Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome

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Background: The genetic etiologies of the hyper-IgE syndromes are diverse. Approximately 60% to 70% of patients with hyper-IgE syndrome have dominant mutations in STAT3, and a single patient was reported to have a homozygous *TYK2* mutation. In the remaining patients with hyper-IgE syndrome, the genetic etiology has not yet been identified.

Objectives: We aimed to identify a gene that is mutated or deleted in autosomal recessive hyper-IgE syndrome.

Methods: We performed genome-wide single nucleotide polymorphism analysis for 9 patients with autosomal-recessive hyper-IgE syndrome to locate copy number variations and homozygous haplotypes. Homozygosity mapping was performed with 12 patients from 7 additional families. The candidate gene was analyzed by genomic and cDNA sequencing to identify causative alleles in a total of 27 patients with autosomal-recessive hyper-IgE syndrome.

Results: Subtelomeric biallelic microdeletions were identified in 5 patients at the terminus of chromosome 9p. In all 5 patients, the deleted interval involved dedicator of cytokinesis 8 *(DOCK8)*, encoding a protein implicated in the regulation of the actin cytoskeleton. Sequencing of patients without large deletions revealed 16 patients from 9 unrelated families with distinct homozygous mutations in *DOCK8* causing premature termination, frameshift, splice site disruption, and single exon deletions and microdeletions. DOCK8 deficiency was associated with impaired activation of $CD4^+$ and $CD8^+$T cells.

Conclusion: Autosomal-recessive mutations in *DOCK8* are responsible for many, although not all, cases of autosomal-recessive hyper-IgE syndrome. *DOCK8* disruption is associated with a phenotype of severe cellular immunodeficiency characterized by susceptibility to viral infections, atopic eczema, defective T-cell activation and T_H17 cell differentiation, and impaired eosinophil homeostasis and dysregulation of IgE. (J Allergy Clin Immunol 2009;124:1289-302.)

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Supported by National Institutes of Health grants 5R01AI065617 and 1R21AI087627 to T.C. and by the EU Marie-Curie grant MEXT-CT-2006-042316 and the European Community's 7th Framework Programme FP7/2007-2013 grant EURO-PADnet HEALTH-F2-2008-201549 to B.G.

Disclosure of potential conflict of interest: K. R. Engelhardt, S. Winkler, and G. Lopez-Herrera are employed on a research grant from the European Union (EU Marie-Curie grant). S. McGhee is a board member of Madison's Foundation. E. R. B. McCabe has received research support from the National Institutes of Health/National Human Genome Research Institute. B. Grimbacher (EU Marie-Curie grant) has received research support from the European Union and the Primary Immunodeficiency Association. The rest of the authors have declared that they have no conflict of interest.

Received for publication September 23, 2009; revised October 22, 2009; accepted for publication October 26, 2009.

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0091-6749/$00.00

Published by Elsevier, Inc on behalf of the American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.10.038

Abbreviations used
AR: Autosomal-recessive
CFSE: Carboxyfluorescein succinimidyl ester
CNV: Copy number variation
DHR: Dedicator of cytokinesis homology region
DOCK: Dedicator of cytokinesis
GEF: Guanine nucleotide exchange factor
HIES: Hyper-IgE syndrome
NIH: National Institutes of Health
NK: Natural killer
STAT3: Signal transducer and activator of transcription 3
WASP: Wiskott-Aldrich syndrome protein

Key words: *Autosomal recessive hyper-IgE syndrome, human gene mutation, DOCK8, primary immunodeficiency, molluscum contagiosum, recurrent infection, T cells, T_H17 cells, eosinophils, IgE regulation, copy number variations, genomic deletions*

The hyper-IgE syndromes (HIES; also called Job syndrome; OMIM: Online Mendelian Inheritance in Man #147060 and #243700) are rare primary immunodeficiencies (estimated prevalence <1:1 million) characterized by the clinical triad of recurrent staphylococcal skin abscesses, recurrent pneumonia, and serum IgE levels >10 times the upper norm. HIESs usually manifest in childhood and have a highly variable expressivity.[1,2] Although most cases are sporadic, both autosomal-dominant and autosomal-recessive (AR) inheritance have been described. The predominant form is autosomal-dominant HIES caused by heterozygous, dominant-negative mutations in signal transducer and activator of transcription 3 *(STAT3)*.[3] In this form of HIES, extraimmune manifestations occur, including skeletal abnormalities such as retained primary teeth and a typical facial appearance. A scoring system for these findings has been developed at the National Institutes of Health (NIH)[4] and refined to a STAT3 score by Woellner et al.[5] In contrast, AR-HIES is characterized by recurrent viral and bacterial infections, extreme eosinophilia, and elevated IgE without skeletal or dental abnormalities.[6] The genetic causes of AR-HIES are largely unknown. Minegishi et al[7] reported a monogenetic defect in the cytoplasmatic tyrosine kinase Tyk2 in a single patient with clinical features of AR- HIES who had consanguineous parents. In a follow-up study, however, additional patients with AR-HIES did not have mutations in the *TYK2* gene.[8] Recently a second patient with a related phenotype has been found to be deficient in Tyk2 (J.-L. Casanova, personal communication, October, 2009).

