TABLE 3 Laboratory findings of Aicardi-Goutières syndrome patients

Patient	Genotype	CSF lymphocytosis	CSF elevated IFN-α	CSF elevated neopterin	Serum elevated IFN-α	Serum elevated autoantibody	Other clinical features
1	*TREX1* AD	n.d.	n.d.	n.d.	Yes (14 years)	None	Moderate hypothyroidism
2	*TREX1* AD	n.d.	n.d.	n.d.	Yes (13 years)	None	None
3	*TREX1* AD	Yes (1 years)	No (7 years)	No (7 years)	Yes (7 years)	None	None
4	*TREX1* AR	Yes (10 months)	Yes (2 years)	n.d.	n.d.	Unknown	Abnormal liver function
5	*TREX1* AR	Yes (23 days)	No (10 years)	n.d.	No (10 years)	Anti-ssDNA, anti-dsDNA	Abnormal liver function
6	*RNASEH2B* ?	Yes (8 months)	Yes (8 months)	n.d.	Yes (8 months)	None	Abnormal liver function
7	*RNASEH2A* AR	Yes (6 months)	Yes (6 months)	Yes (2 years)	No (6 months)	Anti-ssDNA	None
8	*SAMHD1* AR	Yes (2 months)	n.d.	n.d.	No (12 years)	ANA 1:20480, anti-DNA, anti-RNP	Thrombocytopenia, leucocytopenia, anaemia, abnormal liver function, diabetes mellitus
9	*SAMHD1* AR	Yes (9 months)	Yes (9 months)	n.d.	n.d.	ANA 1:640, anti-ssDNA, anti-dsDNA	Thrombocytopenia, abnormal liver function, hypocomplementaemia, hypergammaglobulinaemia, moderate hypothyroidism
10	*SAMHD1* AR	No (16 years)	n.d.	Yes (16 years)	n.d.	ANA 1:1280, anti-ssDNA, anti-RNP, anti-SS-A	Abnormal liver function, hypergammaglobulinaemia, diabetes mellitus
11	ND	No (11 months)	Yes (11 months)	n.d.	n.d.	Anti-LKM1	Thrombocytopenia, abnormal liver function, hypocomplementaemia, hypergammaglobulinaemia
12	ND	No (3 years)	No (3 years)	Yes (3 years)	n.d.	ANA 1:320	None
13	ND	n.d.	n.d.	n.d.	n.d.	Unknown	Thrombocytopenia, abnormal liver function
14	ND	Yes (4 days)	n.d.	n.d.	n.d.	None	Anaemia, abnormal liver function

AR: autosomal recessive; AD: autosomal dominant; ND: not detected; n.d.: not done. Patients 1–14 are the same as those listed in Table 2. The age at which the data were collected is shown in parentheses.

TABLE 4 Summary of Aicardi-Goutières syndrome patients with dominant-type *TREX1* mutations

Patient	Reference	Genotype	Mutation type	Ethnicity	Chilblain lesions	Developmental delay	Seizure	CSF elevated IFN-α	Serum elevated IFN-α
1	19	p.Asp18Asn	Het	Japanese	Yes	Moderate	FC only	n.d.	Yes (14 years)
2	—	p.His195Tyr	Het, *de novo*	Japanese	Yes	Severe	No	n.d.	Yes (13 years)
3	—	p.Asp200Asn	Mos, *de novo*	Japanese	Yes	Severe	No	No (7 years)	Yes (7 years)
—	10	p.Asp200Asn	Het, *de novo*	Scottish	Yes	Severe	No	Yes (3 years)	Undescribed
—	2	p.Asp200His	Het, *de novo*	German	Yes	Undescribed	Undescribed	Undescribed	Undescribed
—	11	p.Asp18Asn	Het, *de novo*	Undescribed	Yes	Relatively mild	No	Yes (14 years)	Yes (14 years)
—	12	p.Asp18His	Het, *de novo*	Undescribed	Yes	Severe	Undescribed	Yes (4 months)	Undescribed

Het: heterozygous; Mos: mosaic; FC: febrile convulsion; n.d.: not done. The age at which the data were collected is shown in parentheses.

molecularly proven AGS that were also diagnosed with SLE, one harbouring a *SAMHD1* mutation and the other a *TREX1* mutation [2, 17]. In the present study, the first AGS patient complicated with SS, which is also known as a type I IFN-related disease [31], was identified, in addition to two AGS patients diagnosed with SLE. All three patients harboured *SAMHD1* mutations and tested positive for multiple autoantibodies. These findings suggest that, of all the genes associated with AGS, *SAMHD1* mutations may be most closely associated with autoimmunity. Further studies of this association might shed light on the pathophysiology of AGS and autoimmune diseases.

All five patients harbouring *TREX1* mutations had chilblain lesions, a frequency significantly greater than that observed for the rest of the cohort (2/9 patients; $P < 0.05$). However, no previous studies have reported that chilblain lesions are more common in AGS patients harbouring *TREX1* mutations than in those harbouring other gene mutations. Thus we paid attention to the three patients harbouring dominant-type *TREX1* mutations. The clinical features of seven AGS patients with dominant-type *TREX1* mutations, who consist of the three reported herein and four additional cases reported in the literature, are presented in Table 4 [2, 10–12]. Notably, all seven cases had chilblain lesions, which is a significantly higher proportion than that observed in AGS patients as a whole (43% of 123 patients; $P < 0.01$) [3]. Besides AGS, heterozygous *TREX1* mutations are also associated with FCL, which presents with skin symptoms alone [10, 18, 19, 32]. Since dominant-type *TREX1* mutations are more likely to cause chilblains, it would be interesting to examine the differences in the underlying molecular mechanisms.

Although IFN-α levels in the CSF of AGS patients usually normalize during the first few years [3], serum IFN-α levels have not been studied extensively. We found that AGS patients harbouring dominant-type *TREX1* mutations tended to show persistent increases in serum IFN-α levels, even after they became older (Table 4). Previous reports have shown that patients receiving type I IFN present with vasculitic lesions that are similar to the chilblain lesions seen in AGS patients [33, 34]. Therefore we speculate that the chilblain lesions observed in AGS patients harbouring dominant-type *TREX1* mutations are related to persistently high serum IFN-α levels. Since the data on serum IFN-α levels of AGS patients are limited, further studies of more AGS cases from different ethnic backgrounds are needed to confirm the relationship between chilblains and IFN-α levels.

The dimeric protein, TREX1, is a major component of the 3′–5′ exonucleases in mammalian cells and functions to eliminate ssDNA and degrade nicked genomic DNA [6, 35, 36]. A previous study showed that *TREX1* mutations causing dominant-type AGS are localized to Asp18 and Asp200, which are highly conserved Mg^{2+}-coordinating aspartate residues required for catalytic function (Table 4) [37]. The heterozygous mutations p.Asp18Asn, p.Asp200Asn and p.Asp200His cause loss of function and exert dominant negative effects on the wild type [38, 39]. We identified a novel heterozygous *TREX1* mutation, p.His195Tyr, in our cohort. The exonuclease assays revealed that TREX1$^{p.His195Tyr}$ showed defective enzymatic activity, similar to TREX1$^{p.Asp18Asn}$ and TREX1$^{p.Asp200Asn}$. Although we did not generate heterodimers to prove the dominant negative effect, we identified a third TREX1 residue, His195, which causes AGS in a dominant-type manner when mutated.

We also identified the first AGS patient harbouring a somatic mosaicism for a *TREX1* mutation, suggesting that mutated cells could cause AGS, even when co-existing with normal cells. This mosaicism is consistent with the hypothesis that IFN-α released from non-hematopoietic cells acts in a paracrine fashion and plays a vital role in the pathogenesis of AGS [40]. The finding that the

frequency of the mosaicism was similar in every cell lineage or tissue tested suggests that neural cells would also show a similar frequency. From a diagnostic point of view, clinical AGS patients showing an even lower rate of mosaicism in *de novo* dominant-type *TREX1* mutations might be missed by conventional direct sequencing, like some reported cases of cryopyrin-associated periodic syndrome [22, 23, 41, 42].

In conclusion, the present nationwide survey identified more sporadic AGS cases harbouring *de novo* dominant-type *TREX1* mutations than expected. By exploring the genotype–phenotype correlations, we also observed a strong association between dominant-type *TREX1* mutations and chilblain lesions, as well as between *SAMHD1* mutations and autoimmunity. These findings need to be confirmed in AGS patients from different ethnic backgrounds. Nonetheless, these findings emphasize that rheumatologists need to pay attention to possible sporadic AGS cases that present with neurological disorders and autoimmune manifestations, even from non-consanguineous families.

Rheumatology key messages

- A strong association between dominant-type *TREX1* mutations and chilblain lesions was observed in Aicardi-Goutières syndrome patients.
- Special attention should be paid to Aicardi-Goutières syndrome patients with *de novo* dominant-type *TREX1* mutations.

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References

1 Chahwan C, Chahwan R. Aicardi-Goutieres syndrome: from patients to genes and beyond. Clin Genet 2012;81:413–20.

2 Ramantani G, Kohlhase J, Hertzberg C *et al.* Expanding the phenotypic spectrum of lupus erythematosus in Aicardi-Goutières syndrome. Arthritis Rheum 2010;62:1469–77.

3 Rice G, Patrick T, Parmar R *et al.* Clinical and molecular phenotype of Aicardi-Goutieres syndrome. Am J Hum Genet 2007;81:713–25.

4 Orcesi S, La Piana R, Fazzi E. Aicardi-Goutieres syndrome. Br Med Bull 2009;89:183–201.

5 Blau N, Bonafé L, Krägeloh-Mann I *et al.* Cerebrospinal fluid pterins and folates in Aicardi-Goutières syndrome: a new phenotype. Neurology 2003;61:642–7.

6 Crow YJ, Hayward BE, Parmar R *et al.* Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 cause Aicardi-Goutières syndrome at the AGS1 locus. Nat Genet 2006;38:917–20.

7 Crow YJ, Leitch A, Hayward BE *et al.* Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection. Nat Genet 2006;38:910–6.

8 Rice GI, Bond J, Asipu A *et al.* Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. Nat Genet 2009;41:829–32.

9 Rice GI, Kasher PR, Forte GM *et al.* Mutations in ADAR1 cause Aicardi-Goutières syndrome associated with a type I interferon signature. Nat Genet 2012;44:1243–8.

10 Rice G, Newman WG, Dean J *et al.* Heterozygous mutations in TREX1 cause familial chilblain lupus and dominant Aicardi-Goutieres syndrome. Am J Hum Genet 2007;80:811–5.

11 Haaxma CA, Crow YJ, van Steensel MA *et al.* A de novo p.Asp18Asn mutation in TREX1 in a patient with Aicardi-Goutières syndrome. Am J Med Genet A 2010;152A:2612–7.

12 Tüngler V, Silver RM, Walkenhorst H *et al.* Inherited or de novo mutation affecting aspartate 18 of TREX1 results in either familial chilblain lupus or Aicardi-Goutières syndrome. Br J Dermatol 2012;167:212–4.

13 Crow YJ. Type I interferonopathies: a novel set of inborn errors of immunity. Ann N Y Acad Sci 2011;1238:91–8.

14 Obermoser G, Pascual V. The interferon-alpha signature of systemic lupus erythematosus. Lupus 2010;19:1012–9.

15 Lee-Kirsch MA, Gong M, Chowdhury D *et al.* Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. Nat Genet 2007;39:1065–7.

