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Combined DOCK8 and CLEC7A mutations causing immunodeficiency in three brothers with diarrhea, eczema and infections

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The study was approved by the Institutional Review Board of Spedali Civili, Brescia, and informed consent was obtained from the patients and/or the parents.

Author Contributions. R.B. obtained the clinical information, led the functional studies and wrote the manuscript. S.C. and F.C. performed Sanger sequencing and immunoblotting studies. G.R. and R.B. were following the child. S.C. has performed DOCK8 and CLEC7A sequence analysis and immunoblotting experiments. Ci.M. and C.S. have studied FOXP3 gene expression. D.M. has performed flow cytometry. S.B. and Ch.M. have performed genome-wide genotyping of single nucleotide polymorphisms and data analysis with GenCol. C.J.B. contributed computer programming and data analysis. D.L.D. made the sequencing libraries, performed target enrichments and data analysis. N.A.M. carried out data pipelining, software development and bioinformatics. S.L.H. carried out literature research and data analysis. L.Z. and G.P.S. performed sequencing. S.F.K. wrote the manuscript and carried out data analysis. Ro.B. and L.P. tested the in vitro function of Treg cells and contributed to editing of the manuscript.

Competing Interests. L.Z. and G.P.S. are employees of Illumina Inc.

Accession Numbers. Variant sequences were deposited in the NCBI dbGAP.

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Keywords

next generation sequencing; *FOXP3*; IPEX; infections; food allergy

To the Editor:

We report four male brothers from a consanguineous Italian family with an immunodeficiency syndrome characterized by severe eczema, milk and egg allergies, recurrent infections, intractable diarrhea, failure to thrive and, in two of the three, lymphoma. One of them (II-2) died at age 12 months of cardiovascular failure due to intractable diarrhea and the other three were evaluated as part of this study (Table 1, Figure 1A). The proband (II-1) presented shortly after birth with severe eczema and intractable diarrhea, without gastrointestinal infection. In the first year of life, he had multiple allergies to milk, soy, and egg proteins. He had repeated episodes of pneumonia leading to bilateral bronchiectasis at 16 years of age. At age 25 years, he developed lymphoma involving the tonsil, lung and spleen. This was defined as a high grade large cell B cell lymphoma (EBV-negative), and was successfully treated with immune-chemotherapy. At age 27, he had *Pseudomonas aeruginosa* sepsis. At age 28, he was successfully transplanted with hematopoietic stem cells (HSC) from a matched unrelated donor (MUD). Likewise, a younger sibling (II-3) presented at 6 months of age with allergy to milk proteins and intractable diarrhea, despite a milk protein-free diet. He had two episodes of pneumonia and several upper respiratory tract infections. At age 15 years, he developed an anaplastic peripheral T cell lymphoma, involving skin, lymph nodes, liver and spleen. It proved refractory to chemotherapy. The patient died of infection while receiving high-dose methotrexate and cytarabine. Sibling II-4 was fed with an extensively hydrolyzed formula, but presented with non-infectious diarrhea as an infant. He developed respiratory infections and otitis. At age 13 he was successfully transplanted with HSC from a MUD.

Immunological studies in the three siblings surviving infancy showed severe T cell lymphopenia (160-1140 cells/l), with normal relative percentage of B and NK cells (Figure 1B). Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome (IPEX) (OMIM #304790), was suspected^{1, 2}, but *FOXP3* sequencing did not reveal any mutation in the coding region except a previously reported, silent single nucleotide polymorphism (SNP) at serine 181 (543C>T, rs2232367 in the NCBI dbSNP) in all of the affected siblings. A previous study had suggested this variant to be associated with IPEX syndrome³. However, analysis of 100 chromosomes from healthy subjects showed the allele frequency of rs2232367 to be 5% in the Italian population. Sequencing of reverse transcribed mRNA did not reveal any splicing variant of *FOXP3* (data not shown) and expression of FOXP3 protein was normal in CD25+/CD4+ T cells in the proband (Figure 1C). Next, we examined T-regulatory functional properties of CD4+CD25hi+ regulatory cells (Tregs) and CD4+CD25-effector cells from two of the siblings surviving infancy (the proband II-1 and sibling II-4). Tregs isolated by magnetic bead cell sorting, in parallel with those of a healthy donor, and stimulated with anti-CD3 mAb in the presence of allogeneic antigen presenting cells strongly suppressed both the proliferation and IFN-gamma secretion of co-cultured autologous effector T cells at a ratio [Responder: suppressor] of 2:1 (Figure 1D). The proliferative capacity of Treg cells from the proband was also examined, revealing them to be anergic. Altogether, these genetic and functional data ruled out the diagnosis of IPEX syndrome due to *FOXP3* mutation. Genome-wide genotyping of SNPs in the three siblings and their mother with the Affymetrix 6.0 microarray and analysis with GenCol⁴ revealed regions with identical genotype, including chromosomes 9p24 and 12p13 (Supp. figures 1A and 3), suggesting an autosomal recessive disorder. Exome enrichment (37.7 million nucleotides, Agilent SureSelect v.1) and next generation sequencing (NGS) was performed

on the proband, as previously described⁵. Mean enrichment of the exome was 44-fold. NGS yielded 171 million sequences each 130 nucleotides long. ~95% of sequences aligned to the reference human genome uniquely, covering each exonic nucleotide ~135 times. ~1.45% of target nucleotides were not sequenced, while ~88.3% had at least 20-fold sequence coverage. A bioinformatic decision tree was used to identify nucleotide variants in the aligned sequences⁵.

