A heterozygous N-terminal truncation mutation of NFKBIA results in an impaired NF-κB dependent inflammatory response

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Abstract  Germline heterozygous gain-of-function (GOF) mutation of NFKBIA, encoding IκBα, would affect the activation of NF-κB 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 NFKBIA/IκBα. She presented recurrent fever, infectious pneumonia and chronic diarrhea with EDA-ID. Impaired NF-κB translocation and IL1R and TLR4 pathway activation were revealed in this patient. The findings suggested that the truncation mutation of IκBα caused medium impaired of activation of NF-κB but the early death. Furthermore, we reviewed all the reported patients with NFKBIA mutation to learn more about this disease.
Introduction

Nuclear factor kappa B (NF-κB) 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 IkBα, encoded by NFKBIA, is one of the inhibitors of the NF-κB activation and binds to the NF-κB proteins p65 (RelA) and p50. Various stimuli could cause activation of the IkB kinase (IKK) complex, which phosphorylates IkBα on serines 32 and 36, leading to ubiquitination of lysines 21 and 22 and the subsequent degradation of IkBα.2 Mutation of NFKBIA causes impaired IkBα 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.1 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 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 IkBα 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 mutation in NFKBIA 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. 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-1β, IL-18, IL-6, IL-8(CXCL8), IL-12p70, MCP-1(CCL2), IFN-γ and TNF-α concentrations, 1 x 10⁶ 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 μg/ml; Sigma), human interferon (IFN)-γ (1000 U/ml; Peprotech), IL-1β (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-κB 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 4–12×10^9/L; RBC 4.0–5.3×10^{12}/L; PLT 100–380×10^9/L; Hb 110–150 g/L; MCV 80–100 fl; MCH 26–32 pg; CRP < 8 mg/L. (B) Family pedigrees of the patient with variant in NFKBIA. (C) Sequence analysis of the NFKBIA gene. The de novo mutation, c.40G>T, in the NFKBIA gene is indicated by the arrow. (D) Schematic domain structure of NFKBIA. 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 NFKBIA. Amino acid sequence of NFKBIA 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 level, while 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 (Fig. 1A). After a combination of antibiotics, antifungal, anti-tuberculous and regular intravenous immunoglobulin (IVG) 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).

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 |             |

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 stimuli that active NF-κB. We examined cytokines produced by PBMCs after stimulation of TLR4 and IL-1 receptor as described by Lopes-Grandomo et al and Yamamoto et al. 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 IkB-α degradation, keeping the nuclear localization signals on the NF-κB subunits masked, 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 IkB (inhibitor of NF-κB) family of proteins includes IkBα, IkBβ, and IkBε. In resting cells, NF-κB proteins, including p65 (RelA), p105/p50, p100/p52, c-Rel, and RelB, are retained in the cytoplasm by the IkB 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 IkB kinase (IKK) complex, which phosphorylates IkBα on serines 32 and 36, leading to ubiquitination of lysines 21 and 22 and the subsequent degradation of IkBα.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 IkBα. The N-terminal sequence of IkBα is highly conserved across species, especially the six amino acid degron (DSDLDS) and the two serine residues located at positions 32 and 36 in humans. As a result, both of the point mutation and truncation mutation which relate to these sites could impair the degradation of IkBα, 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
Figure 2  Impaired NF-κB 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 (1μg/ml), IFN-γ (1000 U/ml), IL-1β (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.
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. Since truncation mutation of IkBα could give rise to re-initiation of translation at downstream ATG sites, severe disease and great impairment of NF-κB activation are more significant in IkBα point mutants versus truncation mutants. 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-κB in this patient was not completely damaged, which was consistent with reported patient (P5: E14X, Table 3) and other truncation mutation patients (P4: W11X, P6: Q9X, Table 3). 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. 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 IkBα degradation and impaired NF-κB translocation. Pathogenicity of these
Table 3: Genetic and clinical features of patients with heterozygous NFKBIA mutations.