16 Cheng MH, Anderson MS. Monogenic autoimmunity. Annu Rev Immunol 2012;30:393–427.

17 Ramantani G, Häusler M, Niggemann P *et al.* Aicardi-Goutières syndrome and systemic lupus erythematosus (SLE) in a 12-year-old boy with SAMHD1 mutations. J Child Neurol 2011;26:1425–8.

18 Lee-Kirsch MA, Gong M, Schulz H *et al.* Familial chilblain lupus, a monogenic form of cutaneous lupus erythematosus, maps to chromosome 3p. Am J Hum Genet 2006;79:731–7.

19 Abe J, Izawa K, Nishikomori R *et al.* Heterozygous TREX1 p.Asp18Asn mutation can cause variable neurological symptoms in a family with Aicardi-Goutieres syndrome/familial chilblain lupus. Rheumatology 2013;52:406–8.

20 Shintaku H, Asada M, Sawada Y. Diagnosis and treatment of 6-pyruvoyl-tetrahydropterin synthase deficiency. Brain Dev 2000;22(Suppl 1):S118–21.

21 Nishikomori R, Akutagawa H, Maruyama K *et al*. X-linked ectodermal dysplasia and immunodeficiency caused by reversion mosaicism of NEMO reveals a critical role for NEMO in human T-cell development and/or survival. Blood 2004;103:4565–72.

22 Izawa K, Hijikata A, Tanaka N *et al*. Detection of base substitution-type somatic mosaicism of the NLRP3 gene with >99.9% statistical confidence by massively parallel sequencing. DNA Res 2012;19:143–52.

23 Tanaka N, Izawa K, Saito MK *et al*. High incidence of NLRP3 somatic mosaicism in patients with chronic infantile neurologic, cutaneous, articular syndrome: results of an international multicenter collaborative study. Arthritis Rheum 2011;63:3625–32.

24 Orebaugh CD, Fye JM, Harvey S *et al*. The TREX1 exonuclease R114H mutation in Aicardi-Goutières syndrome and lupus reveals dimeric structure requirements for DNA degradation activity. J Biol Chem 2011;286:40246–54.

25 Brucet M, Querol-Audí J, Serra M *et al*. Structure of the dimeric exonuclease TREX1 in complex with DNA displays a proline-rich binding site for WW Domains. J Biol Chem 2007;282:14547–57.

26 Brucet M, Querol-Audí J, Bertlik K *et al*. Structural and biochemical studies of TREX1 inhibition by metals. Identification of a new active histidine conserved in DEDDh exonucleases. Protein Sci 2008;17:2059–69.

27 Bailey SL, Harvey S, Perrino FW *et al*. Defects in DNA degradation revealed in crystal structures of TREX1 exonuclease mutations linked to autoimmune disease. DNA Repair (Amst) 2012;11:65–73.

28 Kavanagh D, Spitzer D, Kothari PH *et al*. New roles for the major human 3′-5′ exonuclease TREX1 in human disease. Cell Cycle 2008;7:1718–25.

29 Tan EM, Cohen AS, Fries JF *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.

30 Miyawaki S. Revised Japan criteria for Sjögren syndrome. Ryumachi 2000;40:48–53.

31 Yao Y, Liu Z, Jallal B *et al*. Type I interferons in Sjögren's syndrome. Autoimmun Rev 2013;12:558–66.

32 Lee-Kirsch MA, Chowdhury D, Harvey S *et al*. A mutation in TREX1 that impairs susceptibility to granzyme A-mediated cell death underlies familial chilblain lupus. J Mol Med (Berl) 2007;85:531–7.

33 Bachmeyer C, Farge D, Gluckman E *et al*. Raynaud's phenomenon and digital necrosis induced by interferon-alpha. Br J Dermatol 1996;135:481–3.

34 Campo-Voegeli A, Estrach T, Marti RM *et al*. Acrocyanosis induced by interferon alpha(2a). Dermatology 1998;196:361–3.

35 Stetson DB, Ko JS, Heidmann T *et al*. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell 2008;134:587–98.

36 Chowdhury D, Beresford PJ, Zhu P *et al*. The exonuclease TREX1 is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death. Mol Cell 2006;23:133–42.

37 de Silva U, Choudhury S, Bailey SL *et al*. The crystal structure of TREX1 explains the 3′ nucleotide specificity and reveals a polyproline II helix for protein partnering. J Biol Chem 2007;282:10537–43.

38 Lehtinen DA, Harvey S, Mulcahy MJ *et al*. The TREX1 double-stranded DNA degradation activity is defective in dominant mutations associated with autoimmune disease. J Biol Chem 2008;283:31649–56.

39 Fye JM, Orebaugh CD, Coffin SR *et al*. Dominant mutations of the TREX1 exonuclease gene in lupus and Aicardi-Goutieres syndrome. J Biol Chem 2011;286:32373–82.

40 Gall A, Treuting P, Elkon KB *et al*. Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity 2012;36:120–31.

41 Saito M, Fujisawa A, Nishikomori R *et al*. Somatic mosaicism of CIAS1 in a patient with chronic infantile neurologic, cutaneous, articular syndrome. Arthritis Rheum 2005;52:3579–85.

42 Saito M, Nishikomori R, Kambe N *et al*. Disease-associated CIAS1 mutations induce monocyte death, revealing low-level mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients. Blood 2008;111:2132–41.

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CASE REPORT

Obvious optic disc swelling in a patient with cryopyrin-associated periodic syndrome

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Abstract: Cryopyrin-associated periodic syndrome (CAPS) is a group of rare hereditary autoinflammatory diseases caused by mutations of the *NLRP3* gene, and leads to excessive production of the proinflammatory cytokine, interleukin-1β. A 35-year-old male presented with recurrent symptoms of urticarial-like rash, periodic fever, arthralgia, headache, and eye redness. His best-corrected visual acuity was 1.0 OD and 0.9 OS. Slit-lamp examination showed conjunctival and episcleral injection in both eyes. Ophthalmoscopy revealed obvious bilateral optic disc swelling and retinal vascular sheathing around the optic discs. Spectral domain optical coherence tomography also showed obvious optic disc swelling. Steroid and nonsteroidal anti-inflammatory drugs did not improve these symptoms. Genetic testing detected a heterozygous mutation of c.907G>A. Thus, the patient was genetically confirmed with CAPS. Visual acuity did not decrease for 3 years, although the optic discs became white in color. CAPS should therefore be distinguished from other disorders when examining optic disc swelling and/or uveitis patients with urticarial-like rash and periodic fever.

Keywords: interleukin-1β, chronic infantile cutaneous and articular syndrome, cryopyrin-associated periodic syndrome, leucine-rich repeat-containing protein 3, optic disc swelling

Introduction

Cryopyrin-associated periodic syndrome (CAPS) is a group of rare hereditary autoinflammatory diseases, and includes the familial cold autoinflammatory syndrome, Muckle–Wells syndrome, chronic infantile cutaneous and articular syndrome (CINCA), and neonatal-onset multisystem inflammatory disease (NOMID).[1] Autoinflammatory syndromes are systemic disorders which result in repeated inflammatory symptoms such as fever, rash, and arthritis in the absence of autoantibodies with high titer or antigen-specific T lymphocytes.[1] It is different from infection, autoimmunity, allergy, and immunodeficiency.[1] CAPS is caused by gain-of-function mutations in the nucleotide-binding domain and leucine-rich repeat-containing protein 3 (NLRP3) within the *cryopyrin* gene.[2] *NLRP3* mutations increase activity of the interleukin-1 (IL-1) inflammasome and lead to excessive production of IL-1β, a proinflammatory cytokine.[2] Thus, genetic testing is necessary for diagnosis of CAPS.[2] CAPS is an autosomal dominant disease and the syndrome generally presents in early childhood.[3] Clinical findings include urticarial-like rash, periodic fever, inflammation of the central nervous system, meningitis, optic disc swelling, deafness, and arthropathy.[3–5] Ocular manifestations of CAPS are not fully understood, although it is known to cause optic disc changes, anterior uveitis, and corneal involvement.[4] In this case study, we report CAPS associated with optic disc swelling.

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Case report

In 2009, a 35-year-old Japanese male visited the dermatology clinic of our hospital presenting with urticarial-like rash from infancy, periodic fever, and arthralgia. He also presented with deafness, repeated eye redness, ocular pain, headache, and general fatigue from the time he was a child. He had a previous history of meningitis at the age of 20 years. At first, Behçet's disease was suspected based on his symptoms. Consequently, he was seen for diagnosis in the ophthalmology clinic at our hospital in September 2009. His best-corrected visual acuity was 1.0 OD and 0.9 OS and his manifest refraction was uncorrected OU. Intraocular pressure was 9 mmHg OD and 7 mmHg OS. Slit-lamp examination showed conjunctival and episcleral injection in both eyes, clear and smooth corneas, and infiltration of inflammatory cells without posterior synechia in the anterior chamber. Gonioscopy did not reveal hypopyon, nodules, or peripheral anterior synechia. Ophthalmoscopy and fundus photography examination revealed obvious bilateral optic disc swelling and retinal vascular sheathing around the optic discs, with absence of dilated capillaries on the optic discs, vitreous opacifications, and retinal exudates (Figure 1). Critical flicker frequency was normal (43.8 Hz OD and 47.2 Hz OS). Goldmann perimetry showed only enlarged blind spots in both eyes. The patient had no ocular findings of Behçet's disease. Hematologic examination showed increased inflammatory markers, a white blood cell count of 18,700 cells/μL (neutrophil count 75.5%, lymphocyte count 20.5%), C-reactive protein of 2.94 mg/dL, and an erythrocyte sedimentation rate of 44 mm. Magnetic resonance imaging showed no intracranial space-occupying lesion.

The anterior uveitis was treated with steroid and nonsteroidal anti-inflammatory eye drops. The patient was also treated at the department of rheumatology and clinical immunology with immunosuppressive agents (methotrexate, tacrolimus, azathioprine, cyclophosphamide) and antirheumatic agents (salazosulfapyridine, leflunomide) to reverse systemic symptoms. However, conjunctival and episcleral injection and systemic symptoms played a limited role in these treatments. Three years later, his best-corrected visual acuity was 1.2 OD and 1.2 OS. His conjunctival and episcleral injection remained unchanged (Figure 2). The optic discs became white in color (Figure 1) and fluorescein angiography demonstrated strong staining and weak late phase leakage from the optic disc (Figure 3). Spectral domain optical coherence tomography (Spectralis, Heidelberg Engineering, Heidelberg, Germany) revealed obvious optic disc swelling (Figure 1).

In 2012, his mother was genetically diagnosed with CAPS. Therefore, the patient underwent genetic testing for the diagnosis of CAPS at Kyoto University Graduate School of Medicine after informed consent was obtained, according to the tenets of the Declaration of Helsinki. Genetic tests detected heterozygous mutations in c.907G>A [p.Asp303Asn (D303N)], a previously reported mutation responsible for CAPS. Thus, he received a definitive diagnosis of CAPS.

