

Combined Immunodeficiency due to DOCK8 Deficiency: A Tribute to Prof. Isil Berat Barlan, M.D.

DOCK8 Eksikliğine Bağlı Kombine İmmün Yetmezlik: Prof. Dr. Işıl Berat Barlan Anısına

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doi: 10.5606/tji.2015.428

Received: August 12, 2015

Accepted: August 12, 2015

ABSTRACT

The hyper IgE syndrome (HIES) is an immunodeficiency characterized by recurrent infections, eczema, and elevated serum IgE concentrations. An autosomal dominant form (AD-HIES) is caused by mutations in the transcription factor STAT3 gene. An autosomal recessive form (AR-HIES) was described in 2004, and is particularly over-represented in Turkish population with HIES. Subsequent studies led to the discovery of the gene encoding the Deducator of Cytokines 8 (DOCK8) as the target of mutations in the overwhelming majority of patients with AR-HIES. This review retraces the steps leading to the discovery of DOCK8 deficiency and the critical role played in the process by Prof. Isil Berat Barlan, M.D., as well as detailing our current knowledge of this disorder and future directions in its investigation and therapy.

Keywords: Cdc42, dedicator of cytokines 8; hyper IgE syndrome; immunodeficiency; signal transducer and activator of transcription 3.

ÖZ

Hiper IgE sendromu (HIES) tekrarlayan enfeksiyonlar, egzema ve yüksek serum IgE konsantrasyonları ile karakterize bir immün yetmezliktir. Otozomal dominant (OD-HIES) formuna transkripsiyon faktör Stat3 gen mutasyonları neden olur. Özellikle HIES'li Türk popülasyonunda sık görülen formu olan otozomal resesif formu (OR-HIES), ilk olarak 2004 yılında tanımlanmıştır. Devam eden çalışmalarda AR-HIES'li hastaların büyük bir kısmında Deducator of Cytokines 8 (DOCK8) gen kodlayıcısının hedef mutasyon olduğu keşfedilmiştir. Bu derlemede DOCK8 eksikliğinin keşfine giden aşamalar ve Prof. Dr. Işıl Berat Barlan'ın bu süreçte oynadığı önemli role değinildi, aynı zamanda bu hastalığa ilişkin güncel bilgiler ve araştırma ve tedavi alanındaki gelecekteki beklentiler ayrıntılı olarak ele alındı.

Anahtar sözcükler: Cdc42; dedicator of cytokines 8; hyper IgE syndrome; immunodeficiency; signal transducer and activator of transcription 3.

Hyper IgE Syndrome

Hyper IgE syndrome (HIES) is a primary immune deficiency characterized by recurrent infections, particularly with *Staphylococcus aureus* and *Candida albicans*, leading to frequent skin and lung abscesses.^[1] Additional clinical features include dermatitis, eosinophilia, and high serum levels of IgE. Hyper IgE syndrome is a syndrome with heterogeneous genetic causes, reflected in distinct inheritance patterns. An autosomal dominant form of the disease (AD-HIES) is commonly seen in outbred populations. In addition to the above manifestations, AD-HIES

is further characterized by mesenchymal features including retained primary teeth, recurrent bone fractures and particular susceptibility to pneumatocele formation. In contrast, an autosomal recessive form of HIES (AR-HIES) lacking in mesenchymal features but associated with recurrent viral infections and autoimmunity predominates in populations with high consanguinity rates, prominently the Turkish population.^[2] This review will focus on the discovery of deleterious mutations in the gene encoding the Deducator of Cytokines 8 (DOCK8) as the underlying cause of the majority of cases in AR-HIES, highlighting in the process the critical role played in disease

discovery by the late Prof. Isil Berat Barlan, M.D. It will also detail underlying molecular and immunological mechanisms implicated in the disease pathogenesis, as well as current approaches to diagnosis and therapy.

