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Deficient T Cell Receptor Excision Circles (TRECs) in Autosomal Recessive Hyper IgE Syndrome Caused by DOCK8 Mutation: Implications for pathogenesis and potential detection by newborn screening

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1. Introduction

In 13 patients from six consanguineous families Renner et al. [1] reported the autosomal recessive hyper IgE syndrome (AR-HIES) as a distinct entity from the previously recognized autosomal dominant (AD) HIES, also known as Job's syndrome. Shared clinical features between the two disorders included eczema, recurrent Staphylococcal infections of the skin and respiratory tract, increased serum IgE levels, and eosinophilia. However, patients with AR-HIES did not have connective tissue abnormalities which are usually seen in AD-HIES, caused by heterozygous defects in *STAT3* [1– 4]. Also, pneumonias in AR-HIES did not form abscesses or pneumatoceles characteristic of AD-HIES. Patients with AR-HIES had increased severity and impaired control of viral infections with molluscum contagiosum, varicella zoster and herpes simplex virus (HSV), as well as a higher incidence of central nervous system complications [1]. Recently, Al Khatib et al [5] reported on 25 Turkish children with various forms of hyper IgE syndrome and showed evidence of defects in Th17 differentiation that may underlie genetically distinct forms of HIES. Zhang et al [6],

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Engelhardt et al [7], Gatz et al [8], Bittner et al [9], McDonald et al [10] have now reported homozygous and compound heterozygous recessive mutations in the dedicator of cytokinesis 8 gene, *DOCK8*, in families with AR-HIES from different ethnic backgrounds. B and T cell deficiency found in some patients with *DOCK8* AR-HIES suggests that this disorder may present as a variant of severe combined immunodeficiency syndrome (SCID) [6,7].

Here, we describe a consanguineous Pakistani family with three children affected with AR-HIES due to a novel *DOCK8* gene mutation. TREC analysis identified a deficiency in the production of recent thymic emigrant T cells.

2. Patients & Methods

Institutional review board approved informed consent was obtained for the molecular studies.

2.1. Patient reports

Two brothers and a sister, (Fig. 1a–f) born to consanguineous parents had AR-HIES. The remaining siblings and their parents were healthy. The sister III.6 had severe eczema and recurrent superinfections that started at two months of age. At 22 months of age she was hospitalized with fever, pruritis, and cutaneous ulcers (Fig. 1a, b). Diffusely scattered, eczematous, ulcerated plaques with yellow-brown crusting, excoriations, and post-inflammatory hyperpigmentation were distributed around the mouth and over the face, neck, trunk, genitals, buttocks, and intertriginous areas. Leukocytosis with eosinophilia, elevated ESR (24 mm/h) and CRP and elevated serum IgE were found on admission. Her maximum IgE level was 16,340 IU/mL. Microbiologic cultures of the skin identified herpes simplex, *Staphylococcus aureus* and *Candida albicans*. Other laboratory studies showed a mildly reduced C3 level, normal C4 level, and normal Mannose binding lectin, neutrophil oxidative burst, and pneumococcal antibody titers. Initial quantitative B and T cell analysis by flow cytometry showed low CD3, CD4, CD8 T cells and low CD16/56 NK cells, but high CD19 B cells (Table 1). Lymphocyte proliferative responses to the phytohemagglutinin, pokeweed mitogen, and concanavalin A were markedly reduced (Mayo medical laboratories, Rochester, MN). Serum interleukin (IL)-6 and IL-10 levels were elevated at (4.2, 41.7 pg/mL respectively) while IL-12 level was normal (< 9.8 pg/mL).

The patient's older brother (III.5, Fig. 1d) also had severe eczema with recurrent superficial infections and asthma. His maximal serum IgE level was elevated at 17,920 IU/mL. He had very low anti-pneumococcal antibody titers. He and his sister had multiple food allergies as well.

The youngest sibling began having an eczematoid rash at 1 month of age with normal blood counts and IgE level (III.7, Fig. 1e). However, by 5 months of age, his IgE level and eosinophil counts became elevated.

The HIES severity score for the three affected siblings (III. 5,6,7) were 34, 34 and 24 respectively [11].

