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Exome-first Approach Identified Novel Homozygous Dedicator of Cytokines 8 (*DOCK8*) Mutations in Three Unrelated Iranian Pedigrees Suspected with Hyper-IgE Syndrome

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ABSTRACT

The prevalence of primary immunodeficiency (PID) is rather high in Iran compared to the world average, mainly due to the high rate of consanguineous marriage. Despite that, little genetic information is available about primary immunodeficiencies in Iran. Autosomal recessive hyper IgE syndrome (AR-HIES) is a severe type of immunodeficiency, mainly caused by mutations in the dedicator of cytokines 8 (*DOCK8*). Rapid and precise diagnoses of patients suffering from AR-HIES can help to manage the patients and reach properly the treatment decision. However, in regions with low financial resources and limited expertise, deep phenotyping is uncommon. Therefore, an exome-first approach is helpful to make a genetic-based diagnosis.

In the present study, whole-exome sequencing (WES) was applied to detect causative mutations in three unrelated primary immunodeficient patients with poor clinical information. One of the cases was a deceased patient with suspected hyper IgE syndrome (HIES) whose parents were subjected to WES.

As a result, three novel pathogenic variants were detected in the *DOCK8* gene, including two splicing sites (c.4241+1G>T and c.4886+1G>T) and one-stop-gain (c.4201G>T, p.Glu1401Ter) variants. Sanger sequencing confirmed the mutations' segregation in corresponding families. Further immunological investigations confirmed that HIES in the studied probands. The

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presence of frontal bossing and broad nose in one of the studied cases, in addition to the typical clinical presentation of DOCK8-AR-HIES, is notable.

This work suggests that an exome-first approach can be a valuable alternative strategy for precise diagnosis of primary immunodeficiency patients.

Keywords: Deducator of cytokinesis 8; Exome-first approach; Hyper IgE syndrome; Whole exome sequencing

INTRODUCTION

Hyper IgE Syndrome (HIES) is a genetic disease and one of the most important primary immunodeficiencies, leading to an ineffective immune response. HIES was first described by Deiwis, Schuller, and Wedgewood in 1966.¹ Common symptoms of HIES include recurrent infections, pneumonia, and elevated serum IgE. Autosomal dominant (AD) and autosomal recessive (AR) inheritance patterns have been reported. However, most of the cases are sporadic.

Mutations in signal transducer and activator of transcription-3 (*STAT3*) (ENSG00000168610) are responsible for AD-HIES (Job syndrome). AD-HIES has a higher chance of recurrent fungal and bacterial infections, pneumonia, and pneumatoceles, compared to other forms.² The autosomal recessive form of HIES (AR-HIES, OMIM:243700) is mainly caused by mutations in dedicator of cytokinesis 8 (*DOCK8*) (ENSG00000107099) and is a combined immunodeficiency. The reported symptoms mainly include eczema, pulmonary problems, and persistent viral infections of the skin,^{3,4} emerging in the first months or years of an infant's life. *DOCK8* mutation-related HIES leads to a specific phenotype, viral skin infections (warts and molluscum) and increased risk of cancers in younger ages⁵ with the absence of skeletal involvement.⁶ In addition to *DOCK8*, *TYK2* (tyrosine kinase 2) (ENSG00000105397) gene mutations can also result in AR-HIES.

Due to the high morbidity and mortality rates of HIES in young children, identifying the causative mutation is important to determine the type of syndrome, to make a differential diagnosis, and to properly and timely handle and treat patients.⁷⁻⁹ In most developing countries, detailed phenotyping of primary immunodeficiency patients is difficult to realize, mostly due to the cost and limited expertise. On the other hand, an exome-first approach has been applied for a precise diagnosis of complicated diseases. In this study, we employed the exome-first approach to make

a genetic diagnosis of 3 unrelated patients suspected of having a primary immunodeficiency but with limited clinical and laboratory information.

MATERIALS AND METHODS

Patients

Upon approval of the research plan at the Zanjan University of Medical Sciences Research Ethics Committee (ZUMS.REC.1395.333), the individuals selected for the study were informed of the whole procedure and consented to the study by signature. The study was conducted in accordance with the Helsinki Declaration and the national ethical guidelines of the medical sciences. We studied three unrelated families of patients (proband 1, proband 2 and 3) suffering from primary immunodeficiency who had been referred to the Imam Reza Clinic (Shiraz, Iran) and Research Institute for Primordial Prevention of Non-communicable Disease (Isfahan, Iran). Proband 1, a girl born to consanguineous parents, and suspected to HIES passed away before the study began. Therefore, further genetic testing was performed on samples from parents. Proband 2 and proband 3, both from consanguineous marriage, were suspected to PID with no initial immunological data. The peripheral blood from all samples was collected in EDTA tubes and used for DNA extraction. Four samples (proband 1's parents, proband 2 and 3) were subjected to whole-exome sequencing (WES) in this study.