The Signal Transducer and Activator of Transcription (STAT) 3 mutations in AD-HIES

An insight into the molecular basis of HIES came with the identification of a homozygous mutation in the Tyrosine kinase 2 (TYK2) gene in a patient with an overlapping HIES-like phenotype.^[3] Subsequent investigations led to the identification of heterozygous mutations in the gene encoding the STAT3 in subjects with sporadic, autosomal-dominant (AD) form of HIES.^[4,5] STAT3, a member of the STAT family of transcription factors, mediates gene regulation by multiple cytokine receptors.^[6,7] Upon receptor engagement by the respective cytokine, STAT3 is recruited to the receptor by the action of receptor-associated Janus kinases (JAKs). It is phosphorylated by the JAKs on the regulatory tyrosine 705 residue, leading to its dimerization and translocation to the nucleus to mediate gene transcription. The STAT3 mutations in AD-HIES are typically missense substitutions or in-frame deletions and cluster in the DNA binding and Src homology 2 (SH2) domains.^[4,5,8,9] They cause an expressed protein which acts in a dominant negative manner to antagonize gene expression.^[5] Antagonism of STAT3 function impairs innate and adaptive immune responses. The former include chemokine induction in mononuclear cells by interleukin 6 (IL-6) and suppression of inflammatory cytokine production by IL-10, while the latter include memory T cell and T follicular helper cell differentiation and upregulation of immunoglobulin production in B cells.^[10,11]

The STAT3 regulates multiple cytokine signaling pathways involved in the innate and adaptive immune responses. Many of these cytokines including IL-6, IL-21, and IL-23, are critical to the differentiation of T helper 17 (TH17) CD4⁺ T cells, which as a result is impaired in AD-HIES.^[12-15] These cells are important in protection against bacterial and fungal pathogens, including *Candida* species and *Staphylococcus aureus*, a function which is reflected in the spectrum of infections associated with HIES.^[16,17]

Discovery of DOCK8 Deficiency in AR-HIES patients

Although AD-HIES associated with STAT3 mutations accounts for a substantial subset of HIES, a second group of HIES, mostly Turkish subjects, was described as AR-HIES with a clinically overlapping, but a distinct phenotype.^[2] These patients usually present

with recurrent infections with pathogens associated with AD-HIES, including *Staphylococcus aureus* and *Candida albicans*, but may also have viral infections atypical for AD-HIES such as herpes simplex, herpes zoster, and molluscum contagiosum. In general, they do not suffer from skeletal or dental abnormalities, as do AD-HIES patients. They often have high serum IgE concentrations similar to those with AD-HIES; however, their eosinophil count is typically higher. Many patients have central nervous system abnormalities including cerebral aneurysms, strokes, and infections, leading to death, eventually. Autoimmune phenomena are also common in this patient population including hemolytic anemia, thrombocytopenia, and vasculitis.^[18]

To discriminate the underlying pathophysiological mechanisms operative the AD- versus AR-HIES, and to facilitate the discovery of novel genetic elements as the underlying cause in the latter subgroup, plans were initiated in 2007 by Professor Dr. Isil Berat Barlan to establish a collaborative study on Turkish subjects with HIES (Figure 1). These plans were realized in May 2008 when Professor Dr. Barlan began assembling a cohort of newly diagnosed patients with HIES, eventually involving 13 different medical centers in nine major metropolitan areas in Turkey (Adana, Ankara,



Figure 1. Professor Dr. Isil Berat Barlan at Marmara University, circa 2007.

Antalya, Bursa, Gaziantep, Istanbul, Izmir, Konya and Trabzon).^[9] In the differential diagnosis of the underlying pathophysiological mechanisms implicated in AD- versus AR-HIES and to facilitate the discovery of novel genetic elements as the underlying cause in the latter subgroup, in May 2008 Prof. Isil Berat Barlan, M.D. initiated a series of studies on Turkish patients with newly diagnosed HIES from 13 different medical centers in nine major metropolitan areas in Turkey (Adana, Ankara, Antalya, Bursa, Gaziantep, Istanbul, Izmir, Konya and Trabzon).^[9] To build this cohort, Prof. Barlan, M.D. reached out to her colleagues across Turkey who generously provided access to their patients (A list of collaborators and centers involved in these early studies is presented in the acknowledgements section). In full collaboration with Prof. Barlan, M.D. and her Turkish colleagues and collaborators, we found that the majority of Turkish patients with an HIES phenotype did not suffer from STAT3 deficiency, but nevertheless, they exhibit defective Th17 cell differentiation.^[9] These results, published in 2009, established that disparate genetic mechanisms gave rise to the overlapping phenotypic manifestations of the different forms of HIES. They provided the necessary foundation and patient materials for the subsequent studies that lead to the identification of the genetic cause of AR-HIES.

