



FULL LENGTH ARTICLE

A heterozygous N-terminal truncation mutation of *NFKB1A* results in an impaired NF- κ B dependent inflammatory response

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Abstract Germline heterozygous gain-of-function (GOF) mutation of *NFKB1A*, encoding I κ B α , would affect the activation of NF- κ B pathway and cause an autosomal dominant (AD) form of anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID). Here we reported a Chinese patient with a heterozygous N-terminal truncation mutation of *NFKB1A*/I κ B α . She presented recurrent fever, infectious pneumonia and chronic diarrhea with EDA-ID. Impaired NF- κ B translocation and IL1R and TLR4 pathway activation were revealed in this patient. The findings suggested that the truncation mutation of I κ B α caused medium impaired of activation of NF- κ B but the early death. Furthermore, we reviewed all the reported patients with *NFKB1A* mutation to learn more about this disease.

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Introduction

Nuclear factor kappa B (NF- κ B) plays a vital role in innate and adaptive immunity, reflecting in the signal transduction in response to external stimuli as well as to internal stimuli within the cell.^{1,2} I κ B α , encoded by *NFKBIA*, is one of the inhibitors of the NF- κ B activation and binds to the NF- κ B proteins p65 (RelA) and p50. Various stimuli could cause activation of the I κ B kinase (IKK) complex, which phosphorylates I κ B α on serines 32 and 36, leading to ubiquitination of lysines 21 and 22 and the subsequent degradation of I κ B α .² Mutation of *NFKBIA* causes impaired I κ B α degradation and leads to an autosomal dominant (AD) form of anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID), characterized by sparse hair, conical teeth, reduced number of sweat glands, and susceptibility to severe infections.³ To date, 19 patients have been reported, including 15 point mutations at or adjacent to the S32 and S36 phosphorylation sites, 1 mosaicism point mutation at S32 site and 3 truncation mutations which introduce a premature stop codon and give rise to N-terminally truncated I κ B α proteins through re-initiation of translation at downstream ATG sites.^{4–7}

In this study, we reported the clinical and immunological features of a Chinese patient with a truncation mutation of *NFKBIA* with EDA-ID. Since the disease with germline GOF *NFKBIA* mutation is a kind of rare primary immunodeficiency disease (PID), the clinical phenotypes are variable and the pathogenesis is not fully clear, we summarized the clinical, molecular and cellular phenotypes of the 20 reported patients since 2003 to recognize it better and help doctors to diagnose it as early as possible.

Materials and methods

Patient

The patient enrolled in this study was a 4-month-old girl born in a nonconsanguineous family. Clinical data and blood were collected when she first visited the Children's Hospital of Chongqing Medical University in January 2020. All research practices were approved by the Medical Ethics Committee of the Children's Hospital of Chongqing Medical University (approval number: 030/2013). Informed consent was obtained from guardians.

Genetic studies and conservative analysis of *NFKBIA*

Whole blood samples were sent to MyGenostics (Beijing, China) and subjected to medical whole-exome sequencing. Mutation in the *NFKBIA* gene was verified by Sanger

sequencing. The conservation analysis of *NFKBIA* gene was analyzed on the weblogo.berkeley.edu.

Cell preparation and culture conditions

Peripheral blood mononuclear cells (PBMCs) were obtained from the patient and healthy adult volunteers by centrifugation of heparinized blood over Ficoll-Hypaque density gradient lymphocyte separation medium (GE Healthcare), with standard techniques. To measure IL-1 β , IL-18, IL-6, IL-8(CXCL8), IL-12p70, MCP-1(CCL2), IFN- γ and TNF- α concentrations, 1×10^6 PBMCs were cultured in RPMI 1640 complete medium (1 ml) for 36 h. The following reagents were used: LPS from *E. coli* 01127:B8 (1 μ g/ml; Sigma), human interferon (IFN)- γ (1000 U/ml; Peprotech), IL-1 β (10 ng/ml; Peprotech). After 36 h, supernatants were collected. Cytokines concentrations were determined by Multi-Analyte Flow Assay Kit (Human Inflammation Panel(13-plex), Biolegend, 740118) according to the manufacturer's instructions. Data were collected with a FACS Canto II flow cytometer (BD Bioscience) and analyzed using LEGENDplexv8.0 software (Biolegend).

Immunofluorescence

PBMCs of the patient and healthy donor were stimulated with 100 ng/ml LPS at different time intervals (0, 15, and 30 min). Then incubated by primary antibody P-NF- κ B p65 (Cell Signaling Technology), secondary antibody donkey-anti-rabbit AF488 (Abcam) and DAPI (Beyotime) at the room temperature. Then detected and analyzed by confocal microscope (Nikon C2 plus) and NIS-Elements BR software.

Statistical analysis

All statistical analyses were conducted in GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, CA).