| Patient | Gender | Origin    | Year of birth | Age of onset | Variant | Inheritance | Infection Manifestations                                                                                                                                 |
|---------|--------|-----------|---------------|--------------|---------|-------------|--------------------------------------------------------------------------------------------------|
| P1      | M      | Italian   | nr            | 2 months     | S32I    | De novo     | Recurrent LRTI: *P. aeruginosa*, Klebsiella, Serratia, *S. aureus* CMC, enteritis (Salmonella typhimurium) *S. typhimurium* infection persisted with recurrent manifestations in psoas muscle, pleural cavity, pericardial fluid, and ribs. Meningitis: β-hemolytic group A Streptococcus: sepsis. Respiratory infection pneumonia Pneumocystis jiroveci, mild CMC. |
| P2      | M      | Dutch     | nr            | 2 years      | S32Im   | De novo     | Pneumonia (parainfluenza virus and Pneumocystis carinii). 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. |
| P3      | M      | Dutch     | nr            | 2 months     | S32I    | Inherited from his father | BCG skin infection Haemophilus influenza: pneumonia, CMC Bacterial: pneumonia, Candida parapsilosis, Stenotrophomonas maltophilia, osteomyelitis of skull and limb, CMC LRTI: *P. aeruginosa*: bronchiectasis and sinusitis *Mycobacterium tuberculosis*: abdominal lymphadenopathy *M. abscessus*: septic arthritis of the knee, osteomyelitis Urinary tract infection: K. pneumoniae Salmonella enteritidis: osteomyelitis, hematochezia Candida: esophagitis *Mycobacterium malmoense*: blood and skin Sapovirus and norovirus: stool *S. aureus* sepsis, CMC, Recurrent pneumonia *S. aureus* and *P. aeruginosa* sepsis |
| P4      | F      | American  | nr            | Birth        | W11X    | Mother: WT;Father: nr | De novo |
| P5      | M      | American  | nr            | 1 month      | E14X    | De novo     | Recurrent pneumonia Pneumonia (parainfluenza virus and Pneumocystis carinii). 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. |
| P6      | M      | Japanese  | nr            | 1 month      | Q9X     | nr          | Pneumonia (parainfluenza virus and Pneumocystis carinii). 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. |
| P7      | M      | Japanese  | 2007          | 4 months     | S36Y    | De novo     | Recurrent LRTI Sepsis: Klebsiella pneumonia, Candida parapsilosis, Stenotrophomonas maltophilia, osteomyelitis of skull and limb, CMC LRTI: *P. aeruginosa*: bronchiectasis and sinusitis *Mycobacterium tuberculosis*: abdominal lymphadenopathy *M. abscessus*: septic arthritis of the knee, osteomyelitis Urinary tract infection: K. pneumoniae Salmonella enteritidis: osteomyelitis, hematochezia Candida: esophagitis *Mycobacterium malmoense*: blood and skin Sapovirus and norovirus: stool *S. aureus* sepsis, CMC, Recurrent pneumonia *S. aureus* and *P. aeruginosa* sepsis |
| P8      | M      | German    | nr            | 6 months     | M37K    | WT          | De novo |
| P9      | F      | Italian   | 2012          | 5 months     | M37R    | nr          | De novo |
| P10     | F      | Chinese   | 2004          | 1 month      | S36Y    | De novo     | Recurrent LRTI Sepsis: Klebsiella pneumonia, Candida parapsilosis, Stenotrophomonas maltophilia, osteomyelitis of skull and limb, CMC LRTI: *P. aeruginosa*: bronchiectasis and sinusitis *Mycobacterium tuberculosis*: abdominal lymphadenopathy *M. abscessus*: septic arthritis of the knee, osteomyelitis Urinary tract infection: K. pneumoniae Salmonella enteritidis: osteomyelitis, hematochezia Candida: esophagitis *Mycobacterium malmoense*: blood and skin Sapovirus and norovirus: stool *S. aureus* sepsis, CMC, Recurrent pneumonia *S. aureus* and *P. aeruginosa* sepsis |