2.2. Molecular & Immunologic Studies

Genomic DNA was isolated from peripheral blood samples from the siblings III.4–7, and their parents (Fig. 1f). The *STAT3* gene was sequenced in III.5 (Correlagen, Waltham, MA) while the 105 k oligo-array comparative genomic hybridization (aCGH) analysis (Agilent Technologies, Sanata Clara, CA) was done only in the proband III.6 (CombiMatrix™, Irvine, CA). *DOCK8* gene sequence analysis was performed in all 3 affected siblings and their parents [7].

2.3. Absolute Lymphocyte counts

Lymphocyte subsets in EDTA anticoagulated blood samples were measured using TruCOUNT™ fluorescent beads at known concentration (BD Biosciences, San Jose CA). Briefly, 50 ul of whole blood with a standard number of beads were stained with CD3/CD8/CD45/CD4 (Cat. No. 340499) for T-Cells and CD3/CD16/CD56/CD45/CD19 for B and NK cells (Cat. No. 340500, Multitest™, BD Biosciences). After red cell lysis samples were fixed and analyzed on an LSR-II FACS machine (BD Biosciences). Ratios of cells in each gate to beads were used to derive absolute cell counts.

2.4. Quantification of TRECs by Real time PCR

Dry blood spot (DBS) preparation and DNA isolation—From the same blood samples used for lymphocyte subset determination (above) 50ul was applied to a filter (Ahlstrom 226 specimen collection paper Whatman #BFC18K932661) and air dried. Three 1/8-inch (3.2mm) punches, each equivalent to ~3 uL blood, were used for DNA extraction in an AutoGenprep 965 robot (AutoGen, Inc, Holliston, MA 01746). After individual punches in deep 96-well plates were pre-incubated in 200 uL of proteinase K lysis solution for 16 h at 65°C, the standard DNA extraction program was followed with an extra alcohol wash and final suspension in 50 ul of TE buffer. DNA concentrations were 13 to 250 ng/uL and A_{260}/A_{280} ratios were above 1.6.

TREC real time PCR—Copy number for the δ Rec-ψJα sj TREC and a β-Actin gene segment as a control were determined for duplicate 5 uL samples of the above DNAs using the ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA). Standard curves were generated from serial dilutions of TREC and β-Actin plasmids. PCR reactions (20 uL) contained BSA 0.04% (New England Biolabs, Ipswich, MA), 1X TaqMan Universal Master mix, 150 nmol/L of FAM-TAMRA fluorescent probe and 500 nmol/L of forward and reverse primers (Applied Biosystems, Foster City, CA) [12,13]. After 2 min at 50°C and 10 min at 95°C, 40 cycles were conducted, 30 s at 95°C and 1 min at 60°C. Comparison copy number values for normal infants per 3.2mm punch were TREC's >100, β-Actin >100,000, based on 2000 anonymous newborn DBS samples from the California Department of Public Health [J. Puck and D. Gonzalez, unpublished data]. Samples from T cell deficient patients [9, and unpublished], have <100 TRECs per punch. If β-Actin copies are <100,000, the sample is considered not adequate due to amplification failure.

3. Results and Discussion

Eczema is a very common pediatric dermatologic problem, and it can become secondarily infected. Eczema herpeticum caused by HSV, either type 1 or 2, can simply be a complication of long-standing atopic dermatitis, but, particularly when IgE is elevated, must raise suspicion for one of the of primary immunodeficiency disorders [1]. The differentiation between common atopy and immunodeficiency depends on a clinical evaluation, family history and laboratory studies [14]. Several groups have demonstrated large and small deletions and point mutations involving the *DOCK8* locus in the majority of patients with AR-HIES [6–11]. DNA sequencing of the *STAT3* gene in the proband showed no pathogenic mutations. The severe eczema herpticum and familial pattern with consanguinity made us suspect AR-HIES. Array CGH analysis failed to identify any pathogenic copy number abnormalities across the genome, including at the *DOCK8* locus at chromosome 9p22. However, direct sequencing of the *DOCK8* gene [7] in all three affected siblings showed a novel 2 bp deletion resulting in a frame shift mutation in exon 14 at codon 510 (Fig. 1g). Both parents were heterozygous for this mutation (Fig. 1g). The *DOCK8* protein is member of a family of Rho-Rac GTP-exchange factors. It contains a DHR2 domain which normally binds to Rho-family G proteins, promoting GTP exchange and activating local cytoskeletal changes, integrin-mediated adhesion, lamellipodia formation, cell polarization, and phagocytosis or cell fusion [15,16,17]. The pathogenicity of this mutation was confirmed by Western blot analysis, which showed absent *DOCK8* protein expression (not shown).

