

# Clinical, Immunological and Molecular Characterization of DOCK8 and DOCK8-like Deficient Patients: Single Center Experience of Twenty Five Patients

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Received: 17 May 2012 / Accepted: 13 August 2012 / Published online: 12 September 2012  
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## Abstract

**Purpose** Autosomal recessive hyper-IgE syndrome is a rare combined immunodeficiency characterized by susceptibility to viral infections, atopic eczema, high serum IgE and defective T cell activation. The genetic etiologies are diverse. Null mutations in *DOCK8* and *TYK2* are responsible for many cases. This study aims to provide a detailed clinical and immunological characterization of the disease and explore the underlying genetic defects among a large series of patients followed by a single center. The available data might improve our understanding of the disease pathogenesis and prognosis.

**Methods** Clinical data of twenty-five patients diagnosed with AR-HIES were collected. Seventeen patients screened for *STAT3*, *TYK2* and *DOCK8* mutations.

**Results** Sinopulmonary infections, dermatitis, hepatic disorders, cutaneous and systemic bacterial, fungal and viral infections were the most common clinical features. The rate of hepatic disorders and systemic infections were high. Twelve patients died with a median age of 10 years. CMV infection was the only statistically significant predicting factor for poor prognosis (early death). Three novel *DOCK8* mutations and two large deletions were found in thirteen patients. No mutations found in *STAT3* or *TYK2* genes.

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10875-012-9769-x) contains supplementary material, which is available to authorized users.

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**Conclusion** Autosomal recessive hyper-IgE syndrome is a combined immunodeficiency disease characterized by high morbidity and mortality rate. The different genetic background and environmental factors may explain the more severe phenotypes seen in our series. *DOCK8* defect is the most common identified genetic cause. Patients with no identified genetic etiology are likely to carry mutations in the regulatory elements of genes tested or in novel genes that are yet to be discovered.

**Keywords** DOCK8 · hyper-IgE syndrome · immunodeficiency · Saudi

## Introduction

Hyper IgE syndrome [HIES] is a complex primary immunodeficiency disorder characterized by the clinical triad of chronic eczema, recurrent skin and pulmonary infections and high serum IgE concentration [1]. Two forms of inheritance have been described. An autosomal dominant form caused by dominant-negative mutations in *STAT3* [2, 3], and an autosomal recessive form which seems to include multiple clinical phenotypes caused by different molecular mechanisms [1, 4].

The autosomal dominant hyper IgE syndrome [AD-HIES] is a multisystem disorder that affects skin, skeletal, vascular and immune systems as well as connective tissues. Affected individuals are mainly prone to staphylococcal cutaneous and pulmonary infections which are usually complicated by pneumatoceles. Mucocutaneous candidiasis is common and they are at risk of haematological malignancies [1–3].

Although clinically overlapping, autosomal recessive HIES [AR-HIES] is distinct from AD-HIES. Patients have more severe phenotypes with higher mortality rate at younger age. They have higher incidence of viral cutaneous infections, sepsis and neurological complications. They tend not to develop pneumatoceles after pneumonia and somatic features are rarely reported [4]. *TYK2* null mutation was identified in two patients with AR-HIES associated with mycobacterial and viral infections [5, 6]. Recently, the clinical phenotype in some patients was attributed to loss of function mutations affecting *DOCK8* (dedicator of cytokinesis 8) gene [7, 8]. However, the underlying molecular cause of many patients still not yet identified [8, 9].

Dock8 protein belongs to the DOCK180-related family of atypical guanine nucleotide exchange factors [GEFs] with two conserved Dock homology regions [DHRs]. It is highly expressed in the immune system especially in lymphocytes. It is also expressed in the placenta, kidney, lung, and pancreas [10]. Although the mechanism by which dock8 exerts its function is not

well understood, the DOCK180-related family member activates the Rho GTPases such as CDC42 and/or RAC1 by the removal of GDP. These GTPases play an important role in regulating actin cytoskeletal organization and gene expression [11]. DOCK8-mutant mice have demonstrated failure to sustain antibody response. They do not develop marginal zone B cells and have no germinal center B cell persistence [12]. Both B and T cell immune synapse are defective [12, 13]. In those animal models, T cell lymphopenia is noticed with decrease in mature CD4 thymocytes egress and CD8 T cell memory survival [14]. In DOCK8 knockout mice, the migration of interstitial dendritic cells to lymph nodes is defective which will affect the T cell priming process [15].

Our population is highly inbred with 56 % consanguinity rate which predispose to increasing incidence of autosomal recessive diseases [16]. The aim of this study is to characterize AR-HIES in Saudi patients.

## Methods

Twenty-five patients from fourteen families with AR-HIES who have been followed up at King Faisal Specialist Hospital and Research Centre [KFSH&RC] were enrolled. Patients with recurrent infections, chronic eczema, eosinophilia [eosinophil count > 500 cells/micl] and serum IgE levels  $\geq 2,000$  IU/ml were included. Patients with high serum IgE levels and eczema but no recurrent infections as well as patients with findings suggestive of AD-HIES [i.e. somatic features and pneumatocele] were excluded.

The clinical and immunological characteristics of the patients were recorded. The median of each of the following values was calculated for each patient: the eosinophil count, serum immunoglobulins, the lymphocyte subsets and the in vitro lymphocyte proliferation response. HIES scores were calculated as previously described [17].