Results

Clinical manifestations of patient

A female Chinese infant of two healthy, non-consanguineous parents was born at term after an uncomplicated pregnancy. After birth, she was hospitalized for intrauterine infection and improved after treatment. She presented with sparse hair and no eyebrows with normal nail growth. She was fed initially with breast milk alone and then in combination with artificial milk. She received BCG and Hepatitis B vaccines at birth without complications. At 2 months of age, she began to have recurrent fever and severe infectious pneumonia (*Acinetobacter baumannii*,

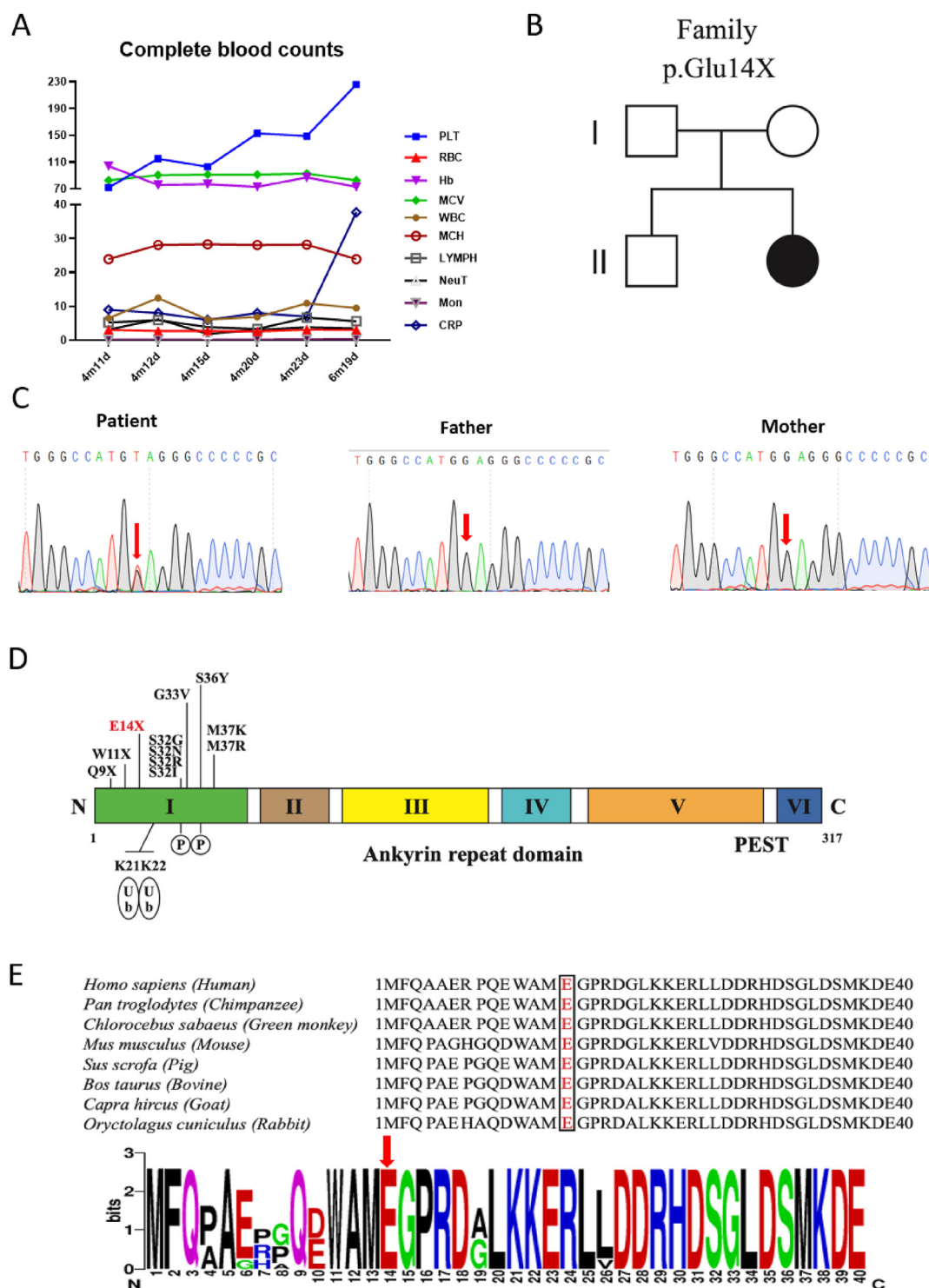


Figure 1 Clinical and genetic characterization of the patient. **(A)** The complete blood counts showed increased CRP (dark blue) and low hemoglobin levels (purple). The normal reference ranges are indicated as following: WBC $4\text{--}12 \times 10^9/\text{L}$; RBC $4.0\text{--}5.3 \times 10^{12}/\text{L}$; PLT $100\text{--}380 \times 10^9/\text{L}$; Hb $110\text{--}150 \text{ g/L}$; MCV $80\text{--}100 \text{ fL}$; MCH $26\text{--}32 \text{ pg}$; CRP $<8 \text{ mg/L}$. **(B)** Family pedigrees of the patient with variant in *NFKB1A*. **(C)** Sequence analysis of the *NFKB1A* gene. The *de novo* mutation, c.40G>T, in the *NFKB1A* gene is indicated by the arrow. **(D)** Schematic domain structure of *NFKB1A*. All the reported mutations are in the upper part of the protein. The different domains are indicated in the lower part of the protein. **(E)** Evolutionary conservation of the site E14 in *NFKB1A*. Amino acid sequence of *NFKB1A* flanking E14 was aligned on the weblogo.berkeley.edu across various species.

enterococcus faecalis, legionella, Klebsiella and fungi). By that time, she had evident growth retardation. During 2–4 months of age, the lab examination suggested that the platelet and hemoglobin were progressive reduction. Her hepatosplenomegaly became more obvious. At 4 months of age, she started suffering from gastrointestinal problem with feeding intolerance, chronic diarrhea and bloody stool. She was also noted to be heat intolerant and unable to sweat. The hemoglobin was persistent below the normal (Fig. 1A). The patient's serum IgG was normal, but the levels of IgA and IgM were below normal (Table 1). Cell counts of CD3⁺CD4⁺ T, NK, CD19⁺ B and the rate of CD4/CD8 were below normal, while cell count of CD3⁺CD8⁺ T was higher than normal (Table 2). After a combination of antibiotics, antifungal, anti-tuberculous and regular intravenous immunoglobulin (IVIG) treatment, the patient did not present significant infectious manifestations but still had gastrointestinal symptoms. At 6.5 months of age, she presented depressed spirit and less moving, about 15 days later, she unfortunately died of cardiac failure.