Homozygous variants were detected in two genes in the proband (Supp. figures 1B, 1C): The first was a known nonsense mutation in the pattern recognition receptor gene (*CLEC7A*, p.Tyr238X, *rs16910526*), that signals via CARD9 (Supp. figure 2C). This mutation has been reported to cause familial chronic mucocutaneous candidiasis (CANDF4, OMIM#613108)⁷. The second was a novel homozygous frameshift variant in exon 27 of the *DOCK8* gene, c.3193delA. *DOCK8* mutations cause autosomal recessive hyper-IgE syndrome (MIM#243700), characterized by T lymphopenia, eosinophilia, elevated IgE, eczema, recurrent infections and severe food allergies^{8,9}. Sanger sequencing confirmed homozygosity for both the *DOCK8* and *CLEC7A* variants in the three siblings and heterozygosity in their parents. The *DOCK8* mutation is predicted to result in a premature stop codon 17 residues downstream of the deletion (Ser1065AlafsX17). However, Sanger sequencing of *DOCK8* mRNA revealed a small number of transcripts that read through the premature stop codon (Supp. figure 2A and data not shown). Indeed, immunoblots of T cells from the three patients showed low level *DOCK8* protein expression (Supp. figure 2B), suggesting residual protein synthesis by alternative splicing. Exome sequencing, when combined with confirmatory testing, has previously shown efficacy for molecular elucidation of Mendelian diseases. Here, we describe another application, namely the identification of potentially causative mutations in two or more loci in inherited diseases. Patients with *DOCK8* mutations display skin infections, pneumonia, elevated serum IgE, but do not usually have diarrhea. The latter is typically associated with IPEX syndrome. The phenotypic severity and enteropathy despite residual *DOCK8* protein expression may reflect the combined defects in two genes acting in parallel immunologic pathways. Interestingly, this unusual phenotype partially resembles IPEX syndrome. The prevalence of mutations in two genes in apparently Mendelian disorders is not known. Such interactions would not be discovered by conventional, serial, univariate molecular testing since the *DOCK8* result would be interpreted as a definitive diagnosis, and further molecular evidence would not be sought. We suggest that exome sequencing be considered in patients with atypical presentations of Mendelian diseases for examination of possible underpinning mutations in more than one locus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Family tree. The proband is indicated by arrow. (B) Scatter plot of absolute counts of T-, B- and NK- cells (◆ II-1, ● II-3, ■ II-4) compared to 20 age-matched HD (○) (mean $\pm$ SD). (C) FOXP3 expression in CD4 of II-1 versus an healthy donor. (D) Capacity of CD4+CD25+ Treg from II-1 and II-4 to suppress autologous CD4+CD25- T cell response as inhibition percentage of 3 H-thymidine incorporation (upper panel) and IFN- γ production (lower panel).

Table 1**Clinical Features of Patients**

	Pt II-1	Pt4 II-2	Pt II-3	Pt II-4	DOCK8 Features	CLEC7A Features	IPEX Features
Age at genetic diagnosis (years)	27	N/A	16	13			
Sex	♂	♂	♂	♂	♂ & ♀	♂ & ♀	♂
Eczema (age of onset)	6 months	Yes	6 months	N/A	Yes	No	Yes
Food allergies	Multiple	N/A	Multiple	Multiple	Yes	No	Rare
Diarrhea (age of onset)	6 months	Yes	6 months	6 months	Rare	No	Yes
Pneumonia	2 episodes	N/A	2 episodes	No	Yes	No	Rare
Meningitis/sepsis	Yes	N/A	Yes	No	Yes	No	Rare
Upper respiratory infections	Yes	N/A	Yes	Yes	Yes	No	Rare
Bronchiectasis	Yes	N/A	No	No	Yes	No	No
Candida infection	Yes	N/A	No	No	Yes	Yes	No
Lymphoma	Yes, B cell	N/A	Yes, cell	TNo	Yes	No	No
Cutaneous viral infections	No	N/A	No	No	Yes	No	No
Early-onset type diabetes	No	N/A	No	No	No	No	Yes
Outcome	HSCT	Deceased	Deceased	HSCT			

HSCT: hematopoietic stem cell transplantation