Using the same cohort in collaboration with Prof. Barlan, M.D. and her colleagues, we next investigated the genetic basis of AR-HIES using high-density single nucleotide polymorphism (SNP) arrays and localized the gene defect to the short arm of chromosome 9. Further collaborative studies on the Turkish cohort by our groups and on a second cohort of AR-HIES like subjects collected by the group of Dr. Bodo Grimbacher in London, localized the gene defect to DOCK8, encoding a guanine nucleotide exchange factor (GEF).^[18] DOCK8 mutations and deletions were identified in most, though not all, subjects with AR-HIES, with a minority suffering from other genetic lesions. Meanwhile, the group by Zhang et al.^[19] also reported on the identification of DOCK8 mutations as a cause of combined immunodeficiency with an identical phenotype to the AR-HIES patients studied by our cohorts. Finally, ethylnitrosourea-induced mutations in murine DOCK8 were found by Randall et al.^[20] to result in crippling of germinal center formation, indicative of impaired humoral immunity and consistent with the combined immunodeficiency phenotype of human DOCK8 deficiency.

Genetic attributes of DOCK8 mutations

The DOCK8 gene encompasses 48 exons spread over 200 kilobases. The overwhelming majority of patients with DOCK8 deficiency among populations with high

a prevalence of consanguinity suffer from homozygous mutations in DOCK8, with a few, particularly from outbred populations, presenting with compound heterozygous mutations. A surprisingly large fraction (>60%) of the genetic defects affecting the DOCK8 locus are deletions, ranging in size from small single exon deletions, to large deletions spanning the entire locus.^[21] The preponderance of deletions as the dominant genetic lesion reflects the large number of repetitive elements found in the DOCK8 locus, many of which come very close to exon boundaries.^[18] Other genetic defects affecting DOCK8 mostly consist of nonsense and splice junction mutations.^[21] Surprisingly, true missense mutations have been very rarely reported in suspected patients with only two such mutations published to date.^[22] Of 58 patients identified by our group to suffer from DOCK8 deficiency whose genetic defect was confirmed by molecular analysis, only one was found to have a novel missense mutation (discussed below). Somatic reversions of splice junction and nonsense mutations are common, leading to partial expression of DOCK8 in some cell lineages, but not others.^[23] Such reversions reflect the competitive advantage of partial recovery of DOCK8 protein expression on cell survival over its complete deficiency.

To date, the majority of patients identified with DOCK8 deficiency are of Turkish, followed by those of Arab descent.^[21,24] Amongst the Turkish population, specific mutant alleles appear common, reflecting founder effects (Keles et al., manuscript in preparation). Similar local spread of mutant alleles is found in other regions in the Middle East.^[25] Heterozygous carriers of mutant DOCK8 alleles are phenotypically normal. Nevertheless, long-term studies on disease risks and clinical outcomes of heterozygous carriers are lacking. As such, whether heterozygosity for DOCK8 mutant alleles poses particular health risks remains an unexplored area of investigation.