Seventeen patients whom their DNA was available were screened for *STAT3*, *TYK2* and *DOCK8* mutations. The dock8 protein expression was assessed whenever it is possible and indicated. The study was approved by the Office of Research Affairs at KFSH&RC, and a written consent was obtained for each of the participating patients.

## Statistical Analysis

We did statistical analysis to find out if CMV infection, bronchiectasis, CNS vasculitis and low IgM level, IgE level, CD3, CD4 and the in vitro proliferative response may predict worse prognosis (early death) in AR-HIES in general and dock8 deficiency (DIDS) in particular. We also

**Table 1** Clinical and laboratory characterization

Patients	Eosinophil/ micl	IgE IU/L	IgG g/l	IgA g/l	IgM g/l	CD3/ mm3	CD4/ mm3	CD8/ mm3	CD19/ mm3	CD16/ 56/ mm3	Lymphocytes proliferatio n response for PHA (CPM)	HIES score/ gene affected	Outcome	Clinical Presentation
F1P1 Boy	870	41,970	On IVIG	1.2	Undetectable	1,742	524	1,142	800	151	20,619	28/ DOCK8	Alive	Recurrent sinopulmonary infections, eczema, skin abscesses, asthma, on IVIG.
F2P1 Girl	1,450	13,630	12.6	1.605	Undetectable	1,365	705	655	502	243	5,536	46/ DOCK8	Died, (sepsis, severe diarrhea)	Recurrent pneumonia, bronchiectasis, sepsis, candidemia, EBV infection, cryptosporidial infections with sclerosing cholangitis, warts, hypothyroidism, adrenal gland leiomyoma, asthma, food allergy, recurrent abscesses, FTT..
F2P2 Girl	1,020	14,275	10.4	0.41	Undetectable	1,866	750	786	826	80	78,092	29/ DOCK8	Alive	Recurrent sinopulmonary infections, EBV infection, viral cutaneous infections (warts and HSV), mucocutaneous candidiasis, asthma, drug allergy and food allergy, recurrent abscesses.
F2P3 Boy	675	10,920	19.2	2.275	0.645	1,185	539	527	531	829	39,135	27/ DOCK8	Alive	Recurrent sinopulmonary infections, bronchiectasis, mucocutaneous candidiasis, recurrent abscesses, scoliosis, hemiparesis, FTT.
F3P1 Girl	1,595	5,720	14.7	3.28	0.19	1,060	298	605	432	108	59,680	28/ DOCK8	Died (sepsis)	Recurrent sino-pulmonary infections, bronchiectasis, eczema, FTT, CMV and EBV infection, impaired liver enzymes, recurrent abscesses, asthma, on IVIG.
F3P2 Boy	1,190	8,670	19.8	2.87	0.2	587	354	89	532	83	100,533	25/ DOCK8	Alive	Recurrent otitis media, eczema, recurrent abscesses, mucocutaneous candidiasis, warts, food allergy, FTT.
F3P3 Girl	8,110	2,764	17.6	Undetectable	0.5	2,386	901	814	1,055	159	75,307	28/ expected DOCK8	Alive	Recurrent sinopulmonary infection, eczema, recurrent abscesses, sclerosing cholangitis, food allergy, asthma.
F3P4 Girl	1,540	6,590	9	1.05	1.18	2,783	1,362	829	2,902	178	58,865	28/ DOCK8	Died (pneumonia, sepsis)	Recurrent sinopulmonary infection, eczema, recurrent abscesses, mucocutaneous candidiasis, nail aspergillus infection, asthma, FTT.
F4P1 Girl	1,580	21,670	30.4	2.89	Undetectable	1,874	390	1,002	564	117	62,970	26/ DOCK8	Alive	Recurrent pneumonia, eczema, recurrent abscesses, viral cutaneous infections (HSV), molloscum contagiosum, perianal carcinoma in situ, food allergy.
F5P1 Boy	4,340	1,590	14.3	0.27	Undetectable	2,890	339	2,244	272	448	3,562	30/ DOCK8		Recurrent sinopulmonary infections, eczema, diarrhea, disseminated

Table 1 (continued)