Genetic analysis revealed NFKBIA mutation in patient

Based on the clinical symptoms and laboratory results, primary immunodeficiency was considered for the patient and whole-exome sequencing was performed. A

heterozygous N-terminal truncation mutation in NFKBIA was found in this patient but not in her parents (Fig. 1B, C). The conservation analysis suggested that the site of 14E in NFKBIA is highly conserved across species (Fig. 1E).

Patient had impaired NF-κB dependent cytokine production

Because NFKBIA is an inhibitor of NF-κB, we evaluated whether the cells of the patient responded normally to the stimulants that active NF-κB. We examined cytokines produced by PBMCs after stimulation of TLR4 and IL-1 receptor as described by Lopes-Grandos et al and Yamamoto et al.^{8,9} The patient's PBMCs showed little response to LPS and IFN-γ in production of IL1β, IL-6, IL-8, IL-12p70, TNF-α, MCP-1 and IFN-γ, although we did detect a low response to IL1β in production of IL-18, IL-6, IL-8, TNF-α, MCP-1 (Fig. 2). These data indicated an impaired signal transduction downstream of the TLR receptor and IL-1 receptor families.

p.E14X leads to reduced nuclear translocation of NF-κB

Since the p.E14X mutation prevents IκB-α degradation, keeping the nuclear localization signals on the NF-κB subunits masked,⁷ we also detected the phosphorylation of RelA (p65) in response to LPS stimulated PBMCs at different time intervals. We found that the level of the patient's phosphorylation of p65 (p-P65) was below the healthy control (Fig. 3A, B). The result showed that the N-terminal truncation mutation in NFKBIA could lead to reduced nuclear translocation of NF-κB.

Discussion

The IκB (inhibitor of NF-κB) family of proteins includes IκBα, IκBβ, and IκBε.¹⁰ In resting cells, NF-κB proteins, including p65 (RelA), p105/p50, p100/p52, c-Rel, and RelB,^{11,12} are retained in the cytoplasm by the IκB family of proteins, which could be activated by a wide variety of cell-surface receptors and finally result in NF-κB activation. Stimuli, including proinflammatory cytokines (TNF-α, IL-1) and Toll-like receptor (TLR) ligands, cause activation of the IκB kinase (IKK) complex, which phosphorylates IκBα on serines 32 and 36, leading to ubiquitination of lysines 21 and 22 and the subsequent degradation of IκBα.² Serines 32 and 36, as well as lysines 21 and 22, are contained within an N-terminal 73-amino-acid sequence and designated the signal response domain because this region regulates the degradation of IκBα. The N-terminal sequence of IκBα is highly conserved across species, especially the six amino acid degron (DSGLDS) and the two serine residues located at positions 32 and 36 in humans.⁴ As a result, both of the point mutation and truncation mutation which relate to these sites could impair the degradation of IκBα, leading to the NF-κB translocation impairment.

In this work we described a patient with heterozygous N-terminal truncation mutation. Our patient had early onset age and presented with typical clinical features of EDA-ID, including sparse hair, susceptibility to severe infections and

Table 1 Immunoglobulin and complement.

	Result	Reference Range
IgG (g/L)	9.96	2.86–16.8
IgA (g/L)	0.097	0.1–1.29
IgM (g/L)	0.151	0.21–1.92
IgE (IU/ml)	<5.00	0–165
C3 (g/L)	1.54	0.74–1.86
C4 (g/L)	0.54	0.11–0.61
ESR (mm/h)	19	0–15

Table 2 Lymphocyte classification.

TBNK	Result	Reference Range
CD3 ⁺ %	92.76	39–73
CD3 ⁺ CD8 ⁺ %	70.14	11–32
CD3 ⁺ CD4 ⁺ %	21.87	25–50
CD3 ⁺ CD4 ⁺ CD8 ⁺ %	0.26	
CD3 ⁺ CD4 ⁺ CD8 ⁺ %	1.01	
NK%	2.20	3–16
CD19%	5.03	7–41
CD4/CD8	0.31	0.98–1.94
CD3 ⁺ # (cell/μl)	3694.10	1400–8000
CD3 ⁺ CD8 ⁺ # (cell/μl)	2793.31	400–2300
CD3 ⁺ CD4 ⁺ # (cell/μl)	870.86	900–5500
CD3 ⁺ CD4 ⁺ CD8 ⁺ # (cell/μl)	10.36	
NK# (cell/μl)	87.49	100–1400
CD19 ⁺ # (cell/μl)	200.30	600–3100
CD45 ⁺ # (cell/μl)	3982.47	