WES and Bioinformatics Analyses

Genomic DNA (gDNA) from peripheral blood was extracted using the innuPREP Blood DNA Mini Kit (Analytic Jena, Germany). DNA library preparation and exome enrichment were performed; using the Sure Select XT Library prep Kit (Agilent Technologies, CA, USA). Exome sequencing was performed by Macrogen (Seoul, South Korea) using Genome Analyzer HiSeq 4000 (Illumina, USA). Reads length was 101 nucleotides. Bioinformatics analyses were performed at

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Zanjan University of Medical Sciences, using the in-house developed pipeline, as previously described.¹⁰⁻¹² The short reads were aligned to the human genome reference version B37; using BWA and duplicate reads were marked using Picard v2.6.0 (<https://broadinstitute.github.io/picard>). GATK and ANNOVAR were used for variant detection and annotation, respectively. High frequent variants within frequency-based databases including 1000 genome, ESP6500 and ExAC were filtered out. Where possible, pathogenic amino acid change variants were evaluated using several predictors such as PolyPhen, SIFT and CADD. A list of around 200 genes relevant to the primary immunodeficiency diseases was used for further filtration.

Segregation Analysis

Conventional PCR on gDNA samples was performed using specific primers for candidate mutations. PCR and purification of the PCR products were performed based on our standard protocols. The PCR products were subjected to Sanger sequencing on an Applied Biosystems 3500G system. All primers were designed by Geneious software (Geneious 10.2.2, Biomatters Ltd. New Zealand). To retrieve protein sequences the NCBI blast was used (www.blast.ncbi.nlm.nih.gov).

RESULTS

Clinical and Immunological Phenotype of the Patients

Three infants, suffering from primary immunodeficiency, were included in the study. They were from three unrelated families with no family history of primary immunodeficiency and born to consanguineous parents. Proband 1, a girl from a 1st cousin marriage passed away at the age of 11 years, before the study began, because of severe primary immunodeficiency. Since the remaining 200 μ L blood sample left from this subject was inadequate, WES analysis was performed on her healthy parents, looking for the common heterozygous deleterious mutation(s). Before death, the girl was suffering from allergy, cutaneous infections, severe eczema, recurrent pneumonia, and otitis media and herpetic encephalitis. Her circulating levels of eosinophilia and IgE were elevated to 2970 cells/ μ L and 2100 IU/mL, respectively. She was also showing CD3⁺ and CD4⁺ T

cell lymphopenia (40 and 12% of lymphocytes, respectively). Other immunological parameters including IgG, IgA, IgM, CD8⁺, CD19⁺, CD16⁺/CD56⁺ were all within the normal range. The patient was primarily diagnosed with HIES, based on clinical manifestations and diagnostic tests.

WES was also performed on proband 2 and proband 3. For both, initially, no immunological data was available. Proband 2, a 14-years old boy from a 1st cousin marriage, presented with asthma, persistent herpetic keratitis, eczema, diarrhea, otitis media, pneumonia, resistant mucocutaneous candidiasis, bilateral wheeze, and chronic hepatitis. His three siblings were already dead due to premature birth and lung disease.

Proband 3, an 11-years old girl from a 2nd cousin marriage, suffered from an allergy, coarse facies (Figure 1), severe generalized eczema, repeated cutaneous abscesses and recurrent infections. In her family, the early death of one sibling with skin problems and lymphoma had been reported.

WES and Pathogenic Variant Detection

The deceased Proband 1 was suspected of HIES based on the clinical presentation and immunological laboratory results. Following the WES of her parents, around 70500 genetic variants were detected. Focusing on the common heterozygous variants in both parents, filtration and prioritization were performed. A novel heterozygous splicing site *DOCK8* variant (c.4241+1G>T) was detected in both parents. Sanger sequencing was performed on the remaining DNA from the deceased proband and confirmed the variant in a homozygous form (Figure 2a).



Figure 1. Representative images of the coarse facies phenotype of proband 3.

For the second and third probands, initially, 108200 and 110400 variants were detected, respectively. Following several steps of filtration, around 45 homozygous variants remained for each proband. In the end, c.4886+1G>T, a novel homozygous splicing site variant in *DOCK8*, was detected in Proband 2 and c.4201G>Tp.Glu1401Ter, a novel homozygous stop-gain variant in *DOCK8* was identified in Proband 3.

The presence of the identified variants was then

confirmed in both unrelated patients (homozygous in Proband 2 and 3 (Figure 2b and 2c, respectively), their healthy parents (heterozygous) and the healthy sibling of Proband 3 (Figure 2). Both splicing sites and stop-gain variants are highly deleterious. The amino acid E1401 which is transformed to a stop codon in *DOCK8* of Proband 3 is highly conserved among various organisms, based on multiple sequencing alignment analyses (Figure 3).