Patients with *DOCK8* mutations have increased morbidity and mortality due to involvement of the central nervous system by vascular lesions (stenosis, occlusion, or aneurysm) and infections, particularly with viruses. They are also at a high risk for cutaneous squamous cell carcinoma [1,8]. To date, these patients have been treated symptomatically with varying success. More recently, successful hematopoietic stem cell transplantation has been reported in three young patients [8,9,10]. The two older siblings reported here are being evaluated for allogeneic bone marrow transplantation, an option which may represent a possibility for cure with acceptable risk.

The mechanism of immunodeficiency in AR-HEIS is not fully understood, but variable abnormalities in cellular immunity are recognized, and were exhibited in our proband, who had reduced T cells and impaired lymphocyte proliferation to mitogen stimulation [5]. In humans, both Zhang et al [6] and Engelhardt et al [7] independently showed that recessive *DOCK8* mutations were associated with impaired T-cell ($CD4^+$ and $CD8^+$) activation and Th17 cell differentiation. In mice, Randall et al [17] demonstrated that Dock8 deletion crippled B cell immunological synapses, germinal centers and long-lived antibody production; *Dock8* appeared to have a critical role in antigen recognition and recruitment of *ICAM-1* to the immunological synapse. Poor in vitro proliferation of *Dock8* deficient T cells may be due to a problem in antigen presentation or in generation of T cells with diverse antigen specificities. Th1 cell deficiency and cytokine abnormalities may result in overactivity of Th2 cells and cytokines, which may then stimulate B-cells to isotype-switch and overproduce IgE [2]. Patient III.6 in this report, indeed had abnormally elevated IL-6 and IL-10 levels. Multiple defects in cytokine signaling pathways have also been recognized

in AD-HIES due to *STAT3* defects, and a Japanese patient with a form of AR-HIES due to homozygous mutation in tyrosine kinase 2 (*Tyk2*) had abnormalities in IFN-gamma, IL-6, IL-10, IL-12, and IL-23 [17]. Defects in Th17 differentiation are also common to all forms of HIES [5–10].

Severe combined immune deficiency (SCID) has been identified as a disorder of high priority for population-based newborn screening using the TRECs assay [17]. Assay for absence or low numbers of TRECs in newborn dried blood spots is coming into use to identify infants born with SCID [20–22]. Since T cell production and function may be impaired in *DOCK8* deficient AR-HIES, we hypothesized that TRECs could be diminished in children affected with this disorder. The original newborn screening blood spots of the children in our family were not available, so the TREC assay was done with fresh blood spotted on a newborn screening filter. The number of TRECs was low in the affected 13 month old child, and TRECs were undetectable in the older two children, at ages 4 and 6 years, suggesting that new T cell production and/or efflux from the thymus might be impaired early in life and perhaps even decline further with age [Table 2]. Interestingly, the high number of T cells in patient III.5 and relatively normal number in III.7 suggest that peripheral homeostatic T cell proliferation and expansion may not be impaired, and may compensate to some degree for decreased thymic output. Defective thymopoiesis and/or efflux of mature thymocytes from the thymus may result in a restricted T cell repertoire diversity in the periphery, with implications for disease pathogenesis. Such a contingency remains however to be established. Finding low to undetectable TRECs in affected children from 1 to 6 years of age suggests that DOCK8 deficient AR-HIES could be a cause of low TREC counts in newborn screening, and this diagnosis should be considered in the evaluation of newborns with abnormal TRECs who do not have typical SCID.

