41 of 60 patients with autosomal-recessive hyper-IgE syndrome carry deletions and point mutations in *DOCK8*

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INTRODUCTION

Background: The Hyper-IgE Syndromes (HIES) are rare primary immunodeficiencies with both autosomal dominant (AD)[1] and autosomal recessive (AR)[2] forms. However, most patients are sporadic cases. Approximately 60-70% of patients with hyper-IgE syndrome have dominant mutations in *STAT3*[3], and a single patient was reported to have a homozygous *TYK2* mutation[4]. In the remaining hyper-IgE syndrome patients, the genetic aetiology has not yet been identified.

Objectives: We aimed to identify a gene that is mutated or deleted in AR-HIES.

Methods: We performed genome-wide single nucleotide polymorphism analysis for nine subjects with AR-HIES to locate copy number variations and homozygous haplotypes, followed by candidate gene sequencing in additional patients. We have now analysed *DOCK8* by homozygosity mapping, PCR analysis and sequencing in a total of 60 patients with AR-HIES.

RESULTS

Subtelomeric homozygous (ARH001-ARH004) or compound heterozygous (ARH005) microdeletions were identified in five patients at the terminus of chromosome 9p. In all patients the deleted interval involved *DOCK8*.

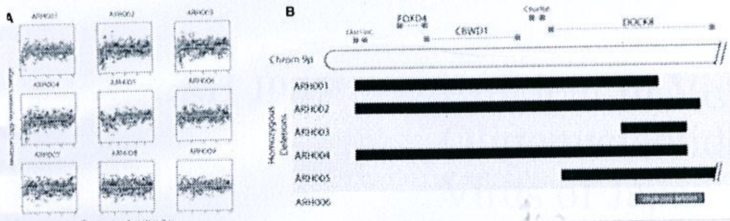


Figure 1. A. ROMA data demonstrating copy number abnormalities consistent with subtelomeric deletions of 9p in AR-HIES. Genome-wide SNP Nsp 250k arrays were used. B. HIES patient deletions and known and predicted genes at the terminus of chromosome 9p. *C9orf66*: open reading frame; *FAM138C*: noncoding RNA gene; *FOXD4*: transcription factor; *CBWD*: protein with cobalamin binding domain and nuclease function; *DOCK8 (dedicator of cytokinesis 8)*: protein implicated in the regulation of the actin cytoskeleton.

Subsequently, 55 more patients were analysed for homozygous or compound heterozygous *DOCK8* deletions and point mutations. Twelve patients from consanguineous parents were excluded from *DOCK8* mutation analysis, because homozygosity mapping with microsatellite markers revealed heterozygosity at the *DOCK8* locus, making *DOCK8* mutations unlikely.

Exonic deletions were shown by the failure to amplify exons from genomic DNA by PCR. Single exon deletions were confirmed by cDNA sequencing.

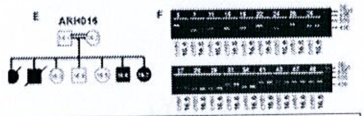


Figure 2. The lack of PCR products from patients' DNA compared to control DNA suggests exonic deletions.

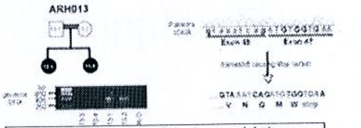


Figure 3. Confirmation of single exon deletion (exon 46) by cDNA sequencing.

Point mutations were detected by genomic DNA and/or cDNA sequencing.

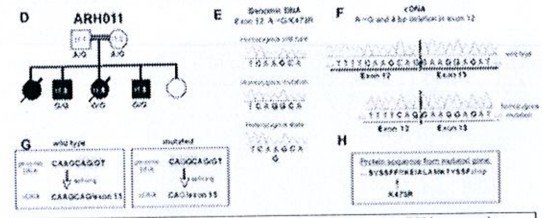


Figure 4. Point mutation in *DOCK8* exon 12 as shown by genomic DNA sequencing (E). cDNA sequencing, however, shows a 4 bp deletion following the point mutation (F). This can best be explained by the generation of a cryptic splice site (G).

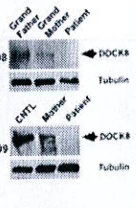


Figure 5. DOCK8 protein expression in PBMCs.