Patients	Eosinophil/ micl	IgE IU/L	IgG g/l	IgA g/l	IgM g/l	CD3/ mm <sup>3</sup>	CD4/ mm <sup>3</sup>	CD8/ mm <sup>3</sup>	CD19/ mm <sup>3</sup>	CD16/ 56/ mm <sup>3</sup>	Lymphocytes proliferatio n response for PHA (CPM)	HIES score/ gene affected	Outcome	Clinical Presentation
F6P1 Girl	870	17,780	13.1	1.47	Undetectable	808	395	327	99 on anti CD20.	99	53,078	30/ DOCK8	Died (refractor seizures, clinical sepsis)	fungal infection, HBV and HCV hepatitis,CMV infection, EBVinfections, brain infarction ? moyanoya, FTT, cutaneous herpetic infection, food allergy, asthma, drug allergy, sepsis
F6P2 Boy	6,030	10,630	12.2	1.35	0.18	759	221	427	554	206	8,540	32/ expected DOCK8	Died with pneumonia and clinical sepsis	Recurrent pneumonia, eczema, FTT, CMV pneumonia, EBV infection, cryptosporidial infections with sclerosing cholangitis, liver fibrosis, esophageal varices, viral cutaneous infection (HSV), recurrent abscesses, citruinemia, oral candidiasis, brain infarction.
F6P3 Boy	1,935	2,587	12.9	1.23	0.37	533	364	182	662	78	57,847	32/ expected DOCK8	Alive	Recurrent pneumonia, bronchiectasis, eczema, sepsis, allergic rhinitis, food allergy, recurrent abscesses.
F7P1 Boy	2,370	3,543	10.8	2.73	0.21	2,071	892	1,139	1,069 J	339	57,612	22/ DOCK8	Alive	Pneumonia, eczema, warts, skin infections, brucellosis, varicella infection, giant aortic aneurysm, drug allergy.
F7P2 Boy	2,600	28,180	26.1	Undetectable	0.35	1,145	319	356	601	33	9,155	18/ expected DOCK8	Died (post HSCT complications: GVHD, sepsis)	CMV and EBV infection, cryptosporidial infections with sclerosing cholangitis, eczema, recurrent abscesses, FTT, food allergy.
F8P1 Girl	4,670	7,298	24.3	Undetectable	1.31	1,119	360	520	720	140	16,926	34/ no mutation was detected	Died (chemotherapy complications: sepsis, herpetic encephalitis)	Recurrent pneumonia, eczema, recurrent abscesses, B cell lymphoma, post chemotherapy CMV and HSV encephalitis, food allergy.
F9P1 Girl	1,220	11,495	26.8	2.9	1.15	1,858	445	1,295	623	149	17,800	36/ ND	Died (pneumonia, sepsis)	Recurrent pneumonia, bronchiectasis, diarrhea, eczema, FTT, HBVand HCV hepatitis, nephrocalcinosis UTI, food allergy.
F9P2 Boy	950	4,421	10.7	2.4	0.48	829	518	237	660	169	61,010	26/ ND	Died (sepsis)	Recurrent pneumonia, sepsis, liver impairment, hepatosplenomegaly, diarrhea, eczema, skin infections.
F10P1 Boy	4,795	6,780	40.9	Undetectable	1.84	2,296	606	1,275	185	186	27,817	28/ DOCK8	Alive	Nail infection, EBV,CMV infection, diarrhea, impaired liver enzymes,

**Table 1** (continued)

Patients	Eosinophil/ mcl	IgE IU/L	IgG g/l	IgA g/l	IgM g/l	CD3/ mm3	CD4/ mm3	CD8/ mm3	CD19/ mm3	CD16/ 56/ mm3	Lymphocytes proliferatio n response for PHA (CPM)	HIES score/ gene affected	Outcome	Clinical Presentation
F11P1 Boy	670	22,815	21.5	5.03	0.81	1,857	1,218	527	467	355	97,449	28/ND	Died (sepsis)	eczema, skin infections, herpetic cutaneous infections, on IVIG.. Otitis media, eczema, skin infection, nail candidiasis
F11p2 Girl	800	8,370	9.9	2.71	0.86	3,470	2,116	997	1,250	625	20,463	28/ND	Died Sepsis, pneumonia	AIHA, CMV,EBVinfection, FTT,viral cutaneous infections (HSV). Sweet syndrome, recurrent pneumonia, eczema, CMV infection, osteomyelitis, AIHA, FTT.
F12P1 Boy	1,650	17,240	18.5	2.17	0.78	2,136	1,132	713	445	100	120,190	28// no mutation was detected	Alive	Recurrent pneumonia, eczema, skin abscesses, FTT.
F13P1 Girl	2,410	5,925	On IVIG		Undetectable	1,107	421	547	320	32	61,523	32/DOCK8	Alive	Sinopulmonary infections, bronchiectasis, eczema, warts, skin infections, CMV and EBV infection, on IVIG, food allergy, asthma
F14P1 Boy	4,300	33,380	8.75	1.26	0.69	2,862	2,096	685	645	242	174,296	26/ no mutation was detected	Alive	Recurrent pneumonia, eczema , abscesses, warts, EBV infection.
F14P2 Boy	1,075	22,280	13.5	0.84	0.96	3,643	1,925	1,509	1,145	208	104,793	24// no mutation was detected	Alive	Abscesses , warts, eczema, allergic rhinitis.

Normal reference values: [CD3(1-11 m: 3,100–4,800, 1-2 years: 2,200–4,100, 3-5 years: 1,600–2,700, 6-13 years: 1,700–1,900 per mm<sup>3</sup>), CD4(1-11 m: 2,200–3,300, 1-2 years: 1,400–2,800, 3-5 years: 1,000–1,700, 6-13 years: 800–1,700 per mm<sup>3</sup>), CD8 (1-11 m: 1,100–1,700, 1-2 years: 800–1,800, 3-5 years: 600–1,000, 6-13 years: 700–1,000 per mm<sup>3</sup>), CD19 (1-11 m: 1,100–1,900, 1-2 years: 700–1,600, 3-5 years: 400–800 per mm<sup>3</sup>), CD16<sup>+</sup> 56<sup>+</sup> (1-11 m: 300–700, 1-2 years: 200–600, 3-5 years: 200–400, 6-13 years: 200–400 per mm<sup>3</sup>) pediatric allergy and immunology 1,998; 9:44–48]. IgG 2.5–9.1 g/L, IgA 0.2–1.2 g/L, IgM 0.2–1.5 g/L, IgE 1.6–30 u/ml and PHA 94,935–171,149 CPM

F family, P patient, EBV Epstein-Barr virus, HSV herpes simplex virus, CMV cytomegalovirus, HBV hepatitis B virus, HCV hepatitis C virus, UTI urinary tract infection, AIHA autoimmune hemolytic anemia, FTT failure to thrive, ND not done

compared between the previously reported DIDS and Saudi DIDS.