Mechanisms of DOCK8 action

DOCK8 encodes a protein with apparent molecular weight of 180-200 kilodaltons (kDa), whose expression, unlike that of STAT3, is restricted to hematopoietic lineages.^[26] DOCK8 is a member of the DOCK180 superfamily of GEFs for Rho GTPases, small Ras-related GTPases which regulate the actin cytoskeleton.^[27] The 11 members of the DOCK family share two conserved domains, termed DOCK homology domains 1 and 2 (DHR1 and DHR2, respectively). DHR1, located upstream of DHR2, binds phosphatidylinositol 3,5 bisphosphate [PtdIns(3,5)P2] and phosphatidylinositol 3,4,5 trisphosphate [PtdIns(3,4,5)P3], and acts to focus the GEF activity at membrane sites of PtdIns(3,5)P2 and PtdIns(3,4,5)

P3 production. The DHR2 domain contains the GEF catalytic center, and mediates interaction with Rho GTPases. The specificity of the DHR2 domain for individual Rho GTPases varies between different DOCK proteins. For instance, whereas DOCK2 activates the Rho GTPase Ras-related C3 botulinum toxin substrate 1 (Rac1), but not cell division cycle 42 (Cdc42), the reverse is true for DOCK8, which specifically activates Cdc42.^[28]

The mechanisms by which DOCK family proteins activate Cdc42 have been elucidated by X-ray crystallographic analysis of DOCK9 in complex with GTP- or GDP-bound Cdc42. A valine at position 1951 of DOCK9, equivalent to valine 1985 in DOCK8, acts to destabilize GDP binding to Cdc42, allowing its release and replacement by GTP.^[29,30] More importantly, our recent studies on a unique Turkish patient (Kindly referred to us Ferah Genel, M.D.) with a missense mutation in the DHR2 domain which inactivated the Cdc42 function of DOCK8, while preserving protein expression, revealed that inactivation of DOCK8 GEF catalytic activity was sufficient to recapitulate the full phenotype of the disease (Keles et al., manuscript submitted for publication).

DOCK8 coordinates the actin cytoskeleton response to mitogenic and chemokine signals through the reversible activation of cell division cycle 42 (Cdc42).^[31-34] DOCK8 deficiency results in impaired actin polarization to the immunological synapses of T cells and natural killer (NK) cells.^[33,35] DOCK8 interacts with the Wiskott-Aldrich Syndrome protein, which depends on Cdc42 for its promotion of actin nucleation.^[34] The failure to mobilize actin at immunological synapses disrupts their formation, reflected in defective accumulation of the lymphocyte function-associated antigen (LFA)-1, and Intercellular Adhesion Molecule (ICAM) 1 at the immunological synapses. Disrupted synapse formation impairs cytotoxic T and NK cell killing, B cell responses, and other attributes of adaptive immune responses. By virtue of its regulation of the actin cytoskeleton, DOCK8 also plays a crucial role in cytotoxic T cells and dendritic cells in three-dimensional (3D) gel matrices, as well as tissues such as the skin.^[32,36] Failure to mediate this function causes paucity of tissue memory T cells and explains in part the particular susceptibility of DOCK8-deficient patients to viral skin infections.^[18,19,21,24]

In addition to regulating the actin cytoskeleton, DOCK8 mediates additional cellular functions. Jabara et al.^[37] showed that DOCK8 linked TLR9-MyD88 signaling in B cells to STAT3 activation via a Src-Syk kinase cascade. This pathway is relevant to the promotion by TLR9 ligands of B cell activation and memory formation. The large size of the DOCK8

protein, its capacity to interact with membranes through the DHR1 domain and its functions both as a GEF and a scaffold for binding other proteins, all predict complex functions which are yet to be fully explored.^[37]

Immunological features of DOCK8 deficiency

DOCK8 is expressed in all key immune cells including T, B and NK cells. Its deficiency profoundly impacts the functions of all three populations. Many DOCK8 deficient patients have lymphopenia involving their CD4 and CD8 compartments. T cell excision circles, markers of recent thymic immigrants, are profoundly decreased in DOCK8-deficient patients.^[38] There is paucity of naive T cells in the periphery,^[35] and CD8⁺ cytotoxic T cells exhibit an exhausted cell phenotype (CD45RA⁺CCR7⁻), have decreased survival, and reduced memory CD8 T cell response. Furthermore, DOCK8-deficient T cells proliferate poorly to mitogens. In addition to T cells, NK cells are frequently decreased in numbers in DOCK8 deficiency and show reduced killing.^[33,34] Nature killer T cell development and the persistence of the NKT cell response are impaired and the latter is associated with reduced survival.