Discussion

CAPS consists of three clinical categories, and the familial cold autoinflammatory syndrome is the mildest condition.[3] The Muckle–Wells syndrome presents an intermediate con-

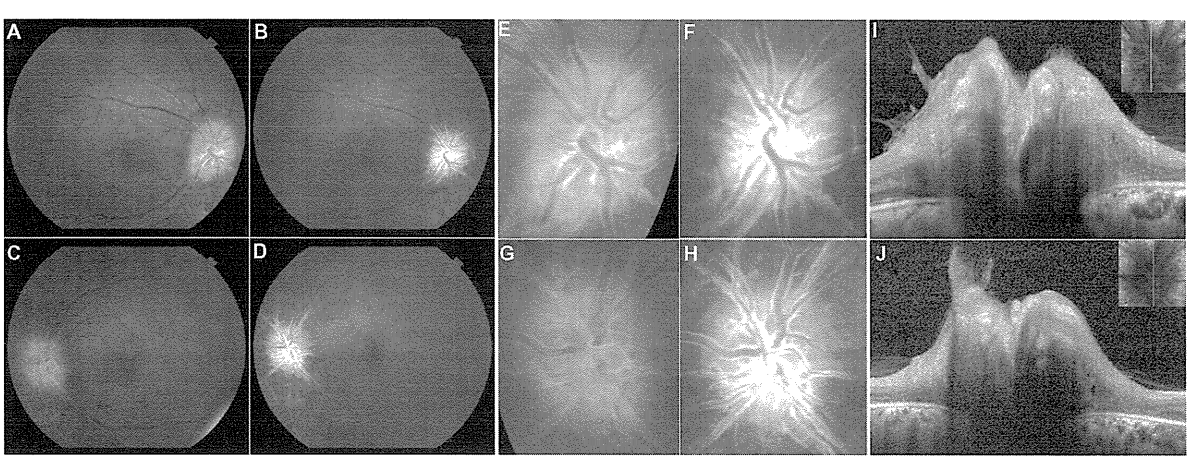

Figure 1 Fundus photographs of the right eye (**A** and **B**) and left eye (**C** and **D**), and photographs focused on the optic disc of the right eye (**E** and **F**) and left eye (**G** and **H**) taken in 2009 and 2012, respectively. Spectral-domain optical coherence tomography scans of the optic disc in the right eye (**I**) and left eye (**J**) in 2012. (**A–D**) Fundus photographs show obvious bilateral optic disc swelling, and absence of vitreous opacifications and retinal exudates. (**E** and **F**) Fundus photographs focused on the optic disc reveal the lack of a sharp outline to the redness of the optic discs, and retinal vascular sheathing around the optic discs. There are no dilated superficial capillaries and hemorrhages on optic discs as was observed in 2009. (**G** and **H**) Both optic discs were white in color and retinal vascular sheathing was increased after 3 years. (**I** and **J**) Longitudinal scans of the optic discs showing obvious optic disc swelling.

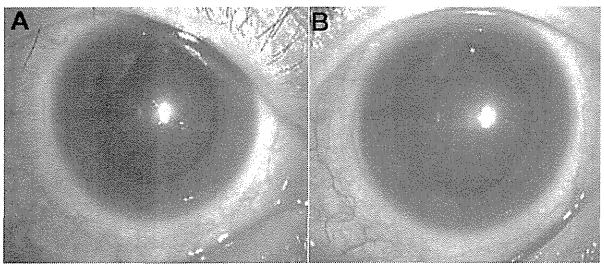

Figure 2 Slit-lamp examinations of the right eye (**A**) and left eye (**B**) in 2012. Slit-lamp biomicroscopy revealed strong conjunctivitis and episcleritis in both eyes treated with mydriatics.

dition between familial cold autoinflammatory syndrome and CINCA/NOMID.[3] CINCA/NOMID is the most severe condition.[3] Common manifestations include urticarial-like rash and periodic fever.[3] Clinical criteria for diagnosis of each individual syndrome have been previously reported.[6–8] According to these reports, CINCA/NOMID presents with urticarial-like rash, periodic fever, inflammation of the central nervous system, and arthropathy.[6–8] Our patient presented with a severe phenotype, including skin erythema, arthralgia, inflammation of the central nervous system, optic disc swelling, meningitis, and deafness. Additionally, alteration of the nucleotide c.907G>A was detected, and previously has been reported as being associated with the phenotypes of CINCA/NOMID and Muckle–Wells syndromes.[9] Consequently, we identified this patient as having CINCA/NOMID or Muckle–Wells syndrome.

The International CINCA/NOMID Ocular Study Group reported ocular manifestations of CINCA/NOMID in 2000.[4] However, at that time CAPS had no known cause. According to the report, of 31 patients, 26 (84%) had optic disc changes, including optic disc edema, pseudopapilledema, and optic atrophy, 13 patients (42%) presented with chronic eye redness, including chronic perilimbal injection and chronic conjunctivitis, 17 patients (55%) had chronic anterior uveitis, and none of the patients had increased intraocular pressure or glaucoma.[4] Our case also demonstrated optic disc changes, chronic conjunctivitis, episcleritis, and anterior uveitis. We think that the optic disc change in our case is optic edema, because fluorescein angiography demonstrated late phase leakage and ophthalmoscopy showed retinal vascular sheathing.

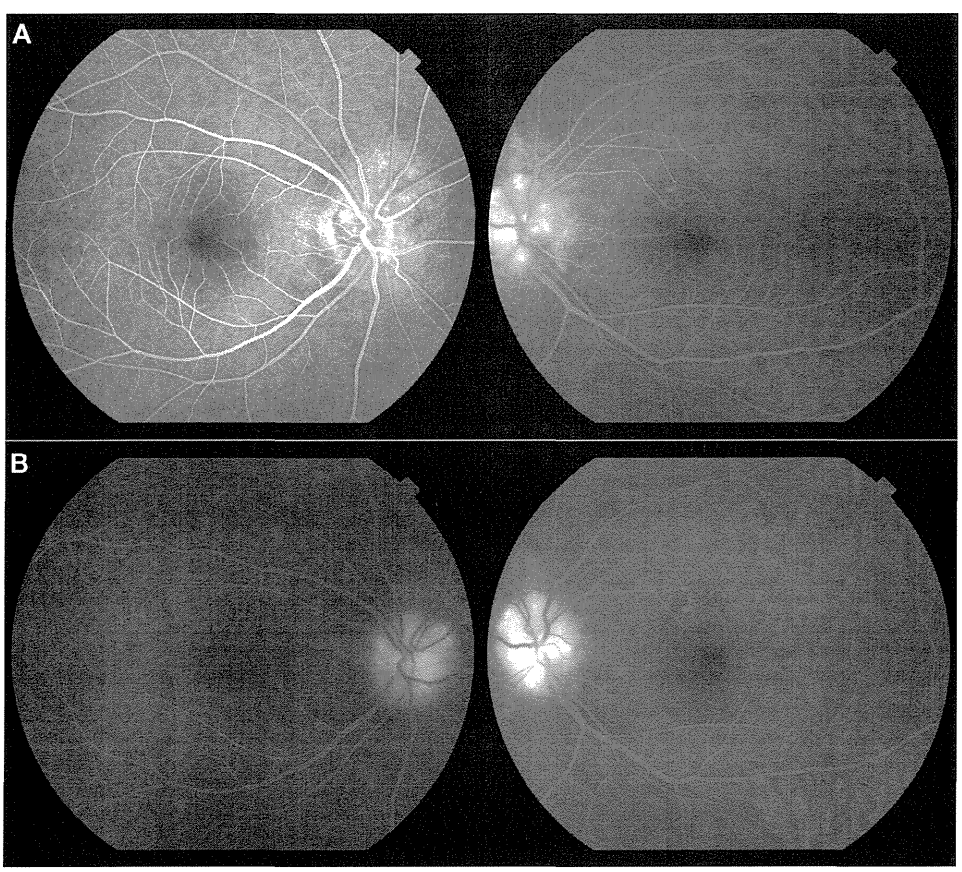

Figure 3 Fluorescein angiography at early phase (**A**) and late phase (**B**) in 2012. Fluorescein angiography showed strong staining and weak late phase leakage from the optic disc in both eyes. There were no dilated superficial capillaries on optic discs.

Terrada et al reported a case of CAPS, and the patient had optic disc changes related to chronic intracranial hypertension due to cerebrospinal fluid inflammation.[10] We do not know whether our patient presented with intracranial hypertension because lumbar puncture was not performed in this case. However, there were no space-occupying brain lesions produced by raised intracranial pressure observed with magnetic resonance imaging.

We speculate two possibilities for optic disc change in our case. First, the optic disc change in our patient may be caused by ocular specific inflammation, because ophthalmoscopy revealed retinal vascular sheathing around the optic discs. Second, inflammation of the central nervous system may lead to optic disc change, because he had a previous history of meningitis. We hypothesize that the optic disc change in CAPS was due to ocular specific inflammation, inflammation of the central nervous system, or raised intracranial pressure.

The CINCA/NOMID Group reported that 19 of 30 patients with CINCA/NOMID had adequate visual acuity or mild visual loss.[4] The patient in our study also had adequate visual acuity. CINCA/NOMID generally occurs with severe systemic symptoms and optic disc changes.[4] However, the report found only eight patients (27%) had severe visual loss.[4] The authors hypothesized that a cause may be the lack of long-term systematic review in many patients.[4] The duration of clinical follow-up in our case was also approximately 4 years. In the future, our patient may have severe visual acuity loss.

Usually steroids, nonsteroidal anti-inflammatory drugs, and immunosuppressive agents are not effective for CAPS.[3] In our study, these drugs had little effect on the patient's symptoms. CAPS is caused by mutations of the *NLRP3* gene that encodes for cryopyrin protein.[2] Cryopyrin protein is part of an inflammasome complex, and regulates production and secretion of the potent proinflammatory cytokine IL-1β in blood myeloid cells.[2] Thus, anti-inflammatory drugs directed at IL-1, such as anakinra, rilonacept, and canakinumab, are used for treatment of CAPS.[11–13]

In conclusion, we showed that the main ocular findings in our case were optic disc swelling, chronic conjunctivitis, episcleritis, and chronic anterior uveitis, without elevated intraocular pressure and peripheral anterior synechia. Steroid and nonsteroidal anti-inflammatory drugs did not improve these symptoms. CAPS is a rare ophthalmologic syndrome and little is known of its cause. Hence, clinicians must distinguish CAPS from other disorders when examining optic disc swelling and/or uveitis patients with urticarial-like rash and periodic fever.

Author contributions

The authors alone are responsible for the content and writing of the paper and agree to allow *Clinical Ophthalmology* to review their data upon request.

Disclosure

The authors report no conflicts of interest.

