

Acute Eosinophilic Pneumonia Occurring in a Deducator of Cytokinesis 8 (DOCK8) Deficient Patient

Ikuya Tsuge, MD, PhD,^{1*} Komei Ito, MD, PhD,² Tamae Ohye, PhD,³ Naoyuki Kando, MD,²
Yasuto Kondo, MD, PhD,¹ Yoichi Nakajima, MD, PhD,¹ Chisato Inuo, MD,¹
Hiroki Kurahashi, MD, PhD,³ and Atsuo Urisu, MD, PhD¹

Summary. Deducator of cytokinesis 8 (DOCK8) deficiency is an autosomal recessive type of combined immunodeficiency with elevated IgE. In this report, we describe a Japanese girl of non-consanguineous family suffering from acute eosinophilic pneumonia (AEP) as a presenting feature of DOCK8 deficiency. Although AEP was self-limiting, consecutively experienced recurrent respiratory infections, severe atopic dermatitis, and vulnerability to viral infections, prompted us to evaluate the possibility of DOCK8 deficiency. Immunological assessments demonstrated decreased IgM, increased IgE, T lymphocytopenia, especially in CD4 T cells, decreased PHA blastogenesis, and decreased CD27⁺CD19⁺ memory B cells. Western blotting revealed the absence of DOCK8 protein. Investigation of genomic DNA by multiplex ligation-dependent probe amplification (MLPA) revealed a heterozygous large deletion of 77 kb spanning from intron 5 to exon 22. DOCK8 cDNA sequencing revealed a nonsense mutation at position 740 (E740X). As far as we know, this is the first Japanese case of DOCK8 deficiency. **Pediatr Pulmonol.**

© 2013 Wiley Periodicals, Inc.

Key words: pulmonary infiltrate; hyper IgE syndrome; lymphocytopenia; atopic dermatitis; food allergy.

Funding source: none reported

INTRODUCTION

Acute eosinophilic pneumonia (AEP) is an acute onset respiratory illness with fever, hypoxemia, diffuse pulmonary infiltrates on chest X-ray and pulmonary eosinophilia.¹ In some cases, AEP is considered as an acute hypersensitivity reaction to unidentified inhaled antigens, but there is also evidence that certain infections can cause AEP. In this report, we describe a Japanese girl suffering from AEP as a presenting feature of dedicator of cytokinesis 8 (DOCK8) deficiency.^{2,3} DOCK8 deficiency is an autosomal recessive type of combined immunodeficiency with typically elevated IgE. Although blood eosinophilia is a common feature of DOCK8 deficiency, this is the first reported case of biopsy-proven AEP occurring in a DOCK8-deficient patient.

CASE REPORT

The patient was a female born to non-consanguineous parents. She had infantile onset atopic dermatitis, which was often complicated by impetiginization and candidiasis. She also suffered from multiple food allergies and experienced two episodes of wheezing bronchitis. At the age of 5 years, she had recurrent respiratory infections, including otitis media, sinusitis and pneumonia, and was finally admitted to Aichi Children's Health and Medical Center with prolonged fever due to perforated otitis media and pneumonia. Although lung auscultation revealed no crackles on initial physical examination, the chest X-ray showed bilateral infiltration in the apical anterior segment of the upper lobes and in the medial segment of the middle lobes. The results of laboratory tests revealed white blood

¹Department of Pediatrics, Fujita Health University, Toyoake, Aichi, Japan.

²Department of Allergy, Aichi Children's Health and Medical Center, Obu-city, Aichi, Japan.

³Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan.

Conflict of interest: None.

*Correspondence to: I. Tsuge, MD, PhD, Department of Pediatrics, School of Medicine, Fujita Health University, 1-98 Dengakugakubo, Kutsukakecho, Toyoake, Aichi-Ken 470-1192, Japan.
E-mail: itsuge@fujita-hu.ac.jp

Received 28 August 2012; Accepted 28 March 2013.

DOI 10.1002/ppul.22814
Published online in Wiley Online Library
(wileyonlinelibrary.com).