Similar to the case of T cells, the B cell compartment also manifests failure of immunological memory formation. Primary B cell responses are preserved in DOCK8 deficiency; however, they decline rapidly. Formation and persistence of memory B cells is profoundly impaired, as is the B cell response to Toll-like receptor 9 (TLR9) stimulation.^[37] DOCK8 deficiency is associated with markedly increased serum IgE level, decreased IgM, and normal or increased serum IgG and IgA concentrations. However, as discussed above, persistence of memory B cell responses is profoundly impaired.^[37]

Patients with DOCK8 deficiency exhibit marked, and frequently striking, eosinophilia, with high frequencies and numbers of circulating eosinophils. As befits HIES patients, their serum IgE concentrations are most often profoundly increased. Furthermore, unlike AD-HIES patients, they commonly suffer food allergy and other allergic diseases. DOCK8-deficient subjects exhibit increased frequencies of circulating T cells expressing Th2 cell cytokines. Furthermore, naive DOCK8-deficient CD4⁺ T cells skew more towards the Th2 cell lineage when cultured in vitro under Th2 cell polarizing conditions as compared to DOCK8-sufficient cells (Sevgi keles et al, manuscript submitted). It was earlier mentioned that Th17 cell differentiation in AR-HIES patients was defective. We have recently demonstrated defective Th17 differentiation in patients with confirmed DOCK8 deficiency (Keles et al., manuscript to be submitted) (Figure 2). Furthermore,

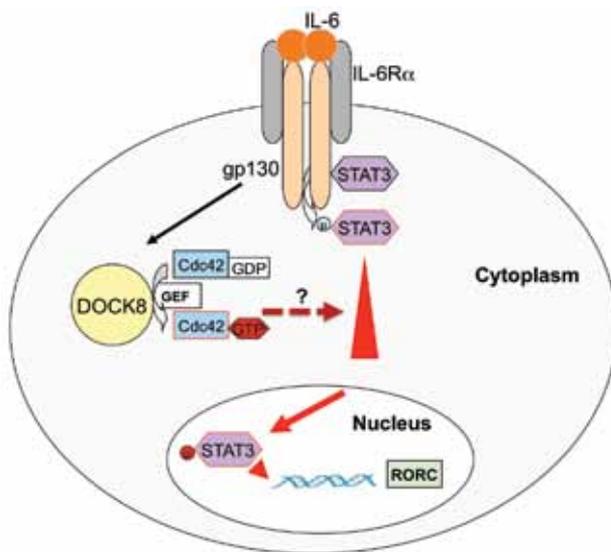


Figure 2. Scheme depicting putative mechanism(s) for the regulation of Th17 cell differentiation by DOCK8. Signaling by IL-6 via the IL-6 receptor alpha chain (IL-6R α)/gp130 tetrameric complex activates STAT3, an essential step in Th17 cell differentiation. IL-6 signaling induces STAT3 phosphorylation through the action of receptor-associated Janus kinases, enabling its translocation to the nucleus. There, STAT3 can induce expression of the Th17 cell master transcription factor gene RORC, in synergy with other signals such as transforming growth factor beta (not shown). DOCK8 may promote STAT3 activation by modulating its phosphorylation and/or enabling its intracellular trafficking in a guanine nucleotide exchange factor (GEF) activity-Cdc42-dependent manner.

the defect in Th17 cell lineage determination extends to IL-17 producing group 3 innate lymphoid cells (ILC3), which require DOCK8 for their function and survival.^[39] It is likely that the defect in Th17 cells and ILC3 differentiation reflects failure of the same fundamental mechanism normally enabled by DOCK8 expression. Furthermore, defective ILC3 function and survival in DOCK8 deficiency may dysregulate Th2 responses by failing to mediate regulation of type 2 immunity by the microbiota.^[40]

Patients with DOCK8 deficiency may develop several autoimmune manifestations including autoimmune cytopenias, hemolytic anemia, vasculitis, and other manifestations. Their sera manifest autoantibodies against a large number of self-antigens.^[41] The mechanisms responsible for autoimmunity reflect both failure of peripheral B cell tolerance, and regulatory T (Treg) cell deficiency characterized by defective suppression.^[41] Treg cell deficiency may also contribute to the increased propensity for food allergy in the context of a skewed Th2 cell environment.^[42]

DOCK8-deficient patients also have decreased plasmacytoid dendritic cells in circulation with

profoundly depressed interferon alpha (IFN- α) production, a phenotype which may possibly contribute to recurrent viral infections.^[43] Accordingly, therapy with IFN- α is of therapeutic benefit in cases of DOCK8 deficiency with severe viral infections refractory to conventional therapies (see below).