References

1. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease (*). *Annu Rev Immunol*. 2009;27:621–668.
2. Hoffman HM, Wanderer AA. Inflammasome and IL-1β-mediated disorders. *Curr Allergy Asthma Rep*. 2010;10:229–235.
3. Yu JR, Leslie KS. Cryopyrin-associated periodic syndrome: an update on diagnosis and treatment response. *Curr Allergy Asthma Rep*. 2011;11:12–20.
4. Chronic Infantile Neurological Cutaneous and Articular/Neonatal Onset Multisystem Inflammatory Disease (CINCA/NOMID) Ocular Study Group. Chronic infantile neurological cutaneous and articular/neonatal onset multisystem inflammatory disease syndrome. *Arch Ophthalmol*. 2000;118:1386–1392.
5. Goldbach RM. Current status of understanding the pathogenesis and management of patients with NOMID/CINCA. *Curr Rheumatol Rep*. 2011;13:123–131.
6. Hoffman HM, Wanderer AA, Broide DH. Familial cold autoinflammatory syndrome: phenotype and genotype of an autosomal dominant periodic fever. *J Allergy Clin Immunol*. 2001;108:615–620.
7. Goldbach RM, Dailey NJ, Canna SW, et al. Neonatal onset multisystem inflammatory disease responsive to interleukin-1 beta inhibition. *N Engl J Med*. 2006;355:581–592.
8. Neven B, Callebaut I, Prieur AM, et al. Molecular basis of the spectral expression of CIAS1 mutations associated with phagocytic cell-mediated autoinflammatory disorders CINCA/NOMID, MWS, and FCU. *Blood*. 2004;103:2809–2815.
9. Dodé C, Le Dû N, Cuisset L, et al. New mutations of CIAS1 that are responsible for Muckle-Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes. *Am J Hum Genet*. 2002;70:1498–1506.
10. Terrada C, Neven B, Boddaert N, et al. Ocular modifications in a young girl with cryopyrin-associated periodic syndromes responding to interleukin-1 receptor antagonist anakinra. *J Ophthalmic Inflamm Infect*. 2011;1:133–136.
11. Hoffman HM, Rosengren S, Boyle DL, et al. Prevention of cold-associated acute inflammatory in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet*. 2004;364:1779–1785.
12. Hoffman HM, Throne ML, Amar NJ, et al. Efficacy and safety of rilonacept(interleukin-1 trap) in patients with cryopyrin-associated periodic syndromes. Results from two sequential placebo-controlled studies. *Arthritis Rheum*. 2008;58:2443–2452.
13. The Canakinumab in CAPS Study Group. Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med*. 2009;360:2416–2425.

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ORIGINAL RESEARCH

Autosomal Dominant Anhidrotic Ectodermal Dysplasia with Immunodeficiency Caused by a Novel NFKBIA Mutation, p.Ser36Tyr, Presents with Mild Ectodermal Dysplasia and Non-Infectious Systemic Inflammation

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Abstract
Purpose Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) is characterized by hypohidrosis, dental abnormalities, sparse hair, and immunodeficiency. Autosomal dominant (AD)-EDA-ID, caused by a heterozygous mutation within *NFKBIA*, is very rare and its clinical features remain largely unknown. This study describes a patient with AD-EDA-ID harboring a novel *NFKBIA* mutation who presented with mild EDA and non-infectious systemic inflammation.
Methods The clinical presentation of an AD-EDA-ID patient was described and immunological, genetic, and biochemical analyses were performed, with a focus on nuclear factor kappa B (NF-κB) activation.
Results The patient presented with symptoms of mild EDA-ID, namely sparse hair and hypohidrosis, although a skin biopsy confirmed the presence of sweat glands. There were no dental abnormalities. The patient also suffered from non-infectious inflammation, which responded to systemic corticosteroid therapy; however, the patient remained ill. Immunological analyses revealed reduced Toll-like receptor/IL-1 (TLR/IL-1) and tumor necrosis factor (TNF) receptor family responses to various stimuli. Genetic analysis identified a de novo heterozygous missense mutation, p.Ser36Tyr, in NFKBIA, resulting in defective NFKBIA degradation and impaired NF-κB activation. The patient was diagnosed with AD-EDA-ID and underwent hematopoietic stem cell transplantation. Engraftment was successful, with few signs of acute graft versus host disease. However, the patient suffered hemolytic anemia and thrombocytopenia, and died from a brain hemorrhage due to intractable thrombocytopenia.
Conclusion AD-EDA-ID patients can present with mild ectodermal dysplasia and non-infectious inflammation, rather than with recurrent infections. Also, hematopoietic stem cell transplantation for AD-EDA-ID is still a clinical challenge.

Keywords EDA-ID · NFKBIA · IκBα · HSCT

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Introduction

Nuclear factor kappa B (NF-κB) is a transcription factor that plays an important role in signal transduction in response to external stimuli as well as to internal stimuli within the cell [1, 2]. It is the key molecule that transduces signals from the Toll-like receptor/IL-1 receptor family, T-cell receptors, B-cell receptors, and the tumor necrosis factor (TNF) receptor

superfamily, all of which are expressed by immune cells [1, 2]. In resting cells, NF-κB forms a complex with inhibitor of NF-κB (IκB) family proteins, which include NFKBIA NFKBIB, and NFKBIE, and the complex is retained within the cytoplasm. Ligand binding to the above receptors activates the IκB kinase (IKK) complex, which phosphorylates IκB, thereby releasing NF-κB, which then translocates to the nucleus to activate the expression of certain genes encoding cytokines, chemokines, adhesion molecules, and acute phase proteins [1, 2]. Two serine residues within NFKBIA, namely, Ser32 and Ser36, are phosphorylated by IKK; this is required for NFKBIA ubiquitination and degradation by the proteasome [3, 4]. Thus, Ser32 and Ser36 are critical for NF-κB activation.

Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) is characterized by anhidrosis, dental abnormalities, sparse hair, and immunodeficiency. EDA-ID is caused by impaired NF-κB signaling, which plays a key role in ectodermal development and alerts the innate immune system to the presence of pathogenic organisms. Mutations in two genes are responsible for EDA-ID: *IKBKG*, which is associated with X-linked EDA-ID (XL-EDA-ID, MIM#300291) [5–7], and *NFKBIA*, which is associated with autosomal dominant EDA-ID (AD-EDA-ID, MIM#612132) [8]. While more than 40 mutations have been identified in *IKBKG* [9], only four have been identified in *NFKBIA* [8, 10–13]. The first case of AD-EDA-ID was reported by Courtois et al. [8]. In this case, the patient harbored a p.Ser32Ile mutation, which abrogates the phosphorylation of NFKBIA Ser32 (required for the ubiquitination and proteasomal degradation of NFKBIA). Other NFKBIA mutations, p.Trp11Ter [12], p.Glu14Ter [11], and p.Gln9Ter [10], are thought to be involved in the early termination and re-start of translation from Met37 of NFKBIA, resulting in a NFKBIA protein that is N-terminally truncated and lacking both of the critical serine residues, Ser32 and Ser36. However, because AD-EDA-ID is such a rare disease, the genotype-phenotype correlation between *NFKBIA* mutations and the underlying molecular mechanism(s) are still not clear.

The present study describes a patient with AD-EDA-ID harboring a de novo missense mutation at Ser36 in NFKBIA. The patient presented with mild ectodermal dysplasia and had experienced fewer bacterial infections than previous AD-EDA-ID cases; however, he also suffered from non-infectious systemic inflammation, which responded to treatment with systemic corticosteroids.

Methods

Case Report

The patient (a 3-year-old boy) was born at term to healthy, non-consanguineous parents. He suffered from a perianal abscess at the age of 4 months, and developed a Bacille Calmette-Guérin (BCG) skin abscess 7 months post-vaccination. The BCG skin infection was controlled by topical treatment and did not require systemic treatment with anti-mycobacterial agents. At this time, primary immunodeficiency was suspected and the patient was referred to a local hospital. Although the patient's serum IgG levels were above normal, intravenous immunoglobulin (IVIG) treatment was initiated due to a suspected defect in humoral immunity. Thereafter, he suffered bouts of gastroenteritis or systemic inflammation, and was admitted to hospital on several occasions; however, no pathogenic organisms were identified by routine bacterial culture of blood, feces, or pharyngeal swabs and he did not respond to empirical antibiotics.

To assess his immunological status further, the patient was referred to Kyoto University Hospital. Physical examination revealed hypohidrosis and sparse hair, but no dental abnormalities, lymphedema, osteopetrosis, or skin lesions suggestive of incontinentia pigmenti. Mild ectodermal dysplasia coupled with a clinical history of bacterial and non-bacterial inflammation was suggestive of EDA-ID. However, skin biopsies did not reveal the loss of sweat glands usually seen in cases of EDA-ID. Immunological, genetic, and biochemical analyses identified a mutation (p.Ser36Tyr) in NFKBIA, indicating that the patient was suffering from AD-EDA-ID. Although his inflammatory conditions were effectively treated with systemic corticosteroids, the patient remained ill and failed to thrive.

At the age of 6 years, the patient received an allogeneic bone marrow transplant from an HLA-DRB1 mismatched unrelated donor to treat the AD-EDA-ID. The conditioning regimen comprised total body irradiation (3 Gy), fludarabine (180 mg/m^2), melphalan (90 mg/m^2), cyclophosphamide (140 mg/kg), and rabbit anti-thymocyte globulin (5 mg/kg). Tacrolimus and short-term methotrexate were used to prevent graft versus host disease (GVHD). An absolute neutrophil count of 0.5×10^9/L was achieved on Day +21 post-transplantation, and a sustained platelet count of $>50\times10^9$/L was achieved on Day +25. No blood transfusions were required after Day +18. Full donor chimerism was confirmed by PCR (using a variable number of long tandem repeats) on Day +27. Cutaneous acute GVHD was noted on Day +18, which was treated with prednisolone. The skin lesions then resolved, allowed tapering of the steroid from Day +25. However, the sudden onset of hemolytic anemia and thrombocytopenia (with no other symptoms) was noted on Day +180. The patient showed a positive Coombs test and did not respond to a platelet transfusion, suggesting autoimmune complications associated with chronic GVHD. Discontinuing tacrolimus, increasing the dose of prednisolone, and including mycophenolate mofetil in the regime yielded a transient improvement. Unfortunately, the severe thrombocytopenia ($2–5\times10^9$/L) returned on Day +194 and the patient died of a cerebellar hemorrhage on Day +211.

Proliferation Assay

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood using Lymphoprep (Axis-Shield, UK) and cultured in RPMI 1640 (Sigma, USA) supplemented with 10 % fetal calf serum (FCS). The cells were plated into 96-well plates coated with increasing concentrations of an anti-CD3 monoclonal antibody (mAb; clone OKT3, Ortho Diagnostics Systems, USA), an anti-CD28 mAb (clone 9.3, BD Biosciences, USA, 2 μg/ml), human recombinant IL-2 (kindly provided by Takeda Pharmaceutical LTD., 100 IU/mL), phorbol myristate acetate (PMA; Sigma, 20 ng/mL), ionomycin (Sigma, 1 μM), phytohemagglutinin (PHA; Life Technologies, USA; 1:100 dilution), purified protein derivative (PPD; Japan BCG Laboratory, Japan, 10 μg/mL), or tetanus toxoid (TT; Sigma, 5 μg/mL). After 36 hours, 0.5 μCi of [^{3}H]-thymidine was added to the culture for 12 hours. The uptake of [^{3}H]-thymidine was measured using a scintillation counter, as previously described [14].

Cytokine Assay

PBMCs were cultured in RPMI 1640 (Sigma) supplemented with 10 % FCS and then stimulated for 24 h with IFN-γ (5000 IU/mL, R&D Systems Inc., USA) plus varied concentrations of lipopolysaccharide (LPS) (Sigma), or with IL-12 (20 ng/mL, R&D Systems Inc.) plus varied concentrations of IL-18 (R&D Systems Inc.). The supernatants were harvested and the concentrations of TNF-α and IFN-γ were measured using the human TNF-α OptEIA set (BD Biosciences) or the human IFN-γ OptEIA set (BD Biosciences), respectively. A primary fibroblast line was derived from a skin biopsy taken from the patient. Healthy control fibroblasts were kindly provided by Dr. Utani (Kyoto University, Japan). Fibroblast cell lines were cultured in DMEM (Sigma) supplemented with 10 % FCS and then stimulated with TNF-α (10 ng/mL; R&D Systems Inc.) or IL-1β (10 ng/mL; R&D Systems Inc.) for 12 h, or with lymphotoxin-α1β2 (LTα1β2) (50 ng/mL; R&D Systems Inc.) for 24 h. The supernatants were harvested and the amount of IL-6 was measured using the human IL-6 OptEIA set (BD Biosciences).