The statistical analysis of the data is done by using the software package SAS version 9.2 [Statistical Analysis System, SAS Institute Inc., Cary, NC, USA]. All continuous variables are compared by using Student's *t*-test and the categorical variables are compared by chi-square test. The level of significance is set at  $p < 0.05$ .

## Results

Twenty-five patients from 14 families not previously reported were fulfilling the criteria [Table I] [online supplementary materials: Figure E1]. The most common manifestations include dermatitis [inclusion criteria] [100 %], sinopulmonary infections [80 %] and failure to thrive [52 %]. The causative organisms included haemophilus influenza (33 %), streptococcal pneumonia (25 %), staphylococcal aureus (25 %) and group A streptococcus (17 %). The recurrent pneumonia led to bronchiectasis in 28 %. Sepsis was documented in 3 patients caused by streptococcal pneumonia, *E. coli*, *klebsiella oxytoca* and methicillin

resistant staphylococcal aureus. Systemic fungal infections were acquired in 2 patients that included disseminated aspergillous infections and candidemia. Mucocutaneous candidiasis and fungal fingernail infections occurred in 28 %. About 44 % acquired cutaneous viral infections caused by human papilloma virus, herpes simplex virus or molluscum contagiosum. EBV and/ or CMV infections were documented in 10 patients [40 %]. Nine patients [36 %] acquired liver diseases; four with cryptosporidial sclerosing cholangitis, two with hepatitis B and C and one with possible viral-induced hepatitis caused by his EBV and CMV infection. The remaining two patients had no identified infectious etiology, however, no further investigation or liver biopsy done to rule out other possibilities. Autoimmune-mediated phenomena occurred in 3 cases, 2 with autoimmune hemolytic anemia and one with hypothyroidism. One patient developed giant thoracoabdominal aortic aneurysm. Three patients had malignancies including anaplastic B cell lymphoma, adrenal leiomyoma and carcinoma in situ of the perianal area. That carcinoma in situ developed on top of extensive human papilloma virus (HPV) infection of the perianal area. Five patients had neurological symptoms

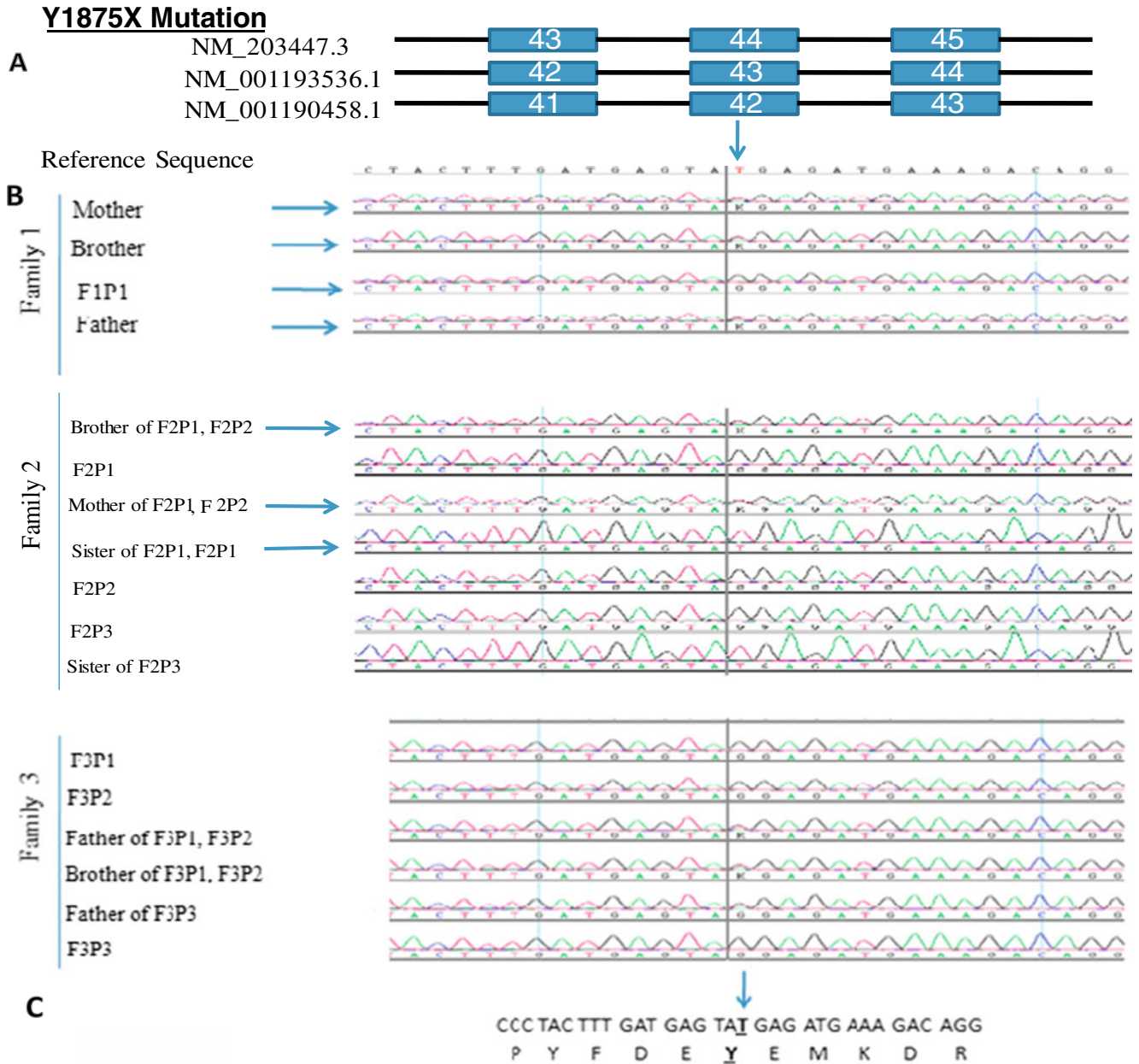
**Table II** Molecular characterization of Saudi AR-HIES