Clinical manifestations, diagnosis and therapy of DOCK8 Deficiency

DOCK8 deficiency is a combined immunodeficiency which entails serious clinical sequelae with high morbidity and mortality. In two large recently published retrospective studies by Engelhardt et al.^[21] and Aydin et al.,^[24] mortality was found to be 34% in one series and 25% in the other, respectively. In the study conducted by Engelhardt et al.,^[21] survival by the age of 10 years was 67% (95% CI, 54 to 83%); however, it decreased to 48% (95% CI, 31 to 73%) by the age of 18 years. Similar results were also reported in the series by Aydin et al.^[24]

Patients with DOCK8 deficiency may present with moderate to severe atopic diathesis. Eczema and highly elevated serum IgE concentrations are nearly universal manifestations.^[21,24] Many patients suffer from food allergy, which is a prominent feature of the disease.^[21,24] Patients also exhibit sensitization to environmental allergens in association with asthma and eczema.^[21,24] DOCK8 is a distinguished form AD-HIES in that the latter, while associated with elevated IgE and eosinophilia, is not associated with food or environmental allergies, reflecting possibly the ineffective degranulation of mast cells precipitated by STAT3 deficiency.^[44] DOCK8-deficient patients share with AD-HIES the susceptibility to recurrent infections with gram-positive bacteria, particularly *Staphylococcus aureus*, and, to a lesser extent, gram negative pathogens. These infections are also contributed by the skin barrier defect associated with eczema, poor antibody responses, and defective provision of helper T cell function. DOCK8 deficiency is also distinguished by a particular susceptibility to viral infections, particularly cutaneous viral infections including Herpes simplex, Varicella zoster, Molluscum contagiosum, and Papilloma viruses.^[18,19,21,24] As detailed above, this unusual susceptibility reflects the defective CD8, NKT, and NK cell killing of virally infected cells, defective formation of memory T cells and plasmacytoid dendritic cells, and defective migration of T cells and dendritic cells in three dimensional connective tissue matrices, such as the skin. DOCK8-deficient patients commonly suffer from mucocutaneous candidiasis, and, at times, more invasive fungal infections related to both failed Th17 cell differentiation and more

widespread immune abnormalities (Keles et al., manuscript to be submitted).

Furthermore, the increased risk entailed by DOCK8 deficiency for the development of autoimmune manifestations may be more common than appreciated, evidenced by the abundance of auto-antibodies in sera of DOCK8 deficient patients.^[41] While autoimmune cytopenias and vasculitis have been reported in patients with DOCK8 deficiency, other manifestations include autoimmune hepatitis and autoimmune enteropathy masquerading as Immune Dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)-like disease (unpublished observations).

Malignancies are common in DOCK8 deficiency, affecting up to 8 to 15% of patients.^[21,24] Malignancies are most frequently virally driven, including Papilloma virus-induced squamous cell carcinomas, and EBV-driven Burkitt lymphoma. The central nervous system is the target of infectious complications including abscesses due to bacteria or fungal infections, and viral encephalitis caused by JC or other viruses. Non-infectious neurological complications are surprisingly common and include vasculitis, aneurysms, and tumor infiltration.^[21,24]

Diagnosis of DOCK8 deficiency is based on the clinical presentation, bolstered by laboratory findings of high IgE and eosinophilia, absent or decreased DOCK8 expression by flow cytometry or immunoblotting, and finally molecular diagnosis with nucleic acid sequencing (genomic and complimentary DNA Sanger sequencing, next generation targeted sequencing and/or whole exome sequencing). Flow cytometric analysis provides a rapid method for screening of DOCK8 deficiency, particularly relevant for screening atypical DOCK8 deficiency and excluding cases of severe eczema or other overlapping phenotypes.^[23,45,46] Due to the potential for somatic reversions for point mutations and small insertions/deletions, care should be taken to screen several immune cell lineages to rule out such a possibility. Although very rare, cases of loss of function missense mutations with normal DOCK8 expression may be missed by flow cytometric screening. In such cases with a high index of suspicion, direct DNA sequencing is indicated.