Flow Cytometry

PBMCs were isolated as described above, suspended in FACS buffer (PBS containing 5 % FCS and 0.05 % sodium azide), stained with monoclonal antibodies (mAbs) specific for CD3 (clone SK7), TCRαβ (clone IP26A), TCRγδ (clone IMMU510), CD4 (clone CK3), CD8 (clone SK1), CD56 (clone B159), CD45RA (clone HI100), CD45RO (clone UCHL1), CCR7 (clone 150503), or CD19 (clone SJ25C1), and then analyzed using a FACSCalibur flow cytometer (BD Biosciences). The data were analyzed using CellQuest software (BD Biosciences). To examine the expression of co-stimulatory molecules on B-cells, PBMCs were stimulated with recombinant human soluble CD40L (rhsCD40L; kindly provided by Dr. Jain, National Institutes of Health, USA) for 48 h and then stained with mAbs against CD23, CD54, CD86, and CD95, as previously described [14]. For isolation of various lineage cells derived from PBMC, flow cytometry sorting was performed by FACSAria II (BD Biosciences) with more than 95 % purity.

Genetic Analysis

After obtaining informed consent from the patient's parents in accordance with the ethics committee of Kyoto University Hospital and the Declaration of Helsinki, genomic DNA and total RNA were isolated from PBMCs and cDNA was generated using the SuperScript II First-Strand Synthesis System for RT-PCR (Life Technologies), as previously described [14, 15]. The exons and intron-exon boundaries, as well as the cDNA for *IKBKG* and *NFKBIA*, were amplified using LATaq (Takara Bio, Japan) and sequenced using the BigDye terminator kit (Life Technologies) and an ABI3100 sequencer (Life Technologies), as previously described [14, 15].

Western Blotting

PHA blasts were obtained by stimulating PBMCs with PHA (Sigma). The PHA blasts were then stimulated with TNF-α (10 ng/mL) and the cells harvested after 0.5, 1, 3, or 5 h. The cell lysates were loaded onto SDS-PAGE gels, transferred to Immobilon-P (EMD Millipore, USA), and immunoblotted with anti-NFKBIA (BD Biosciences), anti-NFKBIB (Cell Signaling Technology, USA), anti-NFKBIE (Cell Signaling Technology), or anti-β actin (Sigma, as a loading control) antibodies, as previously described [14].

Electrophoresis Mobility Shift Assay

Patient-derived fibroblasts were stimulated with TNFα (10ng/mL) for 2 h, cell extracts were prepared, and an electrophoresis mobility shift assay (EMSA) was performed, as previously described [16]. Briefly, consensus oligonucleotides directed against NF-κB and Oct-1 (used as a control) were radiolabeled with T4 polynucleotide kinase and γ-[^{32}P] ATP. The radiolabeled oligonucleotides were isolated on a gel filtration column, boiled, and allowed to cool slowly to facilitate annealing. The annealed oligonucleotides were then incubated for 30 min with the cell extracts as previously described [16]. The mixture was separated in a polyacrylamide gel run in 0.5×TBE. The gel was then dried and subjected to autoradiography.

Transactivation by Mutant NFKBIA

The cDNA construct of *NFKBIA* was cloned into the pcDNA3.1 (+) vector (Invitrogen, USA) and a p.Ser36Tyr mutant plasmid was generated using the QuickChange site-directed mutagenesis kit according to the manufacturer's instructions (Agilent Technologies, USA). 293-hTLR4A-MD2-CD14 cells (HEK 293 cells stably transfected with human *TLR4a*, *MD2* and *CD14*; InvivoGen, USA) were cultured in DMEM supplemented with 10 % FCS. To examine the effect of the p.Ser36Tyr mutation, 1×10^6 293-hTLR4A-MD2-CD14 cells were transfected with a total of 100 ng plasmid DNA containing different amounts of wild-type NFKBIA expression plasmid and mock plasmid, different amounts of mutant NFKBIA expression plasmid and mock plasmid, or mock plasmid alone. Transfections were performed using the TransIT293 Transfection Reagent (Milus Bio, USA). pNF-κB-luc (20 ng; BD Biosciences) was used as a reporter and pRL-TK (5 ng; Toyo Ink, Japan) was used as an internal control. Twenty hours later, the 293-hTLR4A-MD2-CD14 cells were stimulated with LPS (100-ng/ml, InvivoGen, USA) for 3 h and then harvested. NF-κB activation was analyzed in a luciferase assay, as previously described [15].

Results and Discussion

First, we evaluated the immunological features of the patient (Table I). Laboratory examinations showed marked neutrophilia with a slight increase in monocytes and a minimal reduction in the lymphocyte population. IgG, IgA, and IgD levels were increased, although IgM levels were normal. Isohemagglutinin levels were normal, PPD skin reactions were positive, and both NK cell activity and complement function were normal. Flow cytometric analysis of the PBMC population showed a reduction in the TCRγδ+T-cell and effector memory CD8+ T-cell populations with cut-off of mean −1.5 SD (Table II). Total B-cell and CD27+IgD- B-cell numbers were within normal limits. T-cell proliferation in response to the coated anti-CD3 antibody was dose-dependent; reduced T-cell proliferation was observed in response to anti-CD3 at 0.01 μg/ml and 0.1 μg/ml, but normal proliferation was observed in response to anti-CD3 at 1.0 μg/ml and 10 μg/ml (Fig. 1). T-cell proliferation in response to PHA, PMA/ionomycin, PPD, and TT showed that the patient's stimulation indices were comparable with those of the normal controls, indicating that T-cell responses to mitogens and antigen recall stimulation were normal (Fig. 1).

Because the patient had a diagnosis of suspected EDA-ID, we next evaluated whether the patient's cells responded normally to stimulants that activate NF-κB. We examined cytokine production by PBMCs and fibroblasts after stimulation of TLR/IL-1 receptor family or TNF receptor family members as described by Courtois et al. [8, 14]. The patient's PBMCs showed little response to LPS, although we did detect a low response to IL-18 (Fig. 2a and b). Furthermore, the patient's fibroblasts showed reduced responses to TNF-α IL-1β and LTα1β2 (Fig. 2c and d). These data indicated impaired signal transduction downstream of the TLR/IL-1 receptor and TNF receptor families. We also examined the expression of CD23, CD54, CD86, and CD95 on B-cells after stimulation of CD40, which is another member of the TNF receptor family whose signal transduction depends on NF-κB (Fig. 3). Compared with B-cells derived from a healthy control subject, the patient's B-cells showed down-regulated expression of CD23 and CD54; however, CD86 and CD95 levels were similar to those of the control. Taken together, these data indicate that the patient's cells showed reduced responses to CD40 stimulation, strongly suggesting that they harbored a defect in NF-κB-mediated signal transduction.

The genes responsible for EDA-ID are *IKBKG* and *NFKBIA*. Thus, we next performed genetic analyses of these two genes and identified a de novo heterozygous c.107C>A (p.Ser36Tyr) mutation in *NFKBIA*; no mutation was identified in the exons and exon-intron boundaries of the *IKBKG* gene (Fig. 4). This *NFKBIA* mutation was not present in either of the patient's parents, in 50 healthy Japanese controls, or in the 1000 genomes database. Polyphen 2 predicted this mutation to be "probably damaging", with a score of 0.999. The SIFT program predicted it to be "damaging", with a SIFT score of 0. Both Ser36 and Ser32 are well conserved from *Xenopus laevis* to *Homo sapiens* (Fig. 4b). The serine residue (S36) of NFKBIA is phosphorylated by IKK. Indeed, studies of S36 mutations in both mouse and human NFKBIA show that this residue is critical for NFKBIA degradation and NF-κB activation [3, 4]. These data, coupled with the finding that AD-EDA-ID cases show a heterozygous p.Ser32Ile mutation (another serine residue within NFKBIA that is important for degradation by the proteasome), suggest that the p.Ser36Tyr mutation may be the cause of the clinical phenotype observed in our patient, although the ectodermal dysplasia was very mild.

Since it is reported that a case with somatic mosaicism of NFKBIA p.Ser32Ile mutation has less severe clinical phenotype and lacks ectodermal dysplasia, we performed genetic analysis on various cell lineages to examine a possibility of somatic mosaicism. The sequencing chromatograms of CD3+ cells, CD14+ cells, and CD19+ cells of PBMC as well as that of bone marrow-derived stromal cells showed heterozygous c.107C>A mutation, which indicated that somatic mosaicism of the NFKBIA would be less likely (Fig. 4c).

To confirm whether the p.Ser36Tyr mutation in NFKBIA results in defective NF-κB activation, we performed several biochemical analyses. First, PHA blasts were obtained by

Table I Peripheral blood analysis

		Patient (on IVIG)	Healthy controls
Blood counts			
White blood cells	($\times 10^6$/L)	19350	3600–9800
Neutrophils	($\times 10^6$/L)	15848	3000–5000
Lymphocytes	($\times 10^6$/L)	2457	2500–4500
Monocytes	($\times 10^6$/L)	987	200–950
Eosinophils	($\times 10^6$/L)	39	0–700
Basophils	($\times 10^6$/L)	19	0–150
Red blood cells	($\times 10^{12}$/L)	5.06	4.08–5.07
Hemoglobin	(g/L)	97	116–141
Platelets	($\times 10^9$/L)	546	201–409
Serum immunoglobulins			
IgG	(g/L)	19.19	10.79±2.63
IgA	(g/L)	6.29	2.46±0.91
IgM	(g/L)	0.57	0.83±0.21
IgD	(g/L)	0.34	0.055±0.016
IgE	(IU/mL)	6	<170
T-cell proliferation			
None	(cpm)	194	127–456
Phytohemagglutinin	(cpm)	158808	20500–56800

Control values represent the normal range in 95 % of healthy children aged 9–12 years. Control serum immunoglobulin levels are based on samples obtained from children aged 8–10 years and are expressed as the *mean* ± SD. IVIG indicates a monthly intravenous infusion of immunoglobulins (2.5 g)

culturing the patient's PBMCs. The PHA blasts were then subjected to a NFKBIA degradation assay. Figure 5 shows that the NFKBIA protein derived from the patient was not degraded after TNF-α stimulation; however, NFKBIA proteins derived from the normal controls showed evidence of transient degradation. On the other hand, the NFKBIB and NFKBIE proteins derived from the patient and a healthy control were degraded to a similar extent after TNF-α stimulation.

Next, we evaluated NF-κB activation in an EMSA using extracts from stimulated cells (Fig. 6). Fibroblasts obtained from the patient and from normal controls were stimulated with TNF-α, and the EMSA was performed using a consensus oligonucleotide probe against NF-κB. The results demonstrated that, when stimulated with TNF-α, the patient's fibroblasts showed lower levels of NF-κB p50/p65 activation than normal control fibroblasts.