Patient	Gene affected	Type of mutation	Protein expression
F1P1	DOCK8	Non sense Y1875X	Not Done
F2P1	DOCK8	Non sense Y1875X	Not Done
F2P2	DOCK8	Non sense Y1875X	Not Done
F2P3	DOCK8	Non sense Y1875X	Not Done
F3P1	DOCK8	Non sense Y1875X	Not Done
F3P2	DOCK8	Non sense Y1875X	Not Done
F3P3	Expected DOCK8	Presumed Non sense Y1875X	Not Done
F3P4	DOCK8	Non sense Y1875X	Not Done
F4P1	DOCK8	Non sense S1711X	Not Done
F5P1	DOCK8	Non sense S1711X	Not Done
F6P1	DOCK8	Large deletion	Not Done
F6P2	Expected DOCK8	Presumed large deletion	Not Done
F6P3	Expected DOCK8	Presumed large deletion	Not Done
F7P1	DOCK8	Large deletion	No Expression
F7P2	Expected DOCK8	Presumed large deletion	Not Done
F8P1	Unknown	No mutation detected	Not Done
F9P1	Not Done	Not Done	Not Done
F9P2	Not Done	Not Done	Not Done
F10P1	DOCK8	Large deletion	Not Done
F11P1	Not Done	Not Done	Not Done
F11P2	Not Done	Not Done	Not Done
F12P1	Unknown	No mutation detected	Normal Expression
F13P1	DOCK8	Splice site mutation c. 827+6 T>C	No Expression
F14P1	Unknown	No mutation detected	Not Done
F14P2	Unknown	No mutation detected	Not Done



mainly hemiparesis and seizures. Neuroimaging demonstrated evidence of moyamoya disease in one patient, middle cerebral artery syndrome in another one and brain infarctions in the remaining three patients. Allergic disorders were common [70 %]. The commonest was food allergy (70 %) followed by asthma (47 %), drug allergy (17 %) and allergic rhinitis (12 %). No case developed anaphylaxis. Unfortunately 12 patients [48 %] succumbed to severe pneumonia and/ or sepsis with a median age of 10 years [3-16 years].

All patients have eosinophilia with a median of 1,560 cells/micl [range: 670–8,110 cells/micl]. The median serum IgE level was 9,650 IU/ml [IQR 4,632–16,028]. Low serum IgM levels were documented in 8 patients [32 %] in spite of normal number of B cells. Four cases [16 %] have poor antibody response to both pneumococcal and tetanus toxoid vaccine and hence immunoglobulin replacement therapy was commenced. Nine patients showed progressive decline in T lymphocytes count affecting CD4 more than CD8 cells. In vitro lymphocyte proliferation response was depressed in



**Fig. 1** p.Y1875X Mutation: **a** Schematic representation of the exons containing the mutation from each of the protein-coding splice variants. Numbers within the boxes indicate the exon numbers. Vertical arrow point from the affected exon to the electrograms showing the

mutation. **b** Electrograms of the seven family members sequenced as indicated. The line along the electrograms is placed next to the mutation site. ; **[C]** DNA and protein sequences of normal and affected individuals

10 individuals [40 %]. Seventeen patients were *DOCK8* deficient as confirmed by genetic study [13patients] or based on assumption due to similar clinical presentation and confirmed affected siblings [4patients].

One patient, F7P2, underwent hematopoietic stem cell transplantation from genotypical sibling with no conditioning at 8 years of age. Pretransplantation, he was critically ill with eosinophilic pneumonitis, bronchiectasis, hypoxemia, severe diarrhea, cryptococcal sclerosing cholangitis, as well as CMV and EBV infection. He received  $4.1 \times 10^6$ /kg of CD34. Donor cells engraftment using STR showed 13 % myeloid engraftment and 15.6 % lymphocyte engraftment. Although donor cell engraftment resulted in the control of his viral and cryptococcal infections with decreasing IgE level to 330 IU/L, he unfortunately succumbed to severe graft versus host disease of skin and GUT that were proved by tissue biopsy.