Autosomal-recessive HIES has been described predominantly in consanguineous families from Turkey. We investigated, by genome-wide homozygosity mapping and copy number analysis, 16 patients from 14 families with AR-HIES, defined as a positive NIH HIES score, and absence of significant skeletal findings. Eleven additional patients from six families were analyzed after the candidate gene had been identified.

Homozygosity mapping is a method to localize a disease-associated recessive genotype by searching for homozygous haplotypes in consanguineous families; the underlying assumption is that the recessive mutant allele is identical by descent in affected subjects.[9] If the founder mutation is relatively recent, the causative mutation is likely to be found within the largest stretches of homozygosity. The genotyping required for this approach may be accomplished by using high-density oligonucleotide arrays, which have the additional benefit of providing data on copy number at each single nucleotide polymorphism locus on the array. Copy number variations (CNVs) occur as a result of deletions and insertions of variable size in the genome.[10] CNVs are common and may be polymorphic in the population or arise *de novo* in individuals.

Our analysis has demonstrated an AR-HIES genomic locus and made possible detection of mutations in the dedicator of cytokinesis *(DOCK)–8* as the major defect in AR-HIES.

METHODS

Patients and controls

The phenotypes of the patients with AR-HIES analyzed are shown in Table I. Whole blood samples were taken from patients, family members, and healthy volunteers with informed consent. We analyzed DNA from 20 families suspected of having AR-HIES on the basis of clinical assessment. Criteria for AR-HIES were defined as elevated IgE, eczema, hypereosinophilia, and significant infections (particularly with molluscum contagiosum and herpes family viruses; Fig 1). In three kindreds, there were five deceased affected siblings, but all parents were unaffected. There were 13 families from Turkey, 2 from Iran, and 1 each from Lebanon, Oman, Mexico, Italy, and Ireland. All affected individuals had an NIH HIES score[4] > 20. In family AHR019 an elder sibling died following seizures and developing a coma, both characteristic of AR-HIES. Clinical data on families ARH011 and ARH015 were previously published by Renner et al,[6] family ARH011 has also been reported by Zhang et al,[11] family ARH010 is family 18 in the report by Grimbacher et al,[4] and patients ARH001 to ARH004 and ARH006 to ARH009 were reported by Al Khatib et al.[12]

Methods

A detailed description of the methods can be found in this article's Methods section in the Online Repository at www.jacionline.org, including homozygosity mapping and copy number analysis, PCR and sequence analysis, immunoblotting, and proliferation and carboxyfluorescein succinimidyl ester (CFSE) dilution studies.

RESULTS

Search for copy number variations, microdeletions, and homozygosity

Representational oligonucleotide microarray analysis for CNVs was performed on 8 index patients with AR-HIES previously identified among a cohort of Turkish patients[12] and a singleton patient from Italy with HIES (Fig 2, *A*). In the subtelomeric region of chromosome 9p, homozygous deletions (copy number = 0) were identified in 4 patients and a large compound heterozygous deletion was identified in the Italian patient with AR-HIES. In 1 additional patient hemizygosity was identified at this locus. For most patients the deletion extended from the most terminal p locus to within the *DOCK8* gene (Fig 2, *B*). Homozygous deletions were confirmed by PCR of the affected segments of *DOCK8* (see this article's Fig E1 in the Online Repository at www.jacionline.org). The parental origin of the deletion was investigated and is shown in this article's Fig E2 in the Online Repository at www.jacionline.org.

To obtain additional evidence that the terminal region of chromosome 9p is associated with AR-HIES, homozygosity mapping was performed on the four patients that did not have biallelic deletions and on further seven affected subjects. All eleven subjects were identified to have homozygous regions on distal chromosome 9p (data not shown).

Positional identification of candidate genes

Taken together, all 16 affected probands had either deletions or extended homozygous haplotypes at 9p24.3, a region encoding at least 4 genes, the largest of which is *DOCK8*. The other known