Table II Surface markers expressed by peripheral blood mononuclear cells

	Patient ($\times 10^6$/L)	Healthy controls ($\times 10^6$/L)
CD3+	2246	2813±1197
CD4+	1429	1699±850
CD8+	792	972±457
TCRαβ+	2222	2154±1004
TCRγδ+	24	324±182
CD4+CD45RA+CCR7+(naïve CD4+ T-cells)	1171	1290±756
CD4+CD45RA-CCR7+(central memory CD4+ T-cells)	149	264±123
CD4+CD45RA-CCR7-(effector memory CD4+ T-cells)	100	119±29
CD4+CD45RA+CCR7-	9	59±42
CD8+CD45RA+CCR7+(naïve CD8+ T-cells)	772	655±503
CD8+CD45RA-CCR7+(central memory CD8+ T-cells)	6	30±28
CD8+CD45RA-CCR7-(effector memory CD8+ T-cells)	8	132±87
CD8+CD45RA+CCR7-(effector memory CD8+ T-cells)	6	221±95.3
CD19+	1075	1238±605
CD19+CD27+smIgD-	36	86.6±61.3
CD19+CD27+smIgD+	33	172±123
CD3-CD56+	63	271±186

Absolute numbers are shown. Healthy control values are derived from children aged 2–9-years and are expressed as the mean ± SD

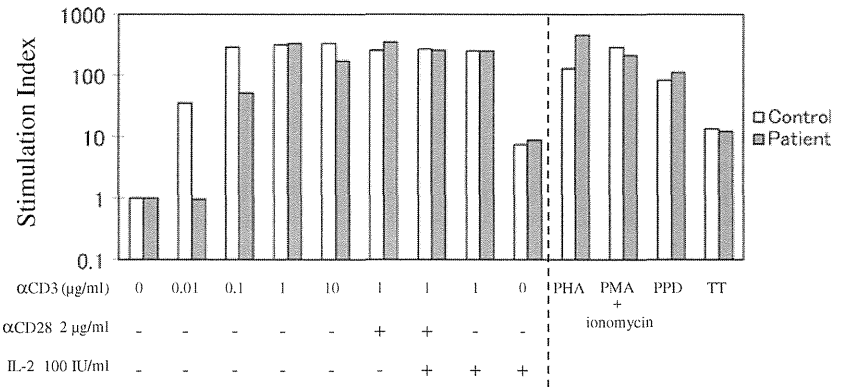

Fig. 1 Proliferation assays using T-cells isolated from the patient. PBMCs were isolated from the patient and from a normal healthy control subject and stimulated with a plate-bound anti-CD3 antibody (at the indicated concentrations), an anti-CD28 antibody, IL-2, PHA, PMA and ionomycin, purified protein derivative (PPD), or tetanus toxoid (TT). The data are expressed as the stimulation index (obtained by dividing the stimulated proliferation counts by the non-stimulated counts). The data represent the mean of duplicate measurements

The mutations in *NFKBIA* that cause AD-EDA-ID are hypermorphic and the mutated NFKBIA protein suppresses NF-κB-mediated signaling [8, 10–12]. Thus, we next examined the ability of the NFKBIA p.Ser36Tyr mutation to suppress the TLR signaling pathway by transiently transfecting HEK293 cells with a plasmid expressing mutant or wild-type cDNA, as previously described (Fig. 7) [10]. We found that NFKBIA p.Ser36Tyr suppressed TLR signaling to a greater extent than wild-type NFKBIA, confirming that the p.Ser36Tyr mutation yielded a hypermorphic protein. Thus, the p.Ser36Tyr NFKBIA mutation resulted in defective proteasomal degradation of NFKBIA and impaired NF-κB activation.

To date, four *NFKBIA* mutations have been reported in six AD-EDA-ID patients: two patients harbored a p.Ser32Ile mutation [8, 13], one harbored a p.Gln9Ter mutation [10], one harbored a p.Trp11Ter mutation [12], one harbored a p.Glu14Ter mutation [11], and one showed p.Ser32Ile mosaicism [13]. All, apart from the patient with p.Ser32Ile mosaicism, suffered from EDA and recurrent bacterial infections. The patient in the present study harbored a p.Ser36Tyr mutation and had mild EDA; however, a skin biopsy showed the presence of sweat glands, his teeth were normal, and he suffered few bacterial infections. One possible explanation, which is not excluded by the present study, is somatic mosaicism in tissues of ectodermal origin. However, the sequencing chromatograms for DNA

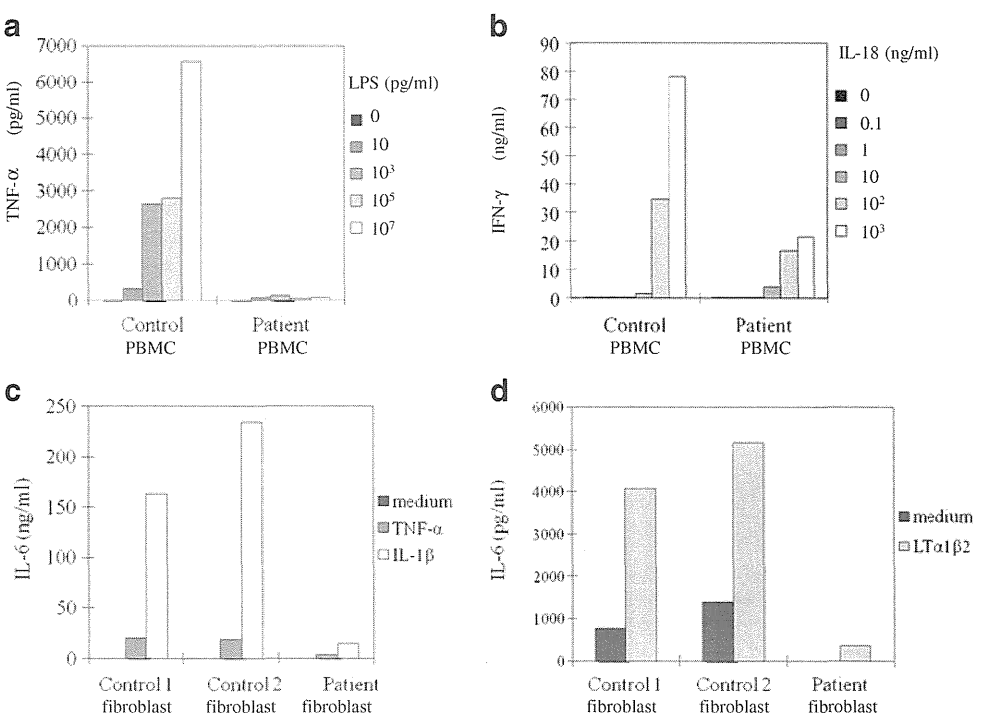

Fig. 2 Defective cytokine production by cells isolated from the patient and stimulated with ligands for TLR/IL-1 and TNF receptor family receptors. PBMCs were stimulated with IFN-γ 5000 IU/mL plus the indicated doses of LPS, and the levels of TNF-α in the supernatant were measured by ELISA 24 h later (**a**). PBMCs were stimulated with IL-12 (20 ng/mL) plus the indicated doses of IL-18, and the levels of IFN-γ in the supernatant were measured by ELISA 24 h later (**b**). Fibroblasts derived from the patient and from healthy donors were stimulated with TNF-α or IL-1β for 12 h (**c**) or with LTα1β2 for 24 h (**d**), and the levels of IL-6 in the supernatant were measured by ELISA

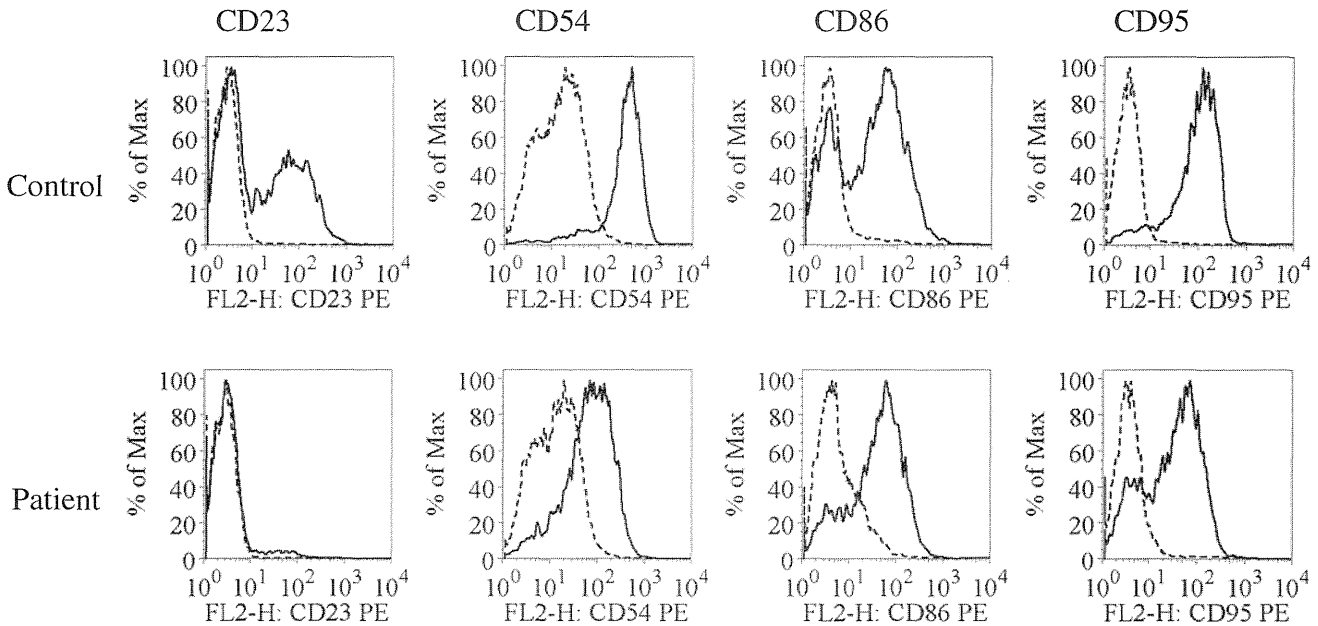

Fig. 3 Down-regulated expression of co-stimulatory molecules on B-cells in response to CD40 stimulation. PBMCs were stimulated with rhsCD40L for 48 h, and the expression of CD23, CD54, CD86, and CD95 on B-cells was analyzed using a flow cytometer. The dotted lines represent non-stimulated cells and the solid lines represent stimulated cells

extracted from the patient's PBMCs, CD3+ cells, CD19 cells, CD14+ cells, and bone marrow-derived stromal cells do not suggest mosaicism, and every cell type examined [T-cells (western blotting), B-cells (surface expression of adhesion molecules), fibroblasts (cytokine secretion), and PBMCs (cytokine secretion)] showed defective NF-κB activation. This