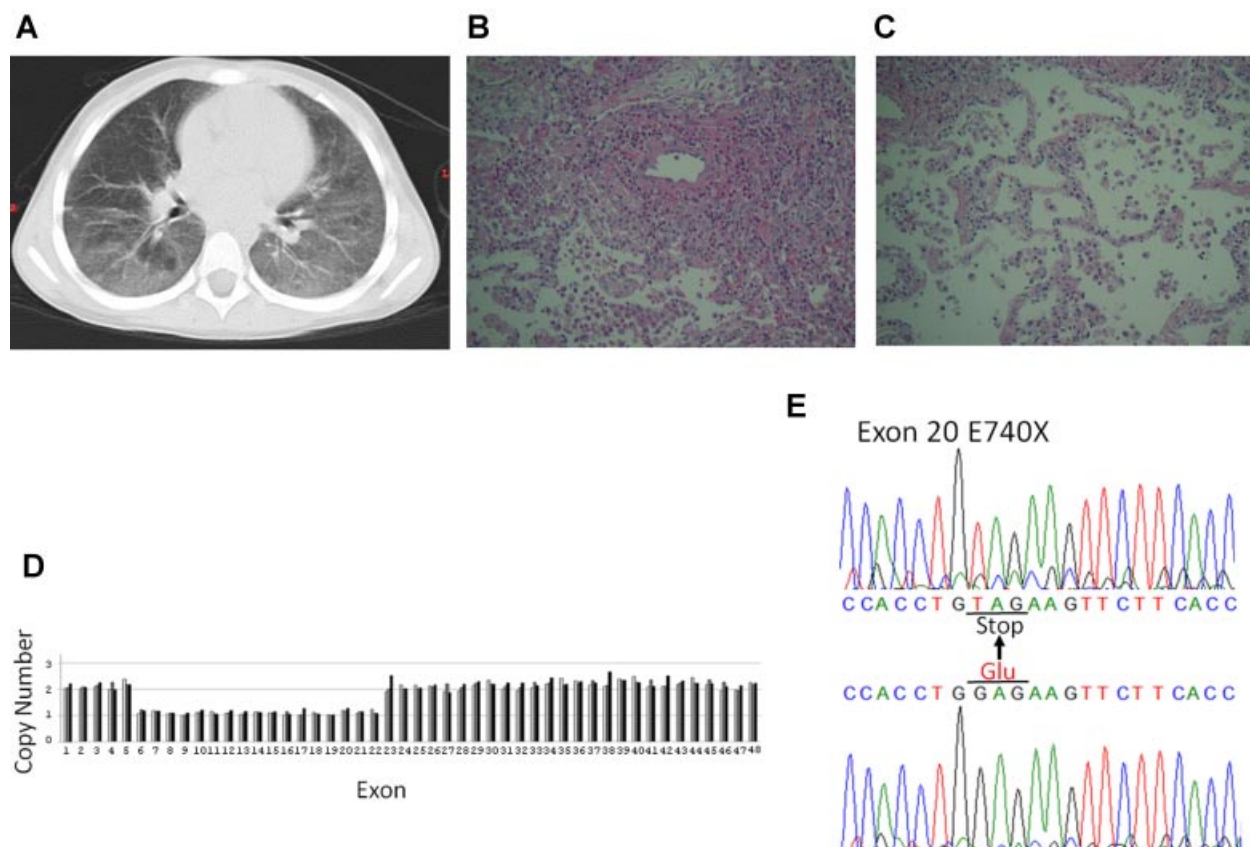


Fig. 1. A: Pulmonary CT on day 9 (at 5 years old) showed ill-defined ground-glass opacities in both lungs. B,C: Hematoxylin and eosin-stained biopsy specimens from left upper lobe. Marked interstitial infiltration of eosinophils and small lymphocytes (B), and macrophages and Masson's body formations (C) were noted. D: Multiplex ligation-dependent probe amplification (MLPA) analysis. Peak area histograms for the patient normalized with a control sample are shown. Triplicate exon dosage is reported on the y-axis as a multiple of one copy. E: cDNA sequencing revealed a nonsense mutation (E740X) corresponding to c.2330G >T at exon 20, which was deleted in another allele.

cells $13.5 \times 10^9/L$ (neutrophils 64.3%, lymphocytes 11.5%, monocytes 2.5%, and eosinophils 18.2%), C-reactive protein 3.9 mg/dl (normal range <0.4 mg/dl), IgG 8.70 g/L (normal range 7.79–15.15 mg/dl), IgA 1.92 g/L (normal range 6.0–23.8 mg/dl), IgM 0.86 g/L (normal range 4.7–21.9 mg/dl), and IgE 14126 IU/mL

