify any of the 48 *DOCK8* exons; in addition we were not able to detect *DOCK8* mRNA suggesting that this patient carries a homozygous deletion of the entire *DOCK8* gene. The second large deletion encompassed almost the whole gene spanning from exons 1 to exon 44 (Patient 2).

The four other patients had smaller deletions: Patient 3 had a deletion of exons 11–13, Patient 4 a deletion of exons 11–14. The deletion of exons 11–13 and 11–14 affected the DOCK8 homology region 1 (DHR1). Both mutations create a frame-shift followed by a premature stop codon, probably leading to nonsense mediated degradation.

The two unrelated Patients 5 and 6 both carried a deletion of exons 25 and 26.

Exon 25 and exon 26 together comprise 264 base pairs; hence their excision leads to an in-frame deletion of 88 amino acids. This in-frame deletion is located between the two DOCK homology regions (DHR) of DOCK8 (Fig. 2).

Although the exact breakpoints of the deletions have not been determined in all six cases, the deletions are likely to result in the loss of normal DOCK8 expression with loss of function, as characteristic of this disease.

Discussion

Like other autosomal recessive diseases, DOCK8 deficiency appears to be most common in populations where the frequency of consanguineous marriages is high [9]. The overall rate of consanguineous marriages in Iran is 38.6%, and with 27.9% first cousin marriages, represents the most common form of consanguineous union [10]. So, in areas with high consanguineous marriages, considering the high rate of related parents in PID patients [11], more attention must be given to importance of mentioned clinical suspicion.

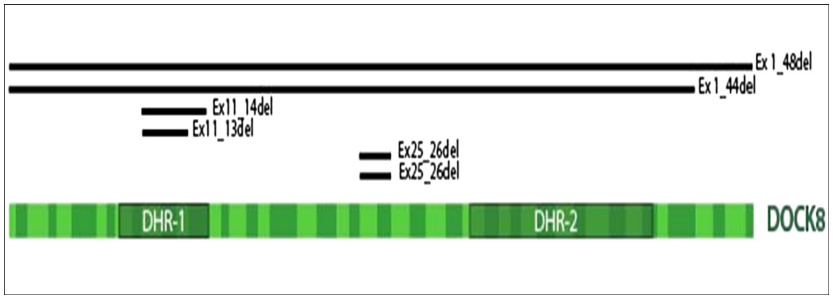

Figure 2. Schematic representation showing DOCK8 mutations of the six patients. The straight lines depict the six multi-exon deletions with undetermined breakpoints. All mutations were homozygous. DHR; DOCK homology region. (Figure modified after Engelhardt et al., 2015).

Here, we identified six patients with DOCK8 deficiency. The diagnosis was made by following the patient management principles proposed in [7]: after the clinical diagnosis of AR-HIES and assessment of the familial history, homozygosity mapping on the DOCK8 locus on chromosome 9 was carried out. Homozygosity in all six patients subsequently led to the amplification of the 48 DOCK8 exons and the diagnosis of DOCK8 deficiency. The method proposed is fast and cost saving.

Two of the patients described had an in-frame deletion of two exons. Although an in-frame deletion suggests a possible residual protein activity, the severe phenotype of both patients makes residual activities unlikely. Moreover, in-frame deletions have been previously described in other severely ill patients [1]. Unfortunately, no assay has been developed yet to test DOCK8 function.

On the whole, the clinical phenotypes of the presented patients is consistent with that of other reports on DOCK8 deficiency and include recurrent sinopulmonary infections which lead to bronchiectasis, generalized skin eczema, and cutaneous viral infections with molluscum contagiosum and herpetic keratitis [12]. Other clinical features such as some degree of allergies and asthma are most important and popular in DOCK8 deficient patients [3]. Nonetheless, the main symptoms of our six patients varied.

Some of the more rare symptoms (gastrointestinal symptoms like diarrhea and hepatomegaly) have already been reported and might be caused by the DOCK8 deficiency. However, as most of the DOCK8-deficient patients are born to consanguineous parents, additional homozygous defects cannot be ruled out.

Unlike the more common AD-HIES, patients with DOCK8 deficiency develop severe allergies, including anaphylaxis to food and environmental antigens, and about 30% have asthma. Three of our patients were diagnosed with moderate and two patients with severe food allergies. In both cases of severe allergies, the extremely limited nutrition led to failure to thrive. In DOCK8 deficiency, the allergic manifestations may reflect the increased numbers of Th2 CD4+ T cells, which express IL-4 or IL-13 and therefore promote allergic disease [6]. However, the exact mechanism responsible for allergy in this form of HIES has not yet been established [5].

DOCK8-deficient patients exhibit multiple abnormalities of the immune system. It has been reported previously that DOCK8-deficient patients display a progressive lymphopenia [13]. The lymphopenia affects both CD4+ and CD8+ T cells and to a lesser extent NK cells. Paradoxically though two patients of our cohort had increased numbers of CD8+ T cells, leading to an inverted CD4+/CD8+ ratio. Although CD19+ B cells have been reported to be decreased in DOCK8 deficiency, two patients of this cohort showed increased counts, all other had normal B-cell counts.

The poor T-cell proliferation observed in our patients, in particular to the mitogen PHA, could be due to defects in T-cell survival, which have been reported previously [14].

Possible somatic reversions of the germline mutations have been described [15]. However, patients with large homozygous deletions, like the ones presented here, are incapable of generating revertants, and are predicted to have more severe disease and early and severe complications.

Among six patients, five of them expired and the parents of the only alive patient did not accept undergoing HSCT; however, there are successful reports of HSCT outcome in DOCK8 patients [16]. Based on Aydin et al. [16] study, early HSCT should be considered strongly due to the higher probability of survival reported for HSCT in DOCK8 patients at lower ages.

As confirmed by our report, DOCK8 deficiency has a high mortality at a young age; therefore, hematopoietic stem cell transplantation (HSCT) should be considered. Because HSCT is best done as early as possible, the early identification of DOCK8 deficiency by molecular diagnosis is essential to manage these patients appropriately.

Acknowledgments

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Conflict of Interest

None declared.

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