29 of 43 families (41 of 60 patients) carry mutations/deletions in *DOCK8*

21 deletions (27 patients)

- Large deletions
  - __Including several genes:__ **4 families** (4 pts)
  - __Including several exons:__ **11 families** (13 pts)
- Single exon deletions: **5 families** (7 pts)
- 2 bp deletion: **1 family** (3 pts)

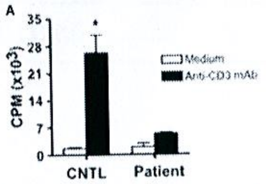
6 point mutations (11 patients)

- Stop codon: **3 families** (3 pts)
- Splice site mutations
  - __Donor:__ **1 family** (4 pts)
  - __Acceptor:__ **1 family** (1 pt)
  - __Cryptic splice site:__ **1 family** (3 pts)

2 other (3 patients)

- **Probably** chromosomal translocation: **1 family** (2 pts)
- Retained intronic bps: **1 family** (1 pt)

DOCK8 deficiency was associated with impaired proliferation of $CD4^+$ and $CD8^+$ T cells.



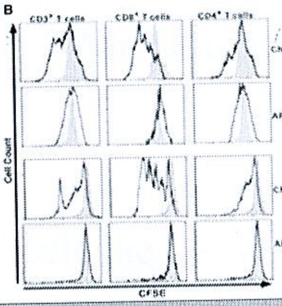


Figure 6. A. Proliferative responses of PBMCs to anti-CD3 mAb treatment (A) and anti-CD3 + anti-CD28 mAb stimulation for 3 days (B). Shaded area: respective unstimulated cell population.

Origin	pts
Turkey	15
Middle East	8
• Lebanon	4
• Kuwait	2
• Egypt	1
• Iran	1
Europe	3
• UK	1
• Italy	1
• Ukraine	1
Asia	2
• Pakistan	1
• India	1
Mexico	1

Origin of DOCK8 deficient patients

THE CLINICAL PHENOTYPE OF DOCK8 DEFICIENCY

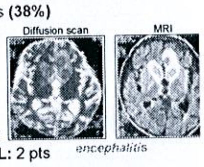
Skin disease

Skin abscesses: 26/36 pts **(72%)**
Candidiasis: 26/31 pts **(84%)**
Severe atopic dermatitis, often colonized with *Staphylococcus aureus*: 36/38 pts **(95%)**

Respiratory - **Upper or lower RTI: 100%**
Upper respiratory tract infections: 33/36 pts **(92%)**
Recurrent pneumonia: 28/35 pts **(80%)**
Bronchiectasis: 11/31 pts **(35%)**

Atopy
Multiple allergies (__food__, environmental, drug): 20/26 pts **(77%)**
Asthma: 11/21 pts **(52%)**

CNS features - 13/34 pts **(38%)**
Meningitis: 4 pts
CNS vasculitis: 3 pts
Encephalitis: 1 pt
Stroke: 3 pt
Fungal abscess: 1 pt
Vascular aneurysm: 1 pt
JC virus-associated PML: 2 pts

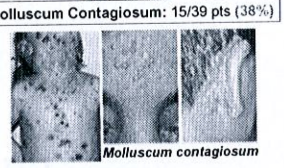


Viral infections - **Severe, recurrent and partially mutilating viral infections:** 34/39 pts **(87%)**

Herpesviridae 24/39 pts (61%)
HSV: 17/39 pts **(44%)**
VZV: 8/39 pts **(21%)**
CMV: 2/39 pts **(5%)**
EBV: 1/39 pts **(3%)**

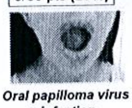
__Herpes:__ skin infection, conjunctivitis, keratitis
__CMV:__ retinitis, meningitis, pneumonia
__EBV:__ pneumonia

Poxviridae
Molluscum Contagiosum: 15/39 pts (38%)



Molluscum contagiosum

Papovaviridae
Papillomaviruses — **HPV:** 9/39 pts **(23%)**



Oral papilloma virus infection

Polyomaviruses — **JC virus:** 2/39 pts **(5%)** → **fatal PML**

Others
Rotavirus: 1pt
HAV: 1pt
HCV: 1pt

Bacterial infections – 15/17 pts **(88%)**

Gram -ve
Proteus mirabilis (3 pts)
E. coli (3 pts)
H. influenzae (2 pts)
Pseudomonas (2 pts)
Klebsiella pneumoniae (2 pts)
Moraxella catarrhalis (1 pt)