### Genetic Analysis

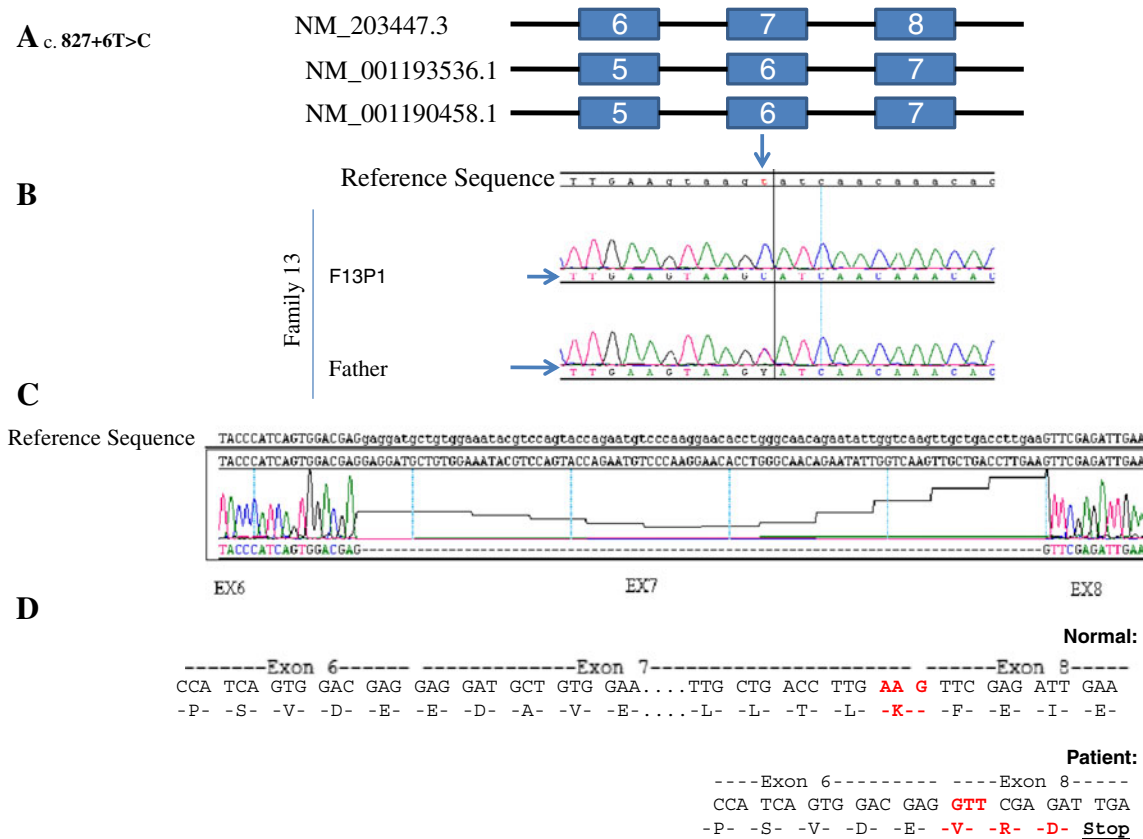
Seventeen patients were screened for mutations in *DOCK8*, *STAT3* and *TYK2* genes (see Table II). No mutations were found in *STAT3* or *TYK2*. *DOCK8* mutations that lead to stop codons were found in 10 patients belonging to 6 families from 5 unrelated tribes. Seven patients [Fig. 1, F1P1, F2P1, F2P2, F2P3, F3P1, F3P2, F3P3] from three unrelated families have an identical mutation in exon 44 [5,625 T>G; Y1875X], two patients [Fig. 2, F4P1 and F5P1] from two

families, belonging to the same tribe, have another unique mutation in exon 40 [5,132 C>A; S1711X] and one patient [Fig 3, F13P1] carry a splice site mutation at the 3' end of exon 7 [c. 827+6 T>C]. The mutation and exon numbers presented above are based on Variant one [NM\_203447.3]. All of the three mutations resulted in a stop codon leading to a truncated protein. The first two mutations (Y1875X and S1711X) are located in the C-terminus of Dock 8 protein between the DHR1 and DHR2 domains and the resulting proteins would lack the DHR2 domain [online supplementary materials: Figure E2]. The third mutation is a splice-site mutation located at the 3' end of exon 7 [c. 827+6 T>C]. To assess the effect of this mutation on the splicing and dock8 protein expression, cDNA from this patient was prepared and sequenced. As indicated in Fig. 3c, the cDNA sequence showed clearly exon 7 skipping. Analysis of the amino acid [aa] sequence that would result from such aberrant splicing would result in three aa change followed by a stop codon [Fig. 3d] resulting in a truncated protein [online supplementary materials: Figure E2]. We expected that this mutation would result in the absence of dock8 protein. To confirm this, we performed western blot analysis [Fig. 5] on this patient and found no Dock8 protein was expressed as compared with her father. The mutations described above have not been described previously and were not listed in the Human Gene Mutation Database [HGMD] [<http://www.hgmd.org/>] web site.

**Fig. 2** p. S1711X Mutation: **a** Schematic representation of the exons containing the mutation from each of the protein-coding splice variants. Numbers within the boxes indicate the exon numbers. Vertical arrow point from the affected exon to the electrogram showing the mutation. **b** Electrogram of the sequences of each family member sequenced as indicated. The line along the electrograms is placed next to the mutation site. **[C]** DNA and protein sequences of normal and affected individuals







**Fig. 3** c. 827+6 T>C Mutation: **a** Schematic representation of the exons containing the mutation from each of the protein-coding splice variants. Numbers within the boxes indicate the exon numbers. Vertical arrow points from the affected exon to the electrogram showing the mutation. **b** Electrogram of the sequences of each family member

sequenced as indicated. The line along the electrograms is placed next to the mutation site. **c** Electrogram of the patient cDNA sequence showing the skipping of exon 7. **d** The cDNA and protein sequences of normal and affected individuals