| P11     | F      | Caucasian /Thai | 2011          | 20 months    | S32G    | De novo     | Recurrent LRTI Sepsis: Klebsiella pneumonia, Candida parapsilosis, Stenotrophomonas maltophilia, osteomyelitis of skull and limb, CMC LRTI: *P. aeruginosa*: bronchiectasis and sinusitis *Mycobacterium tuberculosis*: abdominal lymphadenopathy *M. abscessus*: septic arthritis of the knee, osteomyelitis Urinary tract infection: K. pneumoniae Salmonella enteritidis: osteomyelitis, hematochezia Candida: esophagitis *Mycobacterium malmoense*: blood and skin Sapovirus and norovirus: stool *S. aureus* sepsis, CMC, Recurrent pneumonia *S. aureus* and *P. aeruginosa* sepsis |
| P12     | M      | Japanese  | nr            | 2 months     | S32R    | De novo     | Recurrent LRTI Sepsis: Klebsiella pneumonia, Candida parapsilosis, Stenotrophomonas maltophilia, osteomyelitis of skull and limb, CMC LRTI: *P. aeruginosa*: bronchiectasis and sinusitis *Mycobacterium tuberculosis*: abdominal lymphadenopathy *M. abscessus*: septic arthritis of the knee, osteomyelitis Urinary tract infection: K. pneumoniae Salmonella enteritidis: osteomyelitis, hematochezia Candida: esophagitis *Mycobacterium malmoense*: blood and skin Sapovirus and norovirus: stool *S. aureus* sepsis, CMC, Recurrent pneumonia *S. aureus* and *P. aeruginosa* sepsis |
| P13     | nr     | Japanese  | nr            | Birth        | S32N    | De novo     | Recurrent LRTI Sepsis: Klebsiella pneumonia, Candida parapsilosis, Stenotrophomonas maltophilia, osteomyelitis of skull and limb, CMC LRTI: *P. aeruginosa*: bronchiectasis and sinusitis *Mycobacterium tuberculosis*: abdominal lymphadenopathy *M. abscessus*: septic arthritis of the knee, osteomyelitis Urinary tract infection: K. pneumoniae Salmonella enteritidis: osteomyelitis, hematochezia Candida: esophagitis *Mycobacterium malmoense*: blood and skin Sapovirus and norovirus: stool *S. aureus* sepsis, CMC, Recurrent pneumonia *S. aureus* and *P. aeruginosa* 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 gastrointestinal infections (Shigellosis, C. jejuni), recurrent upper respiratory tract infections (bronchitis, sinusitis, otitis media), recurrent pneumonias (S. pneumoniae, H. influenzae), bronchiectasis with chronic mucoid Pseudomonas aeruginosa infection meningitis (N. meningitidis), CNS tuberculosis with brain abscess, verruca vulgaris (HPV 9 and 57) |
| P16     | M      | Turkish  | 1982          | early childhood | S36A | De novo | Recurrent gastrointestinal infections (Shigellosis, C. jejuni), recurrent upper respiratory tract infections (bronchitis, sinusitis, otitis media), recurrent pneumonias (S. pneumoniae, H. influenzae), bronchiectasis with chronic mucoid Pseudomonas aeruginosa infection meningitis (N. meningitidis), 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, sinusitis, otitis media), recurrent pneumonias, bronchiectasis, Verruca vulgaris |
| P18     | F      | Turkish  | 2015          | early childhood | S36A | Inherited from her father | Recurrent upper respiratory tract infections (bronchitis, sinusitis, otitis media), recurrent pneumonias, bronchiectasis, Verruca vulgaris |
| P19     | M      | Spain    | 2012          | Birth         | D31N   | De novo | Recurrent upper respiratory tract infections (bronchitis, sinusitis, otitis media), recurrent pneumonias, bronchiectasis, Verruca vulgaris |
| P20*    | F      | Chinese  | 2019          | 2 months      | E14X   | De novo | Recurrent bronchopneumonitis (Gram ± 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 eristitis, 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 fever | No | Yes | No | chronic diarrhea | ns | Alive at > 20 years (2004) | 15 |
| P3      | fever, skin rash, fever | Yes | Yes | Retardation | chronic diarrhea | HSCT antibiotics cotrimoxazole IVIG corticosteroid antibiotics | Alive at 22 years (2016) | 13 |
| P4      | | Yes | Yes | nr | nr | HSCT trimethoprim-sulfamethoxazole | Dead (1 year, 2008) | 8 |
| P5      | systemic inflammation | Yes | Yes | nr | chronic diarrhea | HSCT | Alive at 7 years (2016) | 14 |
| P6      | systemic inflammation | Yes | Yes | nr | chronic diarrhea | HSCT | Dead (2013) | 18 |