(normal range <110 IU/mL). Methicillin-resistant *Staphylococcus aureus* (MRSA) was cultured from middle ear exudates, and α -*Streptococcus* sp., *Moraxella* sp., and *Candida* sp. were isolated from sputum culture. Antibiotic therapy with panipenem/betamipron was started and minocycline was subsequently added because of continuing cough and nocturnal fever. Although fever lysis and improvement of respiratory symptoms were obtained by day 6, marked eosinophilia ($26.2 \times 10^9/L$) and exacerbation of interstitial pneumonia on chest X-ray were noted on day 9 of admission. Pulmonary CT scan revealed diffuse interstitial infiltration in both lungs (Fig. 1A). AEP was suspected and closed thoroscopic lung biopsy was performed on day 16 to establish a definitive diagnosis before initiating steroid therapy. Marked interstitial infiltration of eosinophils and small lymphocytes consistent with AEP were found in the biopsy specimen (Fig. 1B), and macrophages and Masson's body formations were noted in alveolar spaces (Fig. 1C). No

ABBREVIATIONS

AEP	Acute eosinophilic pneumonia
DOCK8	Dedicator of cytokinesis 8
FOXP3	Forkhead box P3
MLPA	Multiplex ligation-dependent probe amplification
IPEX	Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome
iT _{reg}	Inducible regulatory T cells
OS	Omenn syndrome
STAT3	Signal transducer and activator of transcription 3

evidence of active fungal infection was observed. Although microbiological culture from the biopsy sample was not performed, MRSA, α -*Streptococcus* sp., *Neisseria* sp., and *Corynebacterium* sp. were isolated from bronchoalveolar lavage fluid (BALF) collected simultaneously. Pneumocystis was not detected in the lung biopsy sample and in smears of BALF. While reviewing the biopsy specimens, however, respiratory symptoms and pulmonary infiltration on chest X-ray improved gradually without corticosteroid therapy and she could be discharged from the hospital on day 26. Although steroid therapy has been used in the reported cases of AEP, spontaneous recovery has also been reported in 6 of the 22 patients in one series.⁴

After her discharge from the hospital, recurrent respiratory infections and severe atopic dermatitis continued, and vulnerability to viral infections such as herpes virus stomatitis, herpes zoster, and molluscum contagiosum gradually became apparent. Characteristic features, such as elevated IgE levels, eosinophilia, multiple allergies, recurrent upper and lower respiratory tract infections, and unusual susceptibility to viral infections, prompted us to evaluate the possibility of DOCK8 deficiency. Immunological assessments demonstrated T lymphocytopenia ($1.2 \times 10^9/L$), especially in CD4 T cells ($0.32 \times 10^9/L$), decreased PHA blastogenesis (14,600 cpm; reference 26,000–53,000 cpm), and decreased CD27⁺CD19⁺ memory B cells (2.8% of CD19⁺ cells; reference 7.5–50%). Th-17 cells, which are reported to be decreased in STAT3-deficient hyper-IgE syndrome (STAT3-HIES),⁵ were not reduced (0.7% of CD4⁺ T cells; reference 0.2–0.7%).

Western blotting of lysates from an EBV-immortalized B-cell line using anti-human DOCK 8 N-terminus antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) revealed the absence of DOCK8 protein (data not shown). Investigation of genomic DNA by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA kit (SALSA MLPA P385 DOCK8 probemix and SALSA MLPA P386 DOCK8-STAT3 probemix; MRC-Holland, Amsterdam, The Netherlands) revealed a heterozygous large deletion of 77 kb spanning from intron 5 to exon 22 (Fig. 1D). DOCK8 cDNA sequencing of an EBV-immortalized B-cell revealed a nonsense mutation at position 740 (E740X) (Fig. 1E), which was located at exon 20 of the undeleted allele.

DISCUSSION

DOCK8 is a kind of atypical guanine exchange factor (GEF), which activate Rho-GTPases. As these GTPases are important in regulating many cellular functions of lymphocytes including actin cytoskeletal organization, cell-cycle progression, and gene expression, deficiency of DOCK8 causes an autosomal recessive type of combined

immunodeficiency with typically elevated IgE. Clinically, DOCK8 deficiency is characterized by recurrent infections of both viral and bacterial etiology, a wide range of allergic manifestations, and early onset malignancy. The laboratory markers include variable degrees of T and B lymphocytopenia, decreased IgM, elevated IgE, and eosinophilia.^{2,3}

The spectrum of pulmonary disease secondary to DOCK8 deficiency includes recurrent pulmonary infections, which sometimes lead to bronchiectasis. A variety of organisms, mostly Gram-positive and Gram-negative bacteria but also fungi and viruses are responsible. IgE mediated asthma is also common, and of the 11 patients reported by Zhang et al.,² 6 had asthma or reactive airway disease. They also observed eosinophilic lung disease in two patients. It has been suggested that AEP results from acute hypersensitivity to inhaled unidentified antigens in otherwise healthy individuals, but there is also evidence that certain infections can cause AEP. Although a number of pathogens common to immunodeficient patients, such as *Pneumocystis* species and *Aspergillus* species, are known to cause lung eosinophilia, none of these pathogens were detected in our case. Her AEP might be attributed to the immune response against usual bacterial infection under Th2-polarizing conditions.