The remaining 7 patients from 6 families, whom no mutations were detected by sequencing, were screened for micro-deletion using 2.7 M Cytogenetic Microarray. Deletions were found in 3 patients [F6P1, F7P1, and F10P1] belonging to 3 families as shown in Fig. 4. Regarding the deletion of F6P1 and F7P1, it starts from codon 209,111 and ends at codon 338,227 with a size of 129 kbp. For F10P1, the deletion starts from codon 209,111 and ends at codon 6,27, 670 with a size of approximately 419 kbp. The marker counts within these regions are 82 [99 % confidence] and 304 [88 % confidence] respectively. Both deletions are located in P24.3 region at the tip of chromosome 9. Unfortunately, the first marker in this region is located at codon 209,111. Hence, the deletion boundary at 5' end cannot be defined accurately. The deletions could extend further 5' making the deletion larger than indicated. The remaining 4 patients have no deletion that we were able to detect [online supplementary materials: Figure E3]. However, one patient [F8P1] has three copies of the region 440829 to 433626. No DNA was available from her parents for similar analysis. This extra copy span

7.2 kb within the DOCK8 gene [online supplementary materials: Figure E3]. It is hard to assess how this extra copy is distributed over the two alleles. Moreover, the sequencing of this region did not indicate such duplication. Unfortunately, this patient was lost to follow up and we were not able to obtain more blood samples from the patient or her family member.

We planned to assess whether the remaining 3 patients [no deletion and no mutations were detected] belonging to two families still express the Dock8 protein by western blot analysis [Fig. 5]. However, we were able to collect blood from only one patient and his family members [F12P1]. The other two patients [F14P1 and F14P2] were lost to follow up. Family 7 is included here as negative control as no Dock8 protein would be expected to be expressed. As expected, F7P1 had no Dock8 expression as compared to his parents. F12P1 expressed Dock8 protein indicating that the genetic cause of HIES in this patient is due to another gene. The patients [F3P3, F6P2, F6P3 and F7P2] were considered Dock8 deficient since they have siblings with confirmed DOCK8 mutation and similar clinical presentation but no available DNA to perform the analysis.



**Fig. 4** ChAS output showing the areas of deletions: The DOCK8 deletions of the patients and their relatives are shown as indicated. F=Family; P=Patient. For each individual, the boxed line with an arrow head in the middle indicates the extent of the deletion. Below the boxed line is 5 rows of dots. Each row represents the copy state from zero to 4 copies. The solid lines connecting the dots over a region

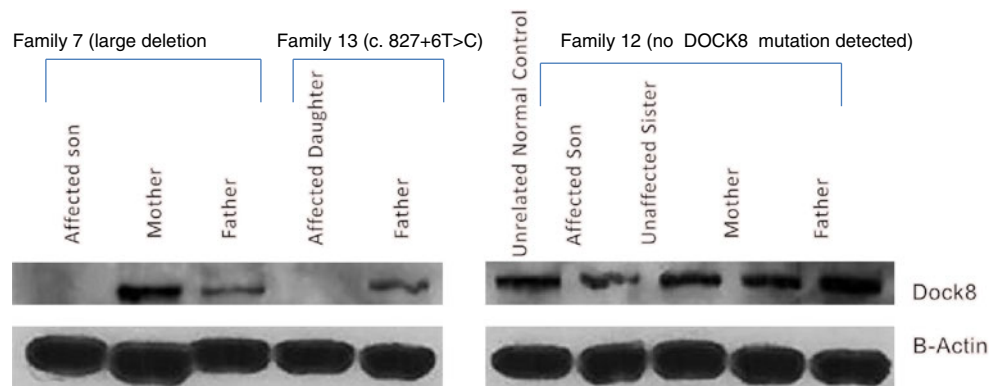
represent the copy state of that region for the indicated individual. At the bottom of the output, the genes in the covered region are mapped. The green boxes indicate whether the gene is reported to associate with disease. Numbers below the green boxes indicate the distance in kilo bases [kb]

In our statistical analysis CMV infection was found to be the only statistically significant factor predicting poor prognosis (early death) [p value 0.0302] in patients with AR-HIES but not in DIDS patients. Comparing Saudi DIDS with previously reported cases [Table III] [10, 18, 19], our patients have less rate of cutaneous viral infections [p value: 0.0019]. However, they have significantly higher susceptibility to systemic viral infections and liver diseases [p value: 0.0008 and 0.0014 respectively]. Low IgM is less frequent in Saudi DIDS [P value: 0.0319].

**Discussion**

This is the largest single center series describing the detailed clinical, immunological and molecular characterization of AR-HIES. Sino-pulmonary infections, eczema as well as cutaneous viral, bacterial and fungal infections were the most common manifestations. Neurological complications, malignancies and immune mediated pathologies including autoimmune hemolytic anemia and vasculitis may develop. These clinical findings are consistent with the previously reported cases [Table II] [4, 7, 8]. However, a noteworthy

**Fig. 5** Western blot analysis for for dock8 protein in three affected families with AR-HIES. Note the absence of protein expression in two patients (family 7 and family 13) while it is present in family 12



**Table III** Clinical and immunological features of Saudi DOCK8 immunodeficiency syndrome (DIDS) in comparison with previously reported cases of DIDS