| P7      | systemic inflammation | Yes | Yes | Retardation | Gastroenteritis, chronic diarrhea | HSCT | Dead (2013) | 19 |
| P8      | fever | Yes | Yes | Retardation | chronic diarrhea | HSCT | Dead (2013) | 19 |
| P9      | fever | Yes | Yes | nr | chronic diarrhea | Subcutaneous IFN-γ (50 μg/m2), cotrimoxazole, IVIG, antituberculosis treatment | Alive at 9 years (2015) | 21 |
| P10     | fever | No | Yes | nr | nr | | | |

(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      | Alive at 6 years (2017) | 22   |
| P12     | fever, skin erythema, systemic inflammation fever | Yes    | Yes         | 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                                                                               | HSCT      | Alive at 10 years (2017) | 7    |
| P15     | fever                                  | Yes    | Yes         | nr                                                                               | HSCT      | Alive at 10 years (2017) | 7    |
| P16     | fever, JRA                              | No     | Yes         | nr bloody stool, inflammatory bowel disease; recurrent intracranial hemorrhage; difficulty in hemostasis | HSCT      | Alive at 37 years (2019) | 5    |
| P17     | No                                     | No     | Yes         | No                                                                               | Co-trimoxazole prophylaxis | Alive at 9 years (2019) | 5    |
| P18     | No                                     | No     | Yes         | 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, bloody stool, chronic diarrhea, hepatosplenomegaly | IVIG chloroquine methotrexate azathioprine azithromycin prophylaxis gentamycin | Dead soon after birth | 6    |
| P20*    | fever                                  | Yes    | Yes         | Retardation                                                                     | 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 NFKBIA mutation.

| Patient | IkB degradation (agonist-cell type) | NF-κB 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 prolif (stimulus) |
|---------|-------------------------------------|------------------------------------------|-------------------------------------------------|---------------------------------------------|-------------------------------------|--------------------------|
| P6-Q9X  | nr                                  | nr                                       | Impaired (LPS -monocyte)                         | nr                                          | Low prolif. (PHA; ConA)            | nr                       |
| P4–W11X | Impaired (LPS-fibroblast)           | Impaired (p50/p65-IL-1β-fibroblast)      | Impaired (IL-1β, LPS-fibroblast)                 | nr                                          | Normal prolif. (low α-CD3, α-CD3/α-CD28, PMA/ino, 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 prolif. (PHA, ConA, and recall Ags) | nr                       |
| P20-E14X | nd                                 | nd                                       | Impaired (LPS,IL-1β-PBMC)                        | nd                                          | Impaired IFN-γ and TNF-α prod. (α-CD3) | nd                       |
| P19-D31N | nr                                 | nr                                       | Impaired (p65-LPS-PBMC)                         | nr                                          | nr                                  | nr                       |
Table 4

| Patient | IkB degradation (agonist-cell type) | NF-κB 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 prolif (stimulus) |
|---------|-------------------------------------|------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------|
| P1−S32I| Impaired (TNF-α, LPS-fibroblast)    | Impaired (p50/p65; p50/p50-TNF-α-fibroblast) | Impaired (LPS-PBMC) | Impaired (TNF-α; LTx1β2-fibroblast) | Absent prolif. (low α-CD3, recall Ags) | Normal (CD40L + IL4) |