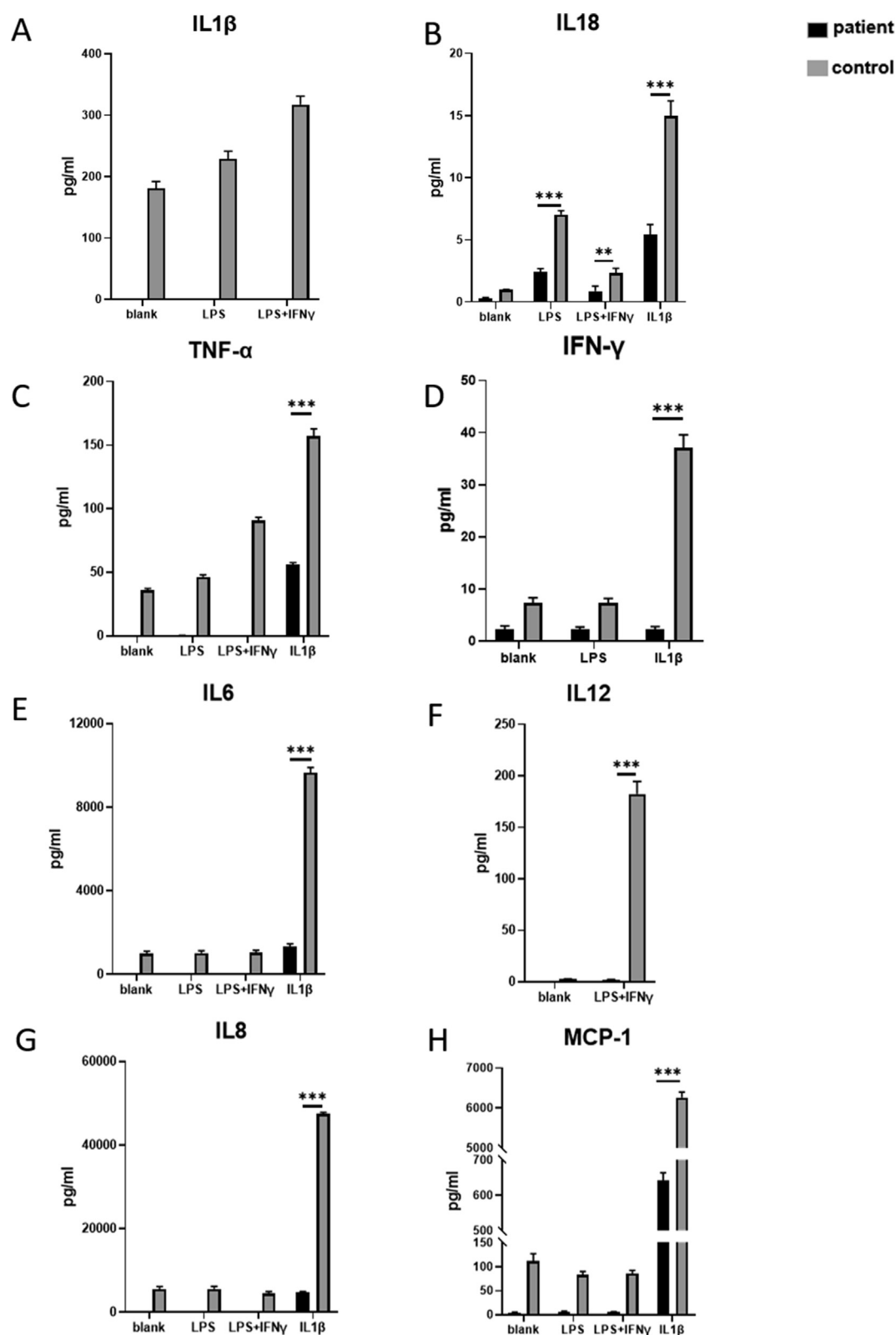


Figure 2 Impaired NF- κ B dependent cytokine production in response to different stimuli by patient's PBMCs. 1×10^6 PBMCs (control, grey bars; patient, black bars) were incubated with medium, LPS (1 μ g/ml), IFN- γ (1000 U/ml), IL-1 β (10 ng/ml); IL- β (A), IL-18 (B), TNF- α (C), IFN- γ (D), IL-6 (E), IL-12 (F), IL-8 (CXCL8) (G) and MCP-1 (CCL2) (H) were quantitated 36 h after stimulation respectively. Data were representative of three independent experiments. ** $P < 0.01$, *** $P < 0.001$.