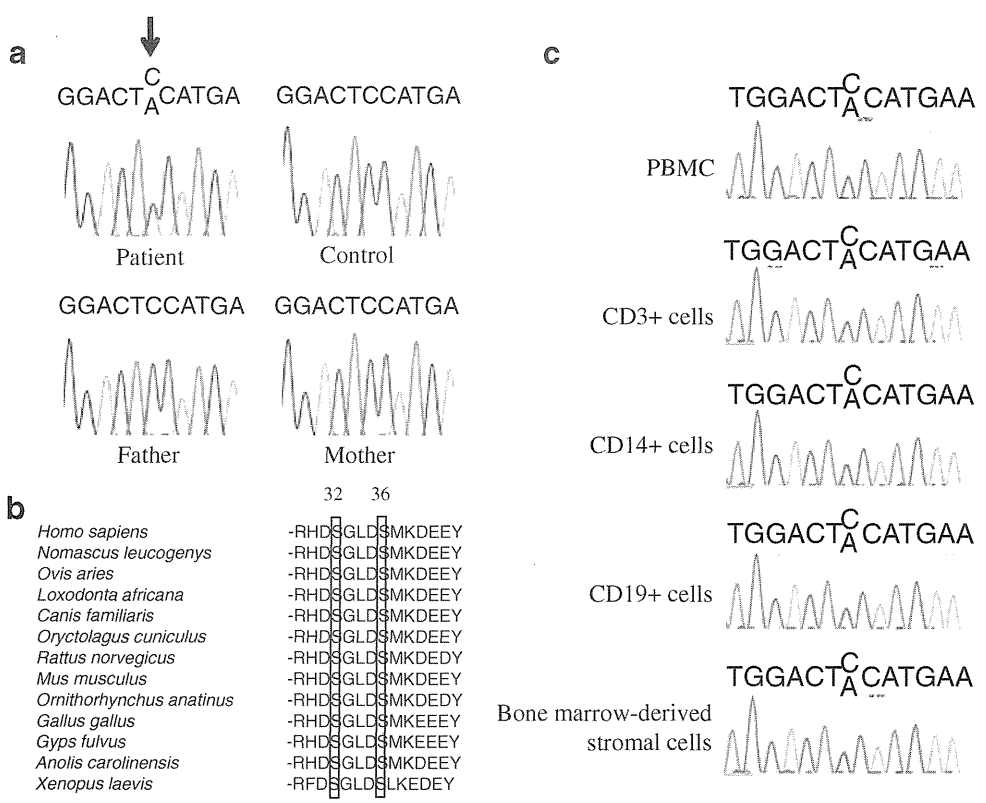

Fig. 4 Sequence analysis of the *NFKBIA* gene. The de novo mutation, c.107C>A, in the *NFKBIA* gene is indicated by the *arrow* (**a**). The primer sequences are available upon request. The conservation of the Ser36 and Ser32 residues within NFKBIA among various species is shown in the right panel (**b**). The sequencing chromatograms of CD3+ cells, CD14+ cells and CD19+ cells of PBMC as well as that of bone marrow-derived stromal cells showed heterozygous c.107C>A mutation (**c**)

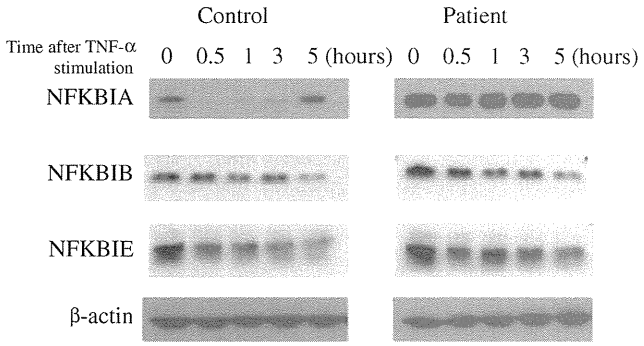

Fig. 5 PHA blasts show defective NFKBIA protein degradation upon stimulation by TNF-α. PHA blasts, derived from PBMCs isolated from the patient and a normal healthy subject, were stimulated for the indicated time and then harvested for Western blot analysis of NFKBIA, NFKBIB, and NFKBIE. β-actin was used as a loading control. The data are representative of two independent experiments

indicates that the majority of the patient's cells are derived from mutated cells. Although it is difficult to draw definite conclusions from a single case and to exclude the possibility of somatic mosaicism in ectodermal tissues, it appears that the p.Ser32Tyr mutation has a less severe clinical impact in terms of the susceptibility to infection. It is also safe to say that AD-EDA-ID patients may show a less marked EDA phenotype, as is reported for some XL-EDA-ID patients [9].

PBMC surface marker analysis demonstrated that, in common with an AD-EDA-ID patient, the patient in the present study showed a reduction in γδ T-cell and memory CD8+ T-cell numbers [8]. It appears that the T-cells derived from some, but not all, AD-EDA-ID patients show a reduction in the memory T-cell population or an increase in the naïve T-cell population. This could explain the reduced responses to low concentrations of anti-CD3 antibody observed in the present study, since T-cells harboring mutations might require stronger stimulation to proliferate effectively. Another feature of T-cell proliferation in AD-EDA-ID is that cells derived from some patients, including the patient in the present study, show

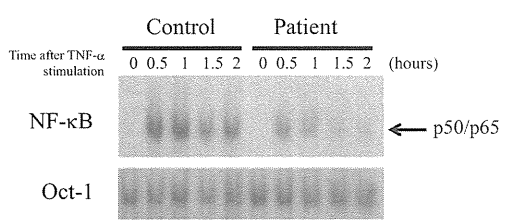

Fig. 6 Patient-derived fibroblasts show defective NF-κB activation upon stimulation by TNF-α. An EMSA of fibroblasts derived from the patient and from a normal healthy subject is shown. The arrow indicates the NF-κβ p50/p65 complex. The results were confirmed in a supershift assay with anti-p50 and anti-p65 antibodies (data not shown). Patient-derived fibroblasts stimulated with TNF-α show reduced activation of the p50/p65 complex. An EMSA for Oct-1 is shown as a control. The data are representative of two independent experiments. Information about the EMSA oligonucleotides is available upon request

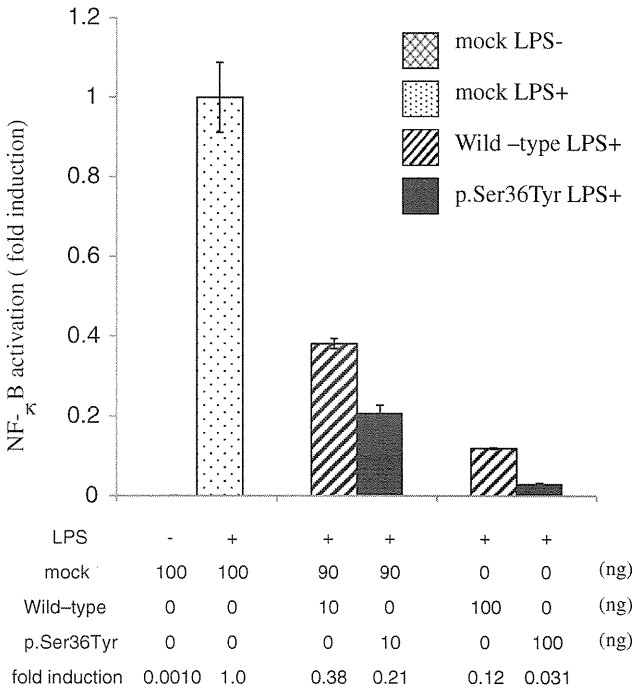

Fig. 7 NFKBIA p.Ser36Tyr suppresses NF-κB activation to a greater extent than wild-type NFKBIA. The suppressive effects of NFKBIA wild-type and NFKBIA p.non NF-κB activation were measured by overexpressing different amounts of the respective NFKBIA expression plasmids in LPS-stimulated 293-hTLR4A-MD2-CD14 cells. Data are expressed as the mean fold increase in activation. Triplicate measurements were made. The mean value for mock-transfected LPS-stimulated cells was set as 1. *Error bars* represent the S.E.M. Data are representative of two independent experiments

higher levels of PHA-induced proliferation than those derived from normal controls (p.Ser32Ile and p.W11Ter patients) [8, 12]. The observed inconsistencies in the proliferative responses of AD-EDA-ID patients may be due to differences in the proliferation assay protocols used by different studies; therefore, further studies that use a consistent T-cell proliferation assay may identify the mechanisms underlying the different T-cell phenotypes in AD-EDA-ID.

The patient suffered from inflammatory episodes, although no pathogens were identified. He was treated effectively with systemic corticosteroids, although we found that it was difficult to taper the drugs. This suggests that the patient had a non-infectious inflammatory condition. In this regard, the patient with p.Ser32Ile mosaicism suffered from systemic-onset juvenile idiopathic arthritis and was diagnosed with rheumatoid factor-positive rheumatoid arthritis in adulthood [13]. In addition, the patient with the p.Gln9Ter mutation suffered from inflammatory bowel disease [10]. The clinical picture for both of these patients, and the patient reported herein, suggest that, similar to patients with XL-EDA-ID, patients with AD-EDA-ID also suffer from inflammatory bowel diseases and other non-infectious inflammatory disorders such as rheumatoid arthritis [9, 15]. Both AD-EDA-ID and XL-EDA-ID are associated with

defects in NF-κB activation; thus, it is likely that a similar mechanism is responsible for the paradoxical phenotypes of immunodeficiency and non-infectious inflammatory disorders. Further studies are needed to identify the mechanisms involved and to develop an effective treatment for patients with EDA-ID.

HSCT is the main treatment used to reconstitute the immune system of patients with primary immunodeficiency diseases, particularly SCID. AD-EDA-ID patients that experience recurrent severe infections and fail to thrive have received HSCT as a curative treatment. Indeed, the two AD-EDA-ID patients described above (one with a p.Ser32Ile mutation and the other with a p.Glu14Ter mutation) received HSCT [17, 18]. The former received HSCT from a haploidentical related donor in combination with T-cell depletion (using a CD34+ cell isolation technique) and survived without GVHD. The latter received two rounds of HSCT with unrelated cord blood cells from 8/10-matched (disparate HLA B and C) and 7/8-matched (disparate HLA A) donors, respectively; the patient experienced graft failure and died. The patient in the present study suffered from non-infectious systemic inflammation and failed to thrive. Therefore, to cure the condition, we decided to perform HSCT. He received an HLA-DRB1 one-locus mismatched unrelated cord blood transplant with a reduced-intensity stem cell transplant regimen. Although the graft survived and 100 % chimerism was achieved, the patient suffered transfusion-resistant thrombocytopenia on Day +180. This caused an intracranial hemorrhage and the patient died. Previous reports suggest that HSCT may not cure XL-EDA-ID; indeed, it may even exacerbate the inflammatory bowel disease [17, 19]. It is possible that pre-existing inflammatory diseases may have affected the course of chronic GVHD after HSCT. Since AD-EDA-ID is very rare, it is important that we collect data regarding the clinical course of these patients to identify the appropriate treatment, including when and how HSCT should be performed.

In conclusion, the present study identified a novel p.Ser36Tyr mutation in NFKBIA, which appears to be responsible for AD-EDA-ID. We also showed that patients with AD-EDA-ID can present with mild EDA and non-infectious systemic inflammation, but may be less susceptible to infection. We also point out that HSCT as a curative treatment for AD-EDA-ID is still a clinical challenge.

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Conflict of Interest The authors report no actual or potential conflicts of interest.