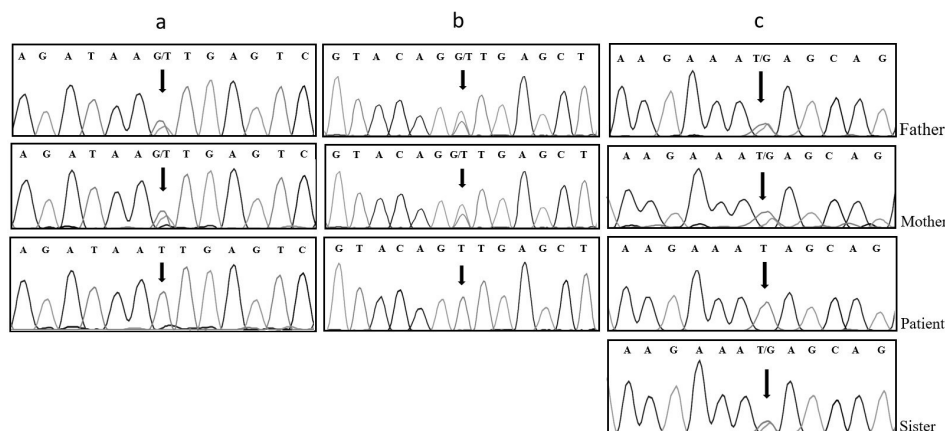


Figure 2. Sanger sequencing of c.4241+1G>T (a), c.4886+1G>T (b) and c.4201 G>T (c) variants in the studied families. The black arrow shows the variant position

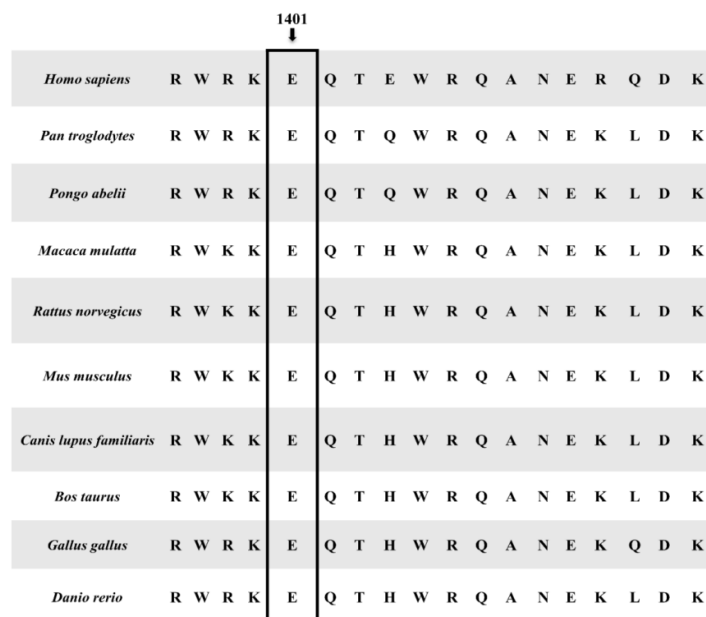


Figure 3. Multiple sequencing alignment of the mutated region (p.Glu1401Ter) in Proband 3, indicating highly conserved amino acid 1401 among various organisms

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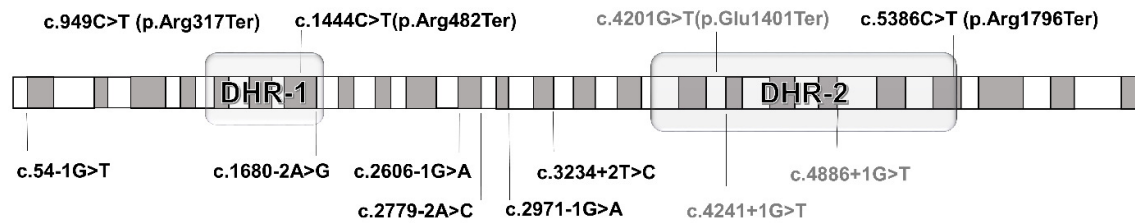


Figure 4. Schematic representation showing the location of previously reported splicing site and stop-gain dedicator of cytokines 8 (*DOCK8*) mutations. Novel mutations found in this study are indicated in grey

All three variants we have found are novel with no frequency recorded in the gnomAD, ExAC, 1000G and ESP6500. Similarly, they were not observed in the Iranome database (<http://www.iranome.ir>), a collection of 800 healthy WES samples from Iran.

DOCK8 protein is mostly expressed in immune system cells,¹³ displaying an important role in the survival and function of B cells, T cells, and NK cells. It has two conserved domains, DOCK Homology Region 1 and 2 (DHR1 and DHR2). The three pathogenic variants detected in this study are notably all located in the DHR2 domain (Figure 4).