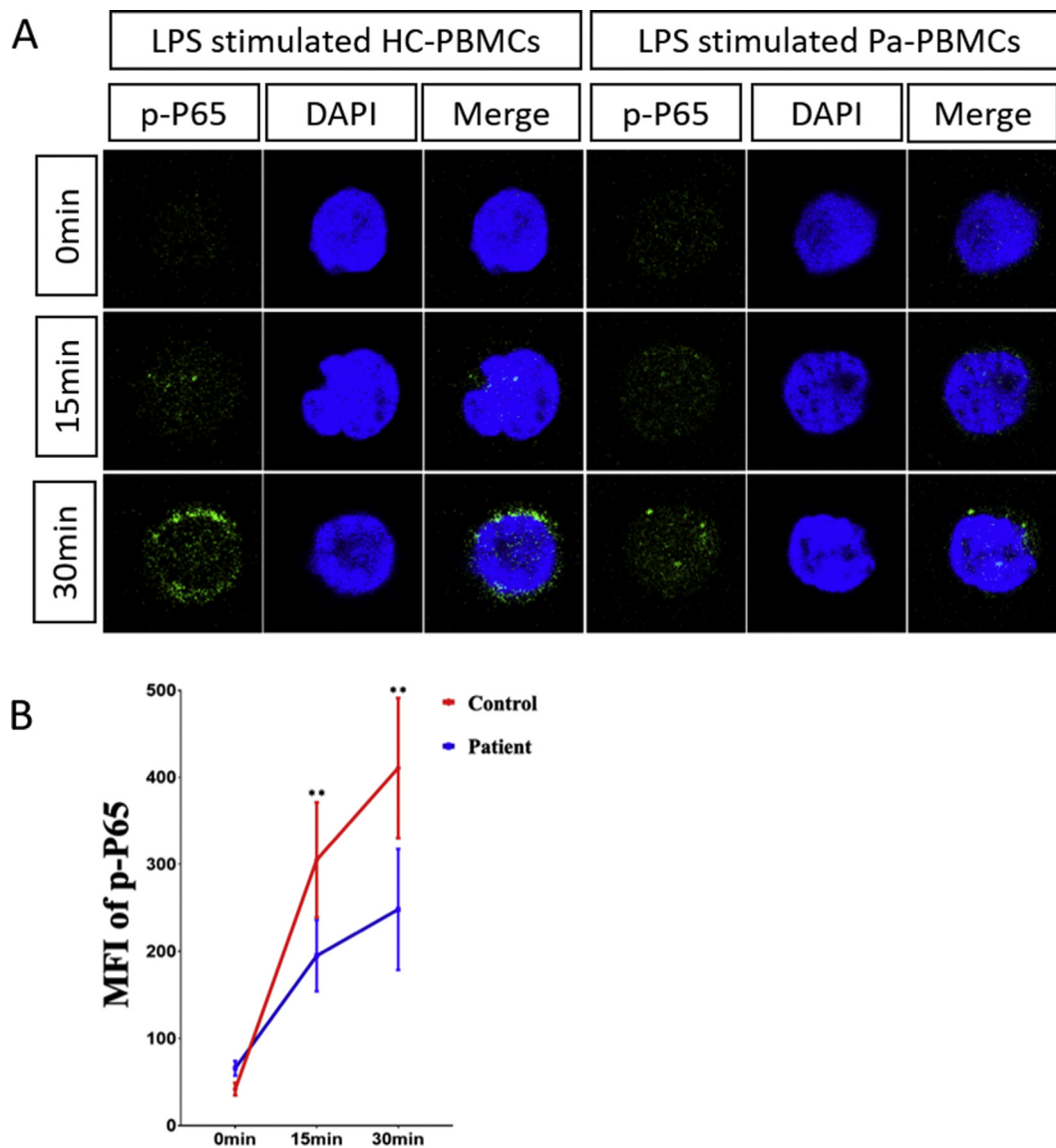


Figure 3 Impaired phosphorylation of RelA (p65) in response to LPS by patient's PBMCs. PBMCs were stimulated with 100 ng/ml LPS at different time intervals (0, 15 and 30 min). (A) Average mean fluorescence intensity (MFI) of p-P65 was analyzed. (B) Shown are representative images and average MFI ($\pm$ SD) from >30 cells for each time interval. $**P < 0.01$.

failure to thrive, however, she was not detected the development of sweat glands. Her high level of CD3⁺CD8⁺ T cell, low levels of CD19⁺ B cell, CD3⁺CD4⁺ T cell and CD4/CD8 suggested that the patient had evident immunodeficiency. She had low level of IgM and IgA with normal IgG, which was different with other patients.⁴⁻⁷ Since truncation mutation of I κ B α could give rise to re-initiation of translation at downstream ATG sites, severe disease and great impairment of NF- κ B activation are more significant in I κ B α point mutants versus truncation mutants.⁷ The patient showed absent or low response to TLR4 and IL-1R in production of inflammatory cytokines and chemokines, and presented with medium impaired phosphorylation of p65. These data suggested that the activation of NF- κ B in this patient was not completely damaged, which was consistent with reported patient (P5: E14X, Table 3)⁸ and other truncation mutation patients (P4: W11X, P6: Q9X, Table 3).^{13,14} However, our patient died at 7 months of age before hematopoietic stem cell

transplantation (HSCT), much earlier than the other three truncation mutation patients. We supposed that the early death was caused by her chronic gastrointestinal syndrome and severe anemia without support treatment because of some special reasons.

Furthermore, we reviewed all the patients with mutation in *NFKBIA* and summarized their clinical features (Table 3), molecular and cellular phenotypes (Table 4). Including our patient, there have been 4 patients with heterozygous N-terminal truncation mutation of *NFKBIA* (P4, P5, P6 and P20). All of them had early onset age and similar infection manifestation with EDA and ID.^{8,13,14} P4 (W11X) was alive over 22 years old without HSCT. P6 (Q9X) was alive at 7 years old after HSCT. However, P5 died after HSCT because of pyogenic bacteria sepsis at 1 year old. These 4 patients had impaired IL-1R/TLR pathway activation, while P4 and P5 were confirmed to have impaired I κ B α degradation and impaired NF- κ B translocation. Pathogenicity of these

Table 3 Genetic and clinical features of patients with heterozygous *NFKB1A* mutations.