Therapy for DOCK8 deficiency includes intravenous or subcutaneous immunoglobulin therapy to compensate for defective B cell immunity, and prophylactic antibiotic, anti-viral, and anti-fungal therapy to ward off infections with the respective pathogens.^[24] Interferon alpha is useful as rescue therapy for severe viral infections, including those due to Herpes simplex and papilloma viruses.^[43,47,48] Bone marrow transplantation is curative

and is the recommended long-term definitive therapy given the significant morbidity and mortality associated with DOCK8 deficiency.^[24,49-51]

Future Directions

Since its discovery in 2009, rapid progress has been made in delineating the clinical features of DOCK8 deficiency and in establishing its underlying pathogenic mechanisms. Much remains to be elucidated, however, in terms of understanding the mechanisms by which DOCK8 regulates the immune response and the relationship between various immunological defects and clinical manifestations of the disease. The Particular elucidation of those mechanisms by which DOCK8 and STAT3 deficiency converge to give overlapping clinical and immunological phenotypes, including the eczema, hyper IgE, eosinophilia, and failure of Th17 cell differentiation is of utmost importance. The long-term clinical outcomes of DOCK8 deficiency also require further investigation, including the clinical impact if any of heterozygous DOCK8 (carrier) mutations. The role of gene therapy for DOCK8 deficiency is also of interest in light of ongoing trials of gene therapy for the related Wiskott-Aldrich syndrome.^[52] Finally, there is an urgent need to optimize clinical therapy for those patients waiting for bone marrow transplantation or those unable to receive such therapy. We believe that further multicenter collaborative studies in Turkey, similar to the one pioneered by Prof. Barlan, M.D., would be instrumental in the realization of such improved therapies.

Acknowledgements

We dedicate this review to the beloved memory of Prof. Isil Berat Barlan, M.D. (1958-2015). Her commitment to the wellness of Turkish children with primary immunodeficiency diseases, particularly those with HIES, was instrumental in initiating the series of studies which shed light into the DOCK8 deficiency. The original studies on the Turkish cohort with HIES were initiated at Marmara University, Istanbul under the leadership of Prof. Isil Berat Barlan, M.D. with the participation of members of her team, including Sevgi Keles, Nerin Bahceciler, and Elif Karakoc-Aydiner.^[9,18] We would like to acknowledge the following investigators from the respective cities/centers whose participation and contribution was essential to the accrual of the cohort and the success of the ensuing studies:

Adana: Mustafa Yilmaz, Cukurova University; Ankara: Aydan Ikinciogullari, Ankara University; Ayse Metin, Diskapi Children's Hospital; Antalya: Olcay Yegin, Akdeniz University; Gaziantep: Ercan Kucukosmanoglu, Gaziantep University; Istanbul:

Yildiz Camcioglu, Haluk Cokugras, Istanbul University Cerrahpasa Medical Faculty; Ayper Somer, Istanbul University Istanbul Medical Faculty; Mutlu Yuksek, Zeynep Kamil State Hospital; Izmir: Necil Kutukculer, Ege University; Konya: Ismail Reisli, Necmettin Erbakan University Meram Medical Faculty; Trabzon: Ali Baki, Karadeniz Technical University.

Finally, we thank all collaborators who participated in our subsequent studies for their support and kind provision of patient materials.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

This work was supported by grants from the National Institutes of Health (5R01AI065617) to T.A.C. and from the Scientific and Technological Research Council of Turkey (1059B191300622) to S.K.

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