| P2−S32Im| nr                                  | nr                                       | Impaired (LPS, PAM3, zymosan-WB) | Impaired (TNF-α; LTx1β2-fibroblast) | Low prolif.a (low α-CD3, PHA) | Normal (CD40L + IL4) |
| P3−S32I| Impaired (LPS-fibroblast)           | Impaired (p65-LPS-MdM)                   | Impaired (LPS, PAM3, LPS-MdM) | Impaired (p65-LPS-MdM) | Low prolif.a (low α-CD3, PHA) | Absence prolif. (recall Ags) |
| P4−S32I| nr                                  | nr                                       | Impaired (LPS—WB)        | Impaired (LPS—WB) | Normal prolif. (PHA) | nr |
| P5−S32G| Impaired (TNF-α-fibroblasts)        | nr                                       | nr                             | Normal prolif. (PHA) | nr |
| P6−S32R| Impaired (TNF-α-fibroblast)         | nr                                       | nr                             | nr |
| P7−S32N| Impaired (CD40L-EBV-B)              | nr                                       | nr                             | nr |
| P8−S36Y| Impaired (LPS-fibroblasts)          | Impaired (p50/p65-TNF-α-fibroblast)     | Impaired (LPS, IL-1β-fibroblast) | Impaired (TNF-α-fibroblast) | Low prolif. (low dose of α-CD3), Normal prolif. (high dose of α-CD3), Normal prolif. (PHA; PMA, recall Ags) | nr |
| P9−S36Y| Impaired (TNF-α, IL-1β-fibroblast)  | nr                                       | Impaired (IL-1β-fibroblast)   | Impaired (TNF-α-fibroblast) | Impaired prolif. (high α-CD3) Normal prolif. α-CD3/α-CD28, PHA, ConA, PMA/iono | nr |
| P10−S36Y| nr                                  | nr                                       | nr                             | nr |
| P11−S36A| nr                                  | nr                                       | nr                             | nr |
| P12−S36A| nr                                  | nr                                       | nr                             | nr |
| P13−S36A| nr                                  | nr                                       | nr                             | nr |
| P14−S36A| nr                                  | nr                                       | nr                             | nr |
| P15−M37K| nr                                  | nr                                       | nr                             | nr |
| P16−M37K| nr                                  | nr                                       | nr                             | nr |
| P17−M37K| nr                                  | nr                                       | nr                             | nr |
| P18−M37K| nr                                  | nr                                       | nr                             | nr |
| P9−M37R| nr                                  | nr                                       | nr                             | Decreased (CpG) |

Note: nr not reported, nd not detected, WB whole blood, MdM macrophage-derived monocytes, Ags antigen, prolif. Proliferation.
three kinds of truncation mutation had been confirmed in cell lines. As for the point mutation patients, all of these patients had ID but P2 (S32Lm), P16, P17, P18(S36A) had no EDA, 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-κB. The pathogenicity of S32G, S32R, S32N and S36A was not confirmed in cell lines, and D31N was found in an infant (P19) who suffered from cutaneous and systemic inflammatory disease resembling the deficiency of interleukin-1 receptor antagonist (DIRA) through clinical exome sequencing screening. 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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References

1. Vallabhapurapu S, Karim M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol. 2009;27:693–733.
2. Karim M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-{kappa}B activity. Annu Rev Immunol. 2000;18:621–663.
3. Picard C, Casanova JL, Puel A. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. Clin Microbiol Rev. 2011;24(3):490–497.