Patient	Gender	Origin	Year of birth	Age of onset	Variant	Inheritance	Infection Manifestations
P1	M	Italian	nr	2 months	S32I	<i>De novo</i>	Recurrent LRTI: <i>P. aeruginosa</i> , <i>Klebsiella</i> , <i>Serratia</i> , <i>S. aureus</i> CMC
P2	M	Dutch	nr	2 years	S32Im	<i>De novo</i>	enteritis (<i>Salmonella typhimurium</i>) <i>S. typhimurium</i> infection persisted with recurrent manifestations in psoas muscle, pleural cavity, pericardial fluid, and ribs
P3	M	Dutch	nr	2 months	S32I	Inherited from his father	Meningitis: β -hemolytic group A <i>Streptococcus</i> : sepsis Respiratory infection pneumonia <i>Pneumocystis jirovecii</i> , mild CMC Recurrent pneumonia
P4	F	American	nr	Birth	W11X	Mother: WT; Father: nr	Pneumonia (parainfluenza virus and <i>Pneumocystis carinii</i>). recurrent episodes of bacteremia oral candidiasis. Pyogenic bacteria sepsis, CMC Bacterial: pneumonia, respiratory syncytial virus. Bronchiolitis, acute otitis media, urinary tract infection. Cytomegalovirus: hepatitis, Rotavirus: enteritis. Bronchiolitis with respiratory syncytial virus BCG skin infection Haemophilus influenza: pneumonia, CMC Recurrent LRTI Sepsis: <i>Klebsiella pneumoniae</i> , <i>Candida parapsilosis</i> , <i>Stenotrophomonas maltophilia</i> , osteomyelitis of skull and limb, CMC LRTI: <i>P. aeruginosa</i> : bronchiectasis and sinusitis <i>Mycobacterium tuberculosis</i> : abdominal lymphadenopathy M. abscessus: septic arthritis of the knee, osteomyelitis Urinary tract infection: <i>K. pneumoniae</i> <i>Salmonella enteritidis</i> : osteomyelitis, hematochezia <i>Candida</i> : esophagitis <i>Mycobacterium malmoense</i> : blood and skin Sapovirus and norovirus: stool <i>S. aureus</i> sepsis, CMC, Recurrent pneumonia <i>S. aureus</i> and <i>P. aeruginosa</i> sepsis
P5	M	American	nr	1 month	E14X	<i>De novo</i>	
P6	M	Japanese	nr	1 month	Q9X	nr	Pneumonia (parainfluenza virus and <i>Pneumocystis carinii</i>). recurrent episodes of bacteremia oral candidiasis. Pyogenic bacteria sepsis, CMC Bacterial: pneumonia, respiratory syncytial virus. Bronchiolitis, acute otitis media, urinary tract infection. Cytomegalovirus: hepatitis, Rotavirus: enteritis. Bronchiolitis with respiratory syncytial virus BCG skin infection Haemophilus influenza: pneumonia, CMC Recurrent LRTI Sepsis: <i>Klebsiella pneumoniae</i> , <i>Candida parapsilosis</i> , <i>Stenotrophomonas maltophilia</i> , osteomyelitis of skull and limb, CMC LRTI: <i>P. aeruginosa</i> : bronchiectasis and sinusitis <i>Mycobacterium tuberculosis</i> : abdominal lymphadenopathy M. abscessus: septic arthritis of the knee, osteomyelitis Urinary tract infection: <i>K. pneumoniae</i> <i>Salmonella enteritidis</i> : osteomyelitis, hematochezia <i>Candida</i> : esophagitis <i>Mycobacterium malmoense</i> : blood and skin Sapovirus and norovirus: stool <i>S. aureus</i> sepsis, CMC, Recurrent pneumonia <i>S. aureus</i> and <i>P. aeruginosa</i> sepsis
P7	M	Japanese	2007	4 months	S36Y	<i>De novo</i>	
P8	M	German	nr	6 months	M37K	Mother: WT Father: nr	
P9	F	Italian	2012	5 months	M37R	nr	
P10	F	Chinese	2004	1 month	S36Y	<i>De novo</i>	Pneumonia (parainfluenza virus and <i>Pneumocystis carinii</i>). recurrent episodes of bacteremia oral candidiasis. Pyogenic bacteria sepsis, CMC Bacterial: pneumonia, respiratory syncytial virus. Bronchiolitis, acute otitis media, urinary tract infection. Cytomegalovirus: hepatitis, Rotavirus: enteritis. Bronchiolitis with respiratory syncytial virus BCG skin infection Haemophilus influenza: pneumonia, CMC Recurrent LRTI Sepsis: <i>Klebsiella pneumoniae</i> , <i>Candida parapsilosis</i> , <i>Stenotrophomonas maltophilia</i> , osteomyelitis of skull and limb, CMC LRTI: <i>P. aeruginosa</i> : bronchiectasis and sinusitis <i>Mycobacterium tuberculosis</i> : abdominal lymphadenopathy M. abscessus: septic arthritis of the knee, osteomyelitis Urinary tract infection: <i>K. pneumoniae</i> <i>Salmonella enteritidis</i> : osteomyelitis, hematochezia <i>Candida</i> : esophagitis <i>Mycobacterium malmoense</i> : blood and skin Sapovirus and norovirus: stool <i>S. aureus</i> sepsis, CMC, Recurrent pneumonia <i>S. aureus</i> and <i>P. aeruginosa</i> sepsis
P11	F	Caucasian / Thai	2011	20 months	S32G	<i>De novo</i>	
P12	M	Japanese	nr	2 months	S32R	<i>De novo</i>	
P13	nr	Japanese	nr	Birth	S32N	<i>De novo</i>	<i>S. aureus</i> and <i>P. aeruginosa</i> sepsis

Table 3 (continued)