Extensive eosinophilia presents a diagnostic challenge for pediatric allergists. Among various causative conditions, hypereosinophilia due to immunodeficiencies such as STAT3-HIES, Omenn syndrome (OS) and immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (IPEX) have been reported. In STAT3-HIES, IL-10 signaling in dendritic cells, which is crucial for generating inducible regulatory T cells (iT_{reg}), was reported to be impaired and accordingly, the appropriate Th2-T_{reg} cell balance to prevent hypereosinophilia might be disturbed.⁶ OS is a form of severe combined immunodeficiency characterized by oligoclonal expansion of T lymphocytes. The oligoclonality of the T cell repertoire in the context of lymphopenia results in severe Th2-associated inflammatory disease, which might be caused by a lack of matching between the T cell repertoire of effector CD4⁺ T cells and T_{reg} cells.⁷ IPEX is caused by mutations in *FOXP3*, a master regulator of T_{reg} cells, and defects in T_{reg} cell function are associated with the development of an unbalanced Th2 response.⁸ Thus, there is a possibility that insufficient T_{reg} cells might be common causes of eosinophilic dysregulation in these immunodeficient conditions. Although the pathogenesis of eosinophilia in DOCK8 deficiency is currently not proven, partial T cell deficiency or oligoclonality⁹ such as OS might induce insufficiency of T_{reg} cells. Further investigation is obviously necessary to elucidate the mechanisms of hypereosinophilia in DOCK8 deficiency and to establish effective strategies for prevention and treatment of these serious complications. Early diagnosis

and treatment with hematopoietic stem cell transplantation (HSCT) is extremely important, as the prognosis of most patients with DOCK8 deficiency is poor due to a high incidence of malignancy and infection. Supportive therapies including prophylactic antibiotics and intravenous immunoglobulins (IVIG) might be beneficial while awaiting HSCT.

ACKNOWLEDGMENTS

This study was approved by the Ethics Committee for Genomic Studies of Fujita Health University. The patient provided written informed consent.

REFERENCES

1. Cottin V, Cordier JF. Eosinophilic pneumonias. *Allergy* 2005;60:841–857.
2. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, Matthews HF, Davis J, Turner ML, Uzel G, et al. Combined Immunodeficiency Associated with DOCK8 Mutations. *N Engl J Med* 2009;361:2046–2055.
3. Engelhardt KR, McGhee S, Winkler S, Sassi A, Woellner C, Lopez-Herrera G, Chen A, Kim HS, Lloret MG, Schulze I, et al. Large deletions and point mutations involving the dedicator of cytokinesis 8 (DOCK8) in the autosomal-recessive form of hyper-IgE syndrome. *J Allergy Clin Immunol* 2009;124:1289–1302.
4. Philit F, Etienne-Mastroianni B, Parrot A, Guérin C, Robert D, Cordier JF. Idiopathic acute eosinophilic pneumonia: a study of 22 patients. *Am J Respir Crit Care Med* 2002;166:1235–1239.
5. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, Kanno Y, Spalding C, Elloumi HZ, Paulson ML, et al. Impaired TH17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* 2008;452:773–776.
6. Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, Agematsu K, Yamada M, Kawamura N, Ariga T, Tsuge I, et al. Defective IL-10 signaling in hyper-IgE syndrome results in impaired generation of tolerogenic dendritic cells and induced regulatory T cells. *J Exp Med* 2011;208:235–249.
7. Milner JD, Ward JM, Keane-Myers A, Paul WE. Lymphopenic mice reconstituted with limited repertoire T cells develop severe, multiorgan, Th2-associated inflammatory disease. *Proc Natl Acad Sci U S A* 2007;104:576–581.
8. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat genet* 2001;27:20–21.
9. Dasouki M, Okonkwo KC, Ray A, Folmsbeel CK, Gozales D, Keles S, Puck JM, Chatila T. 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. *Clin immunol* 2011;141:128–132.