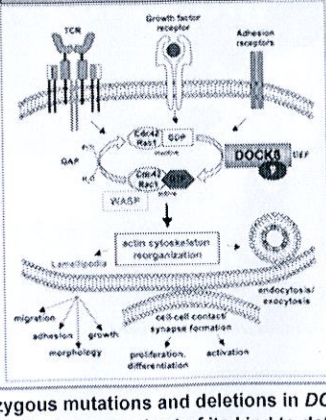
Gram +ve
Staph. aureus (8 pts)
Staph. pyogenes (1 pt)
Staph. epidermidis (1 pt)
Strep. pneumoniae (2 pts)
Listeria monocytogenes (1 pt)

Mycobacteria
Tuberculosis (1 pt)

Others
Poor growth/failure to thrive: 9 pts
Malabsorption and diarrhea: 1 pt
Dental cavities/chronic periodontitis: 2 pts
Squamous cell carcinoma: 2 pts; **Burkitt lymphoma:** 1 pt
Autoimmune hemolytic anemia: 1 pt
Hepatomegaly: 2 pts; **Thrombopenia:** 1 pt
Osteomyelitis: 2 pts

Cell count	decreased	normal	increased
Eosinophils	-	3/35 pts, 9%	32/35 pts; 91%
CD3+ T cells	11/22 pts, 50%	11/22 pts, 50%	-
• CD4+	14/22 pts; 64%	7/22 pts, 32%	1/22 pts, 4%
• CD8+	5/20 pts, 25%	11/20 pts; 55%	4/20 pts; 20%
CD19+ B cells	-	8/21 pts; 38%	13/21 pts; 62%
CD16/56+ NK cells	4/18 pts; 22%	13/18 pts, 72%	1/18 pts; 6%
Serum levels	**decreased**	**normal**	**increased**
IgE	-	1/38 pts; 3%	37/38 pts; 97%
IgG	-	17/21 pts; 81%	4/21 pts; 19%
IgA	4/21 pts; 19%	15/21 pts; 71%	2/21 pts; 10%
IgM	15/21 pts; 71%	6/21 pts; 29%	-

HYPOTHETICAL FUNCTION OF DOCK8



DOCK8 is likely to function as a guanine-nucleotide exchange factor (GEF) for the Rho-GTPases Cdc42 and Rac1, turning them into the active, GTP-bound form upon receptor engagement (e.g. receptor tyrosine kinases, antigen receptors and adhesion receptors)[5,6]. An unknown protein possibly stabilizes the interaction of DOCK8 with Cdc42 and Rac1. GTPase activation induces dynamic filamentous actin rearrangements and lamellipodia formation, possibly *via* WASP, leading to cell growth, migration and adhesion. Given the clinical phenotype of the AR-HIES patients with DOCK8 deficiency, we propose an important role of DOCK8 in T-cell actin dynamics, which might be important for the formation of the immunological synapse, leading to T cell activation, proliferation, and differentiation.

SUMMARY: **We found homozygous or compound heterozygous mutations and deletions in *DOCK8* in 41 out of 60 patients with AR-HIES, originating mainly from Turkey and the Middle East. This is the largest cohort of its kind to date of genetically defined AR-HIES. Our finding is complemented by the work of Zhang and colleagues, who published mutations in *DOCK8* in a cohort of twelve patients with a similar phenotype[7].**

Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, et al. Hyper-IgE syndrome with recurrent infections--an autosomal dominant multisystem disorder. N Engl J Med 1999;340:692-702. 2) Renner ED, Puck JM, Holland SM, Schmitt M, Weiss M, Frosch M, et al. Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. J Pediatr 2004;144:93-9. 3) Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med. 2007;357:1608-19. 4) Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, Tsuchiya S, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity 2006;25:745-55. 5) Ruusala A, Aspenstrom P. Isolation and characterisation of DOCK8, a member of the DOCK180-related regulators of cell morphology. FEBS Lett 2004;572:159-66. 6) Cote JF and Vuori K. Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange. J Cell Sci 2002;115:4901-13. 7) Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ et al. Combined Immunodeficiency Associated with *DOCK8* Mutations. N Engl J Med. 2009 Nov 19;361(21):2046-55.

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