Patient	Gender	Origin	Year of birth	Age of onset	Variant	Inheritance	Infection Manifestations
P14	nr	nr	2007	nr	S32I	?	Recurrent infections
P15	M	nr	2007	nr	G33V	?	Recurrent infections
P16	M	Turkish	1982	early childhood	S36A	<i>De novo</i>	Recurrent gastrointestinal infections (Shigellosis, <i>C. jejuni</i>), recurrent upper respiratory tract infections (bronchitis, sinusitis, otitis media), recurrent pneumonias (<i>S. pneumoniae</i> , <i>H. influenzae</i>), bronchiectasis with chronic mucoid <i>Pseudomonas aeruginosa</i> infection meningitis (<i>N. meningitidis</i>), CNS tuberculosis with brain abscess, verruca vulgaris (HPV 9 and 57)
P17	F	Turkish	2010	early childhood	S36A	Inherited from her father	Recurrent upper respiratory tract infections (bronchitis, otitis media), recurrent pneumonias, bronchiectasis, Verruca vulgaris
P18	F	Turkish	2015	early childhood	S36A	Inherited from her father	Recurrent upper respiratory tract infections (bronchitis, otitis media), two pneumonias within two years
P19	M	Spain	2012	Birth	D31N	<i>De novo</i>	pustular deficiency of interleukin-1 receptor antagonist (DIRA)
P20*	F	Chinese	2019	2 months	E14X	<i>De novo</i>	Recurrent bronchopneumonitis (Gram $\pm$ pyogenic)

Note: nr not report; P2 is father of P3; P16 is father of P17 and P18; P20* is our patient.

Patient	Autoinflammatory Manifestations	EDA	ID	Growth Dev.	Other Phenotype	Treatment	Outcome	Ref.
P1	fever, sterile eriostritis, pustular skin, rash, and soft tissue swelling	Yes	Yes	Retardation	chronic diarrhea; hepatosplenomegaly	HSCT IVIG	Alive at 21 years (2017)	16,17
P2	fever, typical rash, and JIA	No	Yes	No	chronic diarrhea	nr	Alive at > 20 years (2004)	15
P3	fever	Yes	Yes	Retardation	chronic diarrhea	HSCT antibiotics cotrimoxazole	Dead (2005)	15
P4	fever, skin rash,	Yes	Yes	nr	nr	IVIG corticosteroid antibiotics	Alive at 22 years (2016)	13
P5	fever	Yes	Yes	Retardation	feeding intolerance, frequent episodes of diarrhea	HSCT trimethoprim-sulfamethoxazole	Dead (1 year, 2008)	8
P6	systemic inflammation	Yes	Yes	nr	chronic diarrhea	HSCT	Alive at 7 years (2016)	14
P7	systemic inflammation	Yes	Yes	Retardation	Gastroenteritis, chronic diarrhea	HSCT	Dead (2013)	18
P8	fever	Yes	Yes	Retardation	chronic diarrhea	HSCT	Dead (2013)	19
P9	fever	Yes	Yes	nr	chronic diarrhea	nr	Dead (2013)	20
P10	fever	No	Yes	nr	nr	Subcutaneous rIFN- γ (50 μ g/m ²), cotrimoxazole, IVIG, antituberculosis treatment	Alive at 9 years (2015)	21

(continued on next page)

Table 3 (continued)

Patient	Autoinflammatory Manifestations	EDA	ID	Growth Dev.	Other Phenotype	Treatment	Outcome	Ref.
P11	fever	Yes	Yes	No	chronic diarrhea	HSCT IVIG	Alive at 6 years (2017)	22
P12	fever, skin erythema, systemic inflammation	Yes	Yes	nr	nr	HSCT	Dead at 2 years	23
P13	fever	Yes	Yes	nr	bloody stool, inflammatory bowel disease; recurrent intracranial hemorrhage; difficulty in hemostasis	HSCT	Dead at 1.5 years	23
P14	fever	Yes	Yes	nr	nr	HSCT IVIG	Alive at 10 years (2017)	7
P15	fever	Yes	Yes	nr	nr	HSCT IVIG	Alive at 10 years (2017)	7
P16	fever, JRA	No	Yes	No	Warts phimosis	IVIG chloroquine methotrexate azathioprine azithromycin prophylaxis gentamycin	Alive at 37 years (2019)	5
P17	No	No	Yes	No	No	Co-trimoxazole prophylaxis	Alive at 9 years (2019)	5
P18	No	No	Yes	No	Warts	Co-trimoxazole prophylaxis	Alive at 4 years (2019)	5
P19	fever Pustular skin rash soft tissue swelling	nr	Yes	nr	Sterile periostitis Multiorgan failure	nr	Dead soon after birth	6
P20*	fever	Yes	Yes	Retardation	bloody stool, chronic diarrhea; hepatosplenomegaly	IVIG antibiotics	Dead (2020)	

Note: nr not report; P2 is father of P3; P16 is father of P17 and P18; P20* is our patient.

Table 4 Molecular and cellular phenotypes of patients with heterozygous *NFKB1A* mutation.

Patient	I κ B α degradation (agonist-cell type)	NF- κ B translocation (dimer-agonist-cells)	IL-1R/TLR pathway activation (agonist-cell type)	TNFR pathway activation (agonist-cell type)	T-cell response in PBMCs (stimulus)	B-cell prolifer (stimulus)
P6-Q9X	nr	nr	Impaired (LPS—monocyte) Impaired (LPS—fibroblast)	nr	Low prolifer. (PHA; ConA)	nr
P4-W11X	Impaired (LPS-fibroblast)	Impaired (p50/p65-IL-1 β -fibroblast)	Impaired (IL-1 β , LPS-fibroblast) Impaired (poly(I:C), LPS, flagellin, CpG-PBMC)	nr	Normal prolifer. (low α -CD3, α -CD3/ α -CD28, PMA/iono, PHA, recall antigens)	nr
P5-E14X	Impaired (CD40L-EBV-B)	Impaired (p50; p65; c-Rel-CD40L-EBV-B)	Impaired (LPS, SAC OspA-PBMC)	Impaired (CD40L-EBV B cells)	Normal prolifer. (PHA, ConA, and recall Ags) Impaired IFN- γ and TNF- α prod. (α -CD3)	nr
P20-E14X	nd	Impaired (p65-LPS-PBMC)	Impaired (LPS, IL-1 β -PBMC)	nd	nd	nd
P19-D31N	nr	nr	nr	nr	nr	nr