References

1. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol. 2009;27:693–733. doi:10.1146/annurev.immunol.021908.132641.
2. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol. 2000;18:621–63. doi:10.1146/annurev.immunol.18.1.621.
3. Brown K, Gerstberger S, Carlson L, Franzoso G, Siebenlist U. Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. Science. 1995;267(5203):1485–8.
4. Traenckner EB, Pahl HL, Henkel T, Schmidt KN, Wilk S, Baeuerle PA. Phosphorylation of human I kappa B-alpha on serines 32 and 36 controls I kappa B-alpha proteolysis and NF-kappa B activation in response to diverse stimuli. EMBO J. 1995;14(12):2876–83.
5. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. Nat Immunol. 2001;2(3):223–8. doi:10.1038/85277.
6. Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. Nat Genet. 2001;27(3):277–85. doi:10.1038/85837.
7. Zonana J, Elder ME, Schneider LC, Orlow SJ, Moss C, Golabi M, et al. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). Am J Hum Genet. 2000;67(6):1555–62. doi:10.1086/316914.
8. Courtois G, Smahi A, Reichenbach J, Doffinger R, Cancrini C, Bonnet M, et al. A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. J Clin Invest. 2003;112(7):1108–15. doi:10.1172/JCI18714.
9. Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ, et al. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. J Allergy Clin Immunol. 2008;122(6):1169 e16–77 e16. doi:10.1016/j.jaci.2008.08.018.
10. Ohnishi H, Miyata R, Suzuki T, Nose T, Kubota K, Kato Z, et al. A rapid screening method to detect autosomal-dominant ectodermal dysplasia with immune deficiency syndrome. The Journal of allergy and clinical immunology. 2012;129(2):578–80. doi:10.1016/j.jaci.2011.09.042.
11. Lopez-Granados E, Keenan JE, Kinney MC, Leo H, Jain N, Ma CA, et al. A novel mutation in NFKBIA/IKBA results in a degradation-resistant N-truncated protein and is associated with ectodermal dysplasia with immunodeficiency. Hum Mutat. 2008;29(6):861–8. doi:10.1002/humu.20740.
12. McDonald DR, Mooster JL, Reddy M, Bawle E, Secord E, Geha RS. Heterozygous N-terminal deletion of IkappaBalpha results in functional nuclear factor kappaB haploinsufficiency, ectodermal dysplasia, and immune deficiency. The Journal of allergy and clinical immunology. 2007;120(4):900–7. doi:10.1016/j.jaci.2007.08.035.
13. Janssen R, van Wengen A, Hoeve MA, ten Dam M, van der Burg M, van Dongen J, et al. The same IkappaBalpha mutation in two related individuals leads to completely different clinical syndromes. J Exp Med. 2004;200(5):559–68. doi:10.1084/jem.20040773.
14. Nishikomori R, Akutagawa H, Maruyama K, Nakata-Hizume M, Ohmori K, Mizuno K, et al. X-linked ectodermal dysplasia and immunodeficiency caused by reversion mosaicism of NEMO reveals a critical role for NEMO in human T-cell development and/or survival. Blood. 2004;103(12):4565–72. doi:10.1182/blood-2003-10-3655.

15. Kawai T, Nishikomori R, Izawa K, Murata Y, Tanaka N, Sakai H, et al. Frequent somatic mosaicism of NEMO in T cells of patients with X-linked anhidrotic ectodermal dysplasia with immunodeficiency. Blood. 2012;119(23):5458–66. doi:10.1182/blood-2011-05-354167.
16. Nishikori M, Maesako Y, Ueda C, Kurata M, Uchiyama T, Ohno H. High-level expression of BCL3 differentiates t(2;5)(p23;q35)-positive anaplastic large cell lymphoma from Hodgkin disease. Blood. 2003;101(7):2789–96. doi:10.1182/blood-2002-08-2464.
17. Fish JD, Duerst RE, Gelfand EW, Orange JS, Bunin N. Challenges in the use of allogeneic hematopoietic SCT for ectodermal dysplasia with immune deficiency. Bone Marrow Transplant. 2009;43(3):217–21. doi:10.1038/bmt.2008.308.
18. Dupuis-Girod S, Cancrini C, Le Deist F, Palma P, Bodemer C, Puel A, et al. Successful allogeneic hemopoietic stem cell transplantation in a child who had anhidrotic ectodermal dysplasia with immunodeficiency. Pediatrics. 2006;118(1):e205–11. doi:10.1542/peds.2005-2661.
19. Permaul P, Narla A, Hornick JL, Pai SY. Allogeneic hematopoietic stem cell transplantation for X-linked ectodermal dysplasia and immunodeficiency: case report and review of outcomes. Immunol Res. 2009;44(1–3):89–98. doi:10.1007/s12026-008-8085-2.

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MEFV Variants in Patients with PFAPA Syndrome in Japan

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Abstract: *Background*: The pathogenesis of PFAPA (periodic fever, aphthous stomatitis, pharyngitis, adenitis) syndrome is unknown as yet. In order to understand whether genes implicated in other auto-inflammatory diseases might be involved in the pathogenesis of PFAPA, all variants in the genes causing familial Mediterranean fever (FMF), tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), and Hyper IgD syndrome were analyzed in children with PFAPA.

Patients and Methods: All variants in *MEFV*, *TNFRSF1A*, and *MVK* were analyzed in 20 patients with PFAPA. PFAPA were diagnosed by previous published criteria. The findings of all analyses in PFAPA patients were compared with those of unaffected normal subjects (n=62).

Results: In the 13 children of 20 with PFAPA, the heterozygous variants of *MEFV* (5 patients: *E148Q-L110P*, 2 patients: *E148Q*, 1 patient: *E148Q-L110P/E148Q*, 1 patient: *E148Q-P369S-R408Q-E84K*, 1 patient: *E148Q-L110P-P369S-A408G*, 1 patient: *R202Q*, 1 patient: *P115R*) were found. No variants belonging to *TNFRSF1A* or MVK were detected in children with PFAPA. The frequency of the *E148Q-L110P* variants in children with PFAPA was significantly higher than that observed in unaffected normal subjects (7/20 versus 8/62). The duration of the episodes of illness in PFAPA children with *MEFV* variants was shorter than that of patients without variants.

Conclusion: Genes involved in the development and progression of *MEFV* may affect the incidence and the phenotype of PFAPA in children.

Keywords: PFAPA, *MEFV*, FMF, Variant, Japanese.

INTRODUCTION

In 1987 [1], Marshall firstly described, the PFAPA (periodic fever, aphthous stomatitis, pharyngitis and adenitis) syndrome, which is characterized by recurrent episodes of fever associated with cervical adenitis, pharyngitis and aphthous stomatitis. The prognosis of this disease has been reported to be better than that of another autoinflammatory diseases [2]. Corticosteroids are effective in controlling episodes of illness in PFAPA, but they do not cure the ailments or prevent recurrence of the symptoms of this syndrome [3]. Interventions like tonsillectomy and administration of H_2 blockers have been reported to be partially effective for prophylaxis [3]. However the complete pathogenesis of PFAPA is unknown yet, and hence the therapeutic regimens have not yet been established for PFAPA [4].

Familial Mediterranean fever (FMF) is a recessively inherited disorder characterized by acute attacks of fever, and serositis usually lasting for 1–3 days. FMF is caused by mutations in the ME diterranean FeVer gene (*MEFV),* which encodes the protein pyrin (marenostrin)1[5,6]. Colchicine has been shown to be effective for prophylaxis in only 90% of the patients with FMF [7,8].

Recent studies have described that heterozygous variants of the *MEFV* gene were found in patients with PFAPA [9-11]. However, it still remains unclear whether these variants are the causative factors of PFAPA.

The purpose of this study was to understand whether heterozygous variants of *MEFV* may be associated with the onset of PFAPA. We have also tried to understand whether these mutations act as accessory factors and modify the phenotype of patients with PFAPA.

PATIENTS AND METHODS

Twenty children with frequent PFAPA episodes who visited our pediatric outpatient clinic were consecutively selected over a 5-year period (from January 2005 to January 2010). The diagnosis of PFAPA syndrome was established using previously established criteria [1,3,9]. These criteria include recurring fevers associated with exudative tonsillitis, negative throat culture, and possibly, aphthous stomatitis and cervical lymphadenopathy. The additional clinical criteria included completely asymptomatic intervals between the episodes, normal growth and development and exclusion of

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FMF, Behcet' s disease, and cyclic neutropenia. Oral low dose of prednisolone (0.3-0.5mg/kg/dose, 1 or 2 doses per day) was effective on all enrolled patients. *MEFV*, mevalonate kinase (*MVK*), and tumor necrosis factor receptor superfamily, member 1A (*TNFRSF1A*) genes of all enrolled patients were sequenced. After obtaining a written informed consent approved by the Institutional Review Board of Kyoto University, peripheral blood was collected from all patients, and, if needed, their family members. Genomic DNA was extracted, and all the exons including exon-intron junctions of the *MVK, MEFV,* and *TNFRSF1A* genes were amplified by polymerase chain reaction and then sequenced using the ABI3130.

The results are shown as a mean ± SD or proportion, as appropriate. Differences between the groups in discrete variables were evaluated using Fisher's exact test at 5% significance.

Two-sided *P* values were adjusted for multiplicity using Hochberg's method.

Comparisons of continuous variables were done using unpaired Student's *t*-test. All *P* values given are 2-sided. *P* values less than 0.05 were considered significant. Statistical calculation was conducted by SAS version 9.1.3.

RESULTS

Twenty patients (9 boys, 11 girls) diagnosed with PFAPA were followed up in our clinic. Thirteen of these patients had a single *MEFV* (M^+ group). No variant of *TNFRSF1A* and *MVK* was detected in all patients. The genotypes of the *MEFV* gene in the 13 patients are seen in Table **1**. The most common *MEFV* variant patterns seen were *E148Q-L110P* (5 patients) and *E148Q* (2 patients). One patient was homozygous of *E148Q* and heterozygous of *L110P* of *MEFV*. One patient was heterozygous of *E148Q-P369S-R408Q*. One patient was heterozygous for *E148Q-P369S-R408Q-E84K*. One patient had *E148Q-L110P-P369S-R408Q*. The minor variants, *P115R* and *R202Q*, were detected in 2 patients. More than 2 *MEFV variants* were on 1 allele in all PFAPA patients. In 7 patients, no *MEFV* mutations were found. The allele frequencies of *E148Q*, *L110P*, *P369S, R408Q* and G304R in 20 PFAPA patients were 0.3, 0.175, 0.075, 0.075 and 0, respectively (Table **3**).

Table 1. The Genotypes of *MEFV* Genes of 13 Patients with PFAPA

MEFV Variant	No. of Patients
E148Q-L110P	5
E148Q	2
E148Q-L110P/E148	1
E148Q-P369S-R408Q	1
E148Q-P369S-R408Q-E84K	1
E148Q -L110P-P369S-R408Q	1
R202Q	1
P115R	1

Clinical and laboratory data were compared between *MEFV* positive group (n=13) and negative group (n=7) and are presented in Table **2**. Patients carrying an *MEFV* variant showed shorter duration of episodes of illness than patients without variants (3.6±0.86 days versus 5.3±1.89 days,

Table 2. Clinical Characteristics of PFAPA Patients with Variants in the *MEFV* Gene Compared with those of PFAPA Patients without *MEFV* Variants

	Patients with *MEFV* Variants (n=13)	Patients without *MEFV* Variants (n=7)	*P* Value
Age at onset (years)	2.8±1.9	3.2±1.9	NS
Age at Diagnosis (years)	4.3±2.2	4.9±1.9	NS
Male: female ratio	5/8	4/3	NS
Family history of PFAPA	4/9	3/4	NS
Attack duration (days)	3.6±0.86	5.3±1.89	*P*=0.0174
Interval between attacks (weeks)	4.9±1.59	5.5±0.96	NS
Cyclic periodic attacks	5/13	4/7	NS
Pharyngitis	13/13	7/7	NS
Aphthae	7/13	5/7	NS
Enlarged tonsillitis	13/13	7/7	NS
Abdominal pains	1/13	2/7	NS
Musculoskeletal Pains	1/13	2/7	NS
Headaches	4/13	5/7	NS
WBC/μL	143±41	142±41	NS
ESR mm/h	87±23	72±14	NS
CRP levels mg/dL	6.52±3.53	5.87±2.85	NS

NS: not significant.