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Highlights

- Severe combined immune deficiency (SCID) has been identified as a disorder of high priority for population-based newborn screening using the TRECs assay.
- AR-HIES is a form of severe combined immunodeficincy
- Low to undetectable TRECs were found in the three affected children in this family.
- DOCK8 deficient AR-HIES may be a cause of low TREC counts and therefore may be amenable to newborn screening.

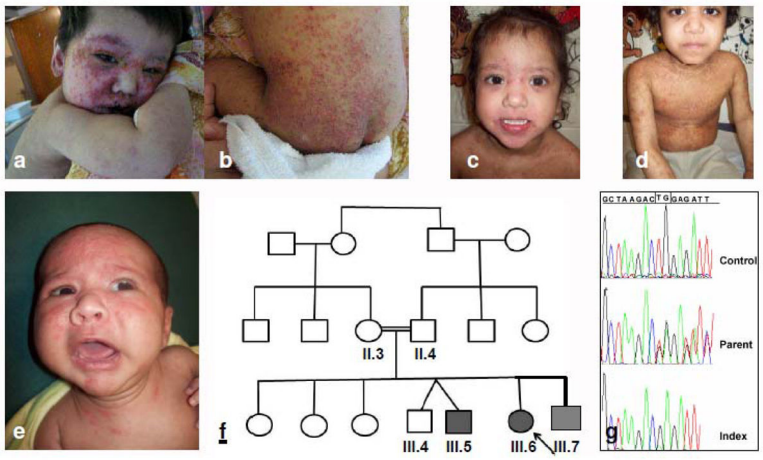

Figure 1.

Face and back views of index case III.6 on admission (a, b) and after discharge (c). Rash upon presentation was diffusely scattered, eczematous plaques with yellow-brown crusting, excoriations, and post-inflammatory hyperpigmentations. (d): older brother (III.5) had similar skin lesions. (e): sibling III.7 at 1 month of age with pusutular, maculopapular, eczematoid rash on the forehead, cheeks and neck. (f) family pedigree showing consanguinity and multiple affected family members (filled symbols, III.5, III.6, III.7) with AR-HIES. (g) Genomic DNA sequencing of DOCK8 shows a homozygous 2 bp deletion in the index case for which parents were heterozygous.

Table 1

Lymphyocyte Subset Analysis in DOCK8 Deficient Patients and Their Parents.

Individual	Age at study*	CD3 cells/uL	CD4 cells/uL	CD8 cells/uL	CD19 cells/uL	CD16/56 cells/uL
II.3	47 y	1656	950	687	261↓	110↓
II.4	42 y	1340	755	506	271↓	133↓
III.5	6 y	470↓	199↓	234↓	412↓	88↓
III.6	4 y	1426	598↓	675↓	1035	567
III.7	5 m	1548	1238	221↓	2344	442

*Age adjusted reference ranges: CD3 adult 530 – 2310 cells/uL, pediatric 1800–3000; CD4 adult 360 – 1180, pediatric 1000 – 1800; CD8 adult 120 – 930, pediatric 800 – 1500; CD19 adult 20 – 490, pediatric 700 – 1300; and CD16/56 adult 20 – 670, pediatric 200 – 600.

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Table 2

T Cell Receptor Excision Circle (TREC) Analysis and T cell number in DOCK8 deficient patients and parents.

Individual	Age at study	TRECs per punch (~3 uL blood)[a]	β-Actin copies per punch[a]	CD3 T cells per uL blood[b]	T cell reference range for age[c]
II.3	47 y	174	446,270	1,451	754–2,764
II.4	42 y	131	278,030	1,474	754–2,764
III.5	6 y	0	612,402	6,009 H	1,610–4,230
III.6	4 y	0	455,600	478 L	1,610–4,230
III.7	13 m	54	797,810	1,499	1,460–5,440

[a] Mean of 3 assays using DNA extracted from 3 separate punches. Normal reference value for newborns, >100 TRECs/punch (mean newborn value 1,000 TRECs/punch); normal β-actin reference range >100,000 copies/punch.

[b] Determined on same blood sample used for TREC and Actin assays, with absolute quantitation by beads.

[c] Stiehm et al., eds. Immunological Disorders in Infants and Children, 5th Ed. Philadelphia: Elsevier Saunders. p 311.