Table 4 (continued)

Patient	I κ B α degradation (agonist-cell type)	NF- κ B translocation (dimer-agonist-cells)	IL-1R/TLR pathway activation (agonist-cell type)	TNFR pathway activation (agonist-cell type)	T-cell response in PBMCs (stimulus)	B-cell prolifer (stimulus)
P1—S32I	Impaired (TNF- α , LPS-fibroblast)	Impaired (p50/p65; p50/p50-TNF- α -fibroblast)	Impaired (LPS-PBMC) Impaired (LPS, IL-1 β -fibroblast)	Impaired (TNF- α ; LT α 1 β 2-fibroblast)	Absent prolifer. (low α -CD3, recall Ags) Normal prolifer. (α -CD3/ α -CD28, PMA, allogeneic cells) Normal IFN- γ prod. (α -CD3, α -CD3/ α -CD28)	nr
P2—S32Im	nr	nr	Impaired (LPS, PAM3, zymosan-WB) Impaired (LPS-MdM) Impaired (LPS-fibroblast)	Impaired (TNF- α ; LT α 1 β 2-fibroblast)	Low prolifer.a (low α -CD3, PHA) Normal prolifer	Normal (CD40L + IL4)
P3—S32I	Impaired (LPS-fibroblast)	Impaired (p65-LPS-MdM)	Impaired (LPS, PAM3, zymosan-WB) Impaired (LPS-MdM) Impaired (LPS-fibroblast)	nr	Low prolifer.a (low α -CD3, PHA) Absence prolifer. (recall Ags)	Normal (CD40L + IL4)
P14—S32I	nr	nr	nr	nr	nr	nr
P11—S32G	Impaired (TNF- α -fibroblasts)	nr	Impaired (LPS—WB)	nr	Normal prolifer. (PHA)	nr
P12—S32R	Impaired (TNF- α -fibroblast)	nr	nr	nr	nr	nr
P13—S32N	Impaired (CD40L-EBV-B)	nr	nr	nr	nr	nr
P15-G33V	Impaired (LPS-fibroblasts)	nr	Impaired (LPS—fibroblast)	nr	nr	nr
P7—S36Y	Impaired (TNF- α -T blast cells)	Impaired (p50/p65-TNF- α -fibroblast)	Impaired (LPS, IL-18—PBMC)	Impaired (TNF- α , LT α 1 β 2-fibroblast) Impaired (CD40L-PBMC)	Low prolifer. (low dose of α -CD3), Normal prolifer. (high dose of α -CD3), Normal prolifer. (PHA; PMA, recall Ags)	nr
P10—S36Y	Impaired (TNF- α , IL-1 β -fibroblast)	nr	Impaired (IL-1 β —fibroblast)	Impaired (TNF- α —fibroblast)	Impaired prolifer. (high α -CD3) Normal prolifer. α -CD3/ α -CD28, PHA, ConA, PMA/iono Impaired (IFN- γ and IL-12 production; BCG, BCG/IL-12; BCG/IFN- γ)	nr
P16—S36A	Impaired (PMA/iono), anti-IgM and LPS-B cell)	nr	nr	nr	nr	nr
P17—S36A	nr	nr	nr	nr	nr	nr
P18—S36A	nr	nr	nr	nr	nr	nr
P8-M37K	Impaired (TNF- α , LPS, PAM3-fibroblast)	Impaired (p50/p65-TNF- α -HeLa cells)	Impaired (LPS, PAM3-fibroblasts) Normal (SAC-WB); impaired (IL-1 β , SAC, LPS, PAM2, PMA/iono-WB)	Impaired (TNF- α -fibroblast) Impaired (TNF- α -WB)	Low prolifer. (OKT3, SAC), Normal prolifer. (PHA, PWM, ConA, recall Ags, diphtheria, tetanus/streptolysin O/mumps)	nr
P9-M37R	nr	nr	nr	nr	Normal prolifer. (PHA, PMA, α -CD3/ α -CD28)	Decreased (CpG)

Note: nr not reported, nd not detected, WB whole blood, MdM macrophage-derived monocytes, Ags antigen, prolifer. Proliferation.

three kinds of truncation mutation had been confirmed in cell lines.⁷ As for the point mutation patients, all of these patients had ID but P2 (S32Im),¹⁵ P16, P17, P18(S36A) had no EDA,⁵ indicating that there was no direct connection between *NFKBIA* mutation and EDA, which has not been studied before. However, these patients showed similar degree of activation of NF- κ B. The pathogenicity of S32G, S32R, S32N and S36A was not confirmed in cell lines,^{5,16–23} and D31N was found in an infant (P19) who suffered from pustular and systemic inflammatory disease resembling the deficiency of interleukin-1 receptor antagonist (DIRA) through clinical exome sequencing screening.⁸ Unfortunately, in addition to symptomatic support treatment, HSCT is the only but not ideal therapy for these patients, which is a problem need to be improved.

Conflict of interests

The authors declare that they have no conflict of interest.

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