4. Boisson B, Puel A, Picard C, Casanova JL. Human IκBα gain of function: a severe and syndromic immunodeficiency. J Clin Immunol. 2017;37(5):397–421.
5. Sogkas G, Adriaan IR, Ringshausen FC, et al. A novel NFKBIA variant substituting serine 36 of IκBα causes immunodeficiency with warts, bronchiectasis and juvenile rheumatoid arthritis in the absence of ectodermal dysplasia. Clin Immunol. 2020;210:e108269.
6. Battle-Masó L, Mensa-Vilaró A, Solís-Moruno M, Marqués-Bonet T, Arostegui JI, Casals F. Genetic diagnosis of auto-inflammatory disease patients using clinical exome sequencing. Eur J Med Genet. 2020;63(5):103920.
7. Petersheim D, Massaad MJ, Lee S, et al. Mechanisms of genotype-phenotype correlation in autosomal dominant anhidrotic ectodermal dysplasia with immune deficiency. J Allergy Clin Immunol. 2018;141(3):1060–1073.
8. Lopez-Granados E, Keenan JE, Kinney MC, et al. A novel mutation in NFKBIA/IKBA results in a degradation-resistant N-terminated protein and is associated with ectodermal dysplasia with immunodeficiency. Hum Mutat. 2008;29(6):861–868.
9. Yamamoto M, Yamazaki S, Uematsu S, et al. Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta. Nature. 2004;430(6996):218–222.
10. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. 2004;25(6):280–288.
11. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 1998;16:225–260.
12. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell. 2002;109(Suppl):S81–S96.
13. 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. J Allergy Clin Immunol. 2007;120(4):907–909.
14. Ohnishi H, Miyata R, Suzuki T, et al. A rapid screening method to detect autosomal-dominant ectodermal dysplasia with immune deficiency syndrome. J Allergy Clin Immunol. 2012;129(2):578–580.
15. Janssen R, van Wagen E, Hoeve MA, et al. The same IkappaBalpha mutation in two related individuals leads to completely different clinical syndromes. J Exp Med. 2004;200(5):559–568.
16. Courtois G, Smahi A, Reichenbach J, et al. A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. J Clin Invest. 2003;112(7):1108–1115.
17. Dupuis-Girod S, Cancrini C, Le Deist F, et al. Successful allogeneic hemopoietic stem cell transplantation in a child who had anhidrotic ectodermal dysplasia with immunodeficiency. Pediatrics. 2006;118(1):e205–e211.
18. Yoshioka T, Nishikomori R, Hara J, et al. 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. J Clin Immunol. 2013;33(7):1165–1174.
19. Schinke LF, Rieber N, Rylaarsdam S, et al. A novel gain-of-function IKBA mutation underlies ectodermal dysplasia with immunodeficiency and polyendocrinopathy. J Clin Immunol. 2013;33(6):1088–1099.
20. Giancane G, Ferrari S, Carsetti R, Papoff P, Iacobini M, Duse M. Anhidrotic ectodermal dysplasia: a new mutation. J Allergy Clin Immunol. 2013;132(6):1451–1453.
21. Lee AJ, Moncada-Vélez M, Picard C, et al. Severe Mycobacterial diseases in a patient with GOF IκBα mutation without EDA [published correction appears in J Clin Immunol. 2016 Apr;36(3):335]. J Clin Immunol. 2016;36(1):12–15.
22. Staples E, Marillo-Gutierrez B, Davies J, et al. Disseminated Mycobacterium malmoense and Salmonella infections associated with a novel variant in NFKBIA. J Clin Immunol. 2017;37(5):415–418.
23. Moriya K, Sahasra Y, Ohnishi H, Kawai T, Kanegane H. IKBA S32 mutations underlie ectodermal dysplasia with immunodeficiency and severe noninfectious systemic inflammation. J Clin Immunol. 2018;38(5):543–545.