Clinical and laboratory features	Reported DIDS (52)	Saudi DIDS (17)	P value
Atopic dermatitis	92 %	100 %	0.5654
Asthma	41 %	47 %	0.6284
Allergies	60 %	64 %	0.7089
Bacterial skin infections	73 %	76 %	0.7821
Mucocutaneous candidiasis	50 %	30 %	0.1385
Cutaneous viral infections	86 %	47 %	0.0019
Respiratory tract infections	96 %	82 %	0.0918
Diarrhea	14 %	17 %	0.6704
Liver disease	6 %	41 %	0.0014
Sepsis	11 %	23 %	0.2228
Systemic viral infection	8 %	47 %	0.0008
Systemic fungal infection	0	11 %	0.058
CNS	4 %	23 %	0.0291
Autoimmune hemolytic anemia	11 %	0	0.1427
Malignancy	17 %	11 %	0.5878
Death	23 %	41 %	0.147
High serum IgE	100 %	100 %	
Eosinophilia	90 %	100 %	0.1843
progressive decreased T cells	65 %	55 %	0.358
low B cells	15 %	0	0.0854
low NK cells	36 %	35 %	0.9262
Low serum IgM	75 %	47 %	0.0319
Poor antibody response	Variable	35 %	–
Lymphocytes proliferation response (PHA)	Variable	41 %	–

observation is the statistically significant high rate of systemic viral infections as well as the tendency to acquire cryptosporidial infections which may indicate more severe T cell dysfunction. This could be related to the different genetic background. Environmental factors may also have a role but unfortunately we do not have data regarding the prevalence of such infections in our community. Although the clinical phenotype and the high mortality rate point to a significant combined cellular and humoral immune defect, variable immunological findings were observed. Although it is difficult to conclude from this small cohort, CMV infection seems to predict poor prognosis (early death) in patients with AR-HIES probably indicating more severe T cell dysfunction. We failed to prove a similar role for CMV infection in documented Dock 8 deficient patients. One explanation is that our untested AR-HIES patients had other genetic defects that predisposed to uncontrolled CMV infection.

Dock8 deficiency is the most common identified genetic cause of AR-HIES in Saudi patients. We have identified

three novel mutations that introduce a stop codon two of which lie between DHR1 and DHR2 domains. Any protein made from transcripts carrying these mutations would be truncated to exclude the DHR2 domain. While DHR1 is required for downstream signaling and biological activity, DHR2 domain was shown to contain the actual catalytic sites for GEF [11]. We conclude that the lack of the catalytic site would impair the Dock8 function and all downstream signaling. The lack of DHR2 domain in the mutant DOCK8 would result in the failure to activate the Rho GTPases. The third mutation was a splice site mutation and was located upstream of DHR1. We showed by cDNA sequencing that this mutation resulted in exon 7 skipping which led to a stop codon. We showed the absence of any Dock8 protein expression in the patient carrying this mutation.

Moreover, we detected large deletion in three patients [Fig. 4 and 5] all of which start upstream of DOCK8 gene eliminating the promoter and the 5' exon or the entire *DOCK8* gene in one family. This deletion will abolish any Dock8 protein expression. In addition, in one patient, we observed a duplication that may affect protein expression if part of the inserted DNA located on the two alleles. However, we have no means of assessing its effect in that regard.

Biological data was available from only one patient with undetected DOCK8 mutation. In that patient, we showed normal Dock8 protein expression indicating the possible involvement of other gene downstream of Dock8 signaling pathway. We planned to follow up this family to recruit more affected individuals as they become available for linkage and homozygosity studies.

The fact that none of our patient harbor a *TYK2* mutation is in favor of the argument that Tyk2 deficiency is one of IL-12 and INF- $\alpha$  signaling defects with inherited susceptibility to viral and mycobacterial disorders rather than primarily AR-HIES etiology [1]. Those patients whom we failed to detect mutations on them may have sequence alterations that cannot be detected by sequence analysis not covered by our test [e.g. deep intronic mutations]. Another explanation is the presence of other molecular defects that may operate in the remaining cases pointing to the possible genetic heterogeneity of this disorder.

How *DOCK8* defect can explain the immunological derangement in DIDS is not fully understood. In humans, investigators demonstrated the association between *DOCK8* mutations and impaired T cell activation [7]. It has critical role in T cells survival and persistence of CD8 memory cells [14]. The number of TRECs was found low in DIDS patients which point to possible impaired thymopoiesis and efflux from the thymus. This may result in a restricted diversity of peripheral T cell repertoire [20]. Patients exhibit defective TH17 differentiation that involves the terminal steps as well as their long term persistence [21].

The risk of malignancy in DIDS could be explained by failure of immune surveillance with decreased CD8 function and chronic antigenic stimulation. However, in human, there is evidence that Dock8 may act as tumor suppressor factor since loss of Dock8 expression in different malignancies may contribute to carcinogenesis [11].

With the recent realization of the combined immune defect nature of AR-HIES, there is a tendency to offer HSCT as definite treatment for the affected patients. Five patients successfully engrafted after conditioned hematopoietic stem cell transplantation are reported in the literature [22–26]. Our patient, who turned to be Dock8 deficient, was unlucky because of pretransplantation morbidities as well as the severe GVHD he developed. However, the documented engraftment with no conditioning as well as the development of GVHD deliver another strong evidence of the severity of T cell dysfunction in those patients.

One valuable observation in patients with autosomal recessive hyper IgE syndrome in general and DOCK8 deficiency in particular is the high incidence rate of atopy and food allergy. This may base further exploration for the role of DOCK8 in allergic disorders. Indeed, a recent study by Al-Herz et al. demonstrated a selective Th2 bias in Dock8 deficiency which may explain the high rate of atopy [19].

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