As previously mentioned, no immunological laboratory data were available for Proband 2 and 3 upon initiation of the study. However, the genetic diagnosis of *DOCK8*-related primary immunodeficiency prompted us to perform immunological tests. As expected, both patients displayed eosinophilia and elevated IgE levels (Proband 2: around 2200 cells/ μ L and 1000 IU/mL, respectively; Proband 3: about 3345 cells/ μ L and 3200 IU/mL, respectively). Moreover, both patients displayed CD3⁺ and CD4⁺ T cell lymphopenia (Proband 2: 45 and 10% of lymphocytes, respectively; Proband 3: 38 and 11% of lymphocytes, respectively). Other immunological parameters were in the normal range.

DISCUSSION

Precise diagnosis of the type of primary immunodeficiency requires a comprehensive set of tests, which are not routinely performed in developing countries where financial expenses are only partially covered by insurance. In this situation, alternatively, the genome-first approach and in particular, exome-first approach could be of great help for a precise genetic diagnosis.¹⁴⁻¹⁸

In this study, we attempted to identify the genetic

cause of three unrelated patients suspected of primary immunodeficiency using the exome-first approach. In all selected cases, the type of primary immunodeficiency was unknown except for one patient suspected for hyper IgE syndrome (HIES). The scenario could be more complex when the proband is deceased, which is not uncommon in primary immunodeficiency patients due to the life-threatening nature of the disease. For such a case in this study (Proband 1), the carrier consanguineous parents were subjected to an exome-first approach.

The identified three novel *DOCK8* pathogenic variants in this study suggest that our patients were suffering from AR-HIES. Further clinical and laboratory investigations confirmed the diagnosis. Unlike most western communities, AR-HIES is rather prevalent among primary immunodeficiencies in societies with a high rate of consanguineous marriages.^{19,20} As such, the prevalence of *DOCK8* deficiency in Iran is expected to be high.²¹

The studied patients presented the typical clinical signs of *DOCK8*-deficient patients including recurrent infections, eczema, elevated serum IgE, and eosinophil levels.^{13,22}

Human *DOCK8* is a large gene with 48 exons, spanning over 200 kb of chromosome 9.²³ In this study, three variants, c.4241+1G>T, c.4886+1G>T, and c.4201G>Tp.Glu1401Ter were identified in *DOCK8*, all are notably affecting the DHR2 domain of the protein. To the best of our knowledge, only six splice site mutations have been reported in *DOCK8* so far, none of them located in the DHR2 domain. Moreover, only three pathogenic stop-gain mutations have been reported in HIES patients of which the only one affecting the DHR2 domain. The involvement of this domain in the regulation of the immune system has been already predicted based on its guanine-nucleotide

exchange factor activity.^{7-9,13,14} This is consistent with the occurrence of three DHR2-domain mutations in our patients, suggesting the important role of DHR2 in the function of DOCK8 protein.

Another interesting finding of this study is the identified controversy in clinical features of *DOCK8* mutation-related HIES compared to the previous reports. It's been previously reported that unlike AD-HIES patients, the *DOCK8* deficient AR-HIES patients do not present coarse facies as their skeletal features will not be affected by *DOCK8* mutations.⁶ However, recently two different studies reported the coarse facial features in two unrelated *DOCK8* deficient patients,^{24,25} in contradiction to classical presumption. The effect of other genetic mutations or severe eczema as a reason for coarse facies in these two patients could not be excluded. Here, the Proband 3 of this study presented frontal bossing and broad nose in addition to the typical clinical presentation of *DOCK8*-AR-HIES including the increased serum IgE, eosinophilia, eczema, and recurrent infections.

These observations confirm the importance of accurate genetic diagnosis in the identification of primary immunodeficiency disease type, which provides a ground for a rapid and appropriate treatment strategy. Hematopoietic stem cell transplantation (HSCT) has proved to be the only standard medical care and treatment for *DOCK8* immunodeficiency syndrome (DIDS) and the prevention of the high mortality rate of this immunodeficiency.^{9,26,27} Rapid and precise diagnosis of DIDS and differential diagnosis with other phenotypically similar HIES is crucial considering that these patients pass away because of infections, lymphoma, and squamous cell carcinoma^{9,28} when the diagnosis is delayed. Death of proband 1 in our study due to the late diagnosis underpins the importance of rapid diagnosis.

In Iran, newborns receive BCG, polio, and hepatitis B vaccines before discharging from the hospital. If they suffer from primary immunodeficiencies, they will be at risk of systemic infections especially when they receive a live attenuated vaccine. All three probands of our study were vaccinated.

Our study suggests an exome-first approach for precise diagnosis of primary immunodeficiency in Iran. This approach could be of great help in regions where detailed phenotyping is unpractical mainly due to cost limitations.

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