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ORIGINAL PAPER

A Chinese DADA2 patient: report of two novel mutations and successful HSCT

Luyao Liu[1] · Wenjie Wang[1] · Ying Wang[1] · Jia Hou[1] · Wenjing Ying[1] · Xiaoying Hui[1] · Qinhua Zhou[1] · Danru Liu[1] · Haili Yao[1] · Jinqiao Sun[1] · Xiaochuan Wang[1]

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Abstract

Deficiency of adenosine deaminase 2 (DADA2) is an autoinflammatory disease caused by autosomal recessive mutations in *Cat Eye Syndrome Chromosome Region 1* (*CECR1*) gene. In this report, we aimed to describe the clinical manifestations, immunological features, genotype, and treatments of one Chinese patient with novel *CECR1* gene mutations. This patient initially presented with recurrent fever and rashes from the age of 3 months, but no pathogen was found. She then developed dry gangrene of the fingers at 5 months of age. Laboratory examinations revealed elevated levels of C-reactive protein and thrombocytes. The expression of interleukin-6 (IL-6) and IL-8 were both elevated. Sequencing results revealed that she had compound heterozygous mutations in *CECR1* gene (c.1211T>C, p.Phe404Ser and c.1114 G>A, p.Val372Met). Subsequently, treatment with anti-IL-6 (tocilizumab) was started. However, she developed blurred vision in the right eye with occlusion of the central retinal artery, accompanied by unsteady gait. Magnetic resonance imaging (MRI) showed infarction of the right thalamus. Finally, she underwent hematopoietic stem cell transplantation (HSCT) and is currently in remission. Our findings suggest that HSCT could cure this disease.

Keywords Adenosine deaminase 2 deficiency · *CECR1* gene · Novel mutations · Hematopoietic stem cell transplantation

Introduction

Deficiency of adenosine deaminase 2 (DADA2) is an autoinflammatory disease, caused by autosomal recessive mutations in *Cat Eye Syndrome Chromosome Region 1* (*CECR1*) gene (Zhou et al. 2014; Navon Elkan et al. 2014). *CECR1* encodes the adenosine deaminase 2 (ADA2) protein, a secreted protein in monocytes, macrophages and dendritic cells, associated with the proliferation and differentiation of monocytes to macrophages (Caorsi et al. 2016; Nanthapisal et al. 2016). ADA2 also maintains the balance between the pro-inflammatory (M1) and the anti-inflammatory (M2) macrophages (Caorsi et al. 2016; Savic and McDermott 2015; Meyts and Aksentijevich 2018).

DADA2 has a wide spectrum of phenotypes, characterized by recurrent fevers, livedo reticularis, lacunar ischemic stroke, intracranial bleeding/hemorrhagic stroke, and systemic vasculopathy, frequently with mild immunodeficiency and hypogammaglobulinemia (Zhou et al. 2014; Navon Elkan et al. 2014). The clinical features of DADA2 are characterized by a highly variable age of onset (ranging from 2 months to 59 years), severity, organ involvement, and outcome (Fellmann et al. 2016; Meyts and Aksentijevich 2018). Treatments with anti-TNF-α agents, anti-interleukin-1 agents, immunosuppressive drugs and steroids might partially reverse clinical manifestations. Hematopoietic stem cell transplantation (HSCT) might cure this deficiency; however, relevant reports have been limited to date (Van Eyck et al. 2014; Debatin 2017). Here, we reported the clinical characteristics, immunological features, and treatments in a 13-month-old Chinese patient of DADA2 with novel *CECR1* gene

Luyao Liu and Wenjie Wang contributed equally to this work.

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✉ Jinqiao Sun
jinqiaosun@fudan.edu.cn

✉ Xiaochuan Wang
xchwang@shmu.edu.cn

[1] Department of Clinical Immunology, Children's Hospital of Fudan University, 399 Wanyuan Road, Shanghai 201102, China

mutations. These results expand the genetic spectrum and treatments of DADA2.

Patient and methods

The study was approved by the Ethics Committee of the Children's Hospital of Fudan University. The patient's parents gave written informed consent.

Clinical data

We retrospectively summarized the clinical data of one Chinese DADA2 patient.

Immunological function evaluation

As previously reported (Sun et al. 2012), IgG, IgA, and IgM were measured by nephelometry. Lymphocyte subsets were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

We used a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) to measure cytokine expression using a BD cytometric bead array (CBA) human soluble protein master buffer kit. We first added 50 μL of flex set standard dilutions to the first 10 tubes (no standard dilution, 1:256, 1:128, 1:62, 1:32, 1:16, 1:8, 1:4, and 1:2, and top standard). We then added 50 μL of each unknown sample to the appropriate assay tubes and added 50 μL of the mixed capture beads to each assay tube. The tubes were incubated for 1 h at room temperature. Fifty microliters of the mixed PE detection reagent was added to each assay tube. The tubes were incubated for 2 h at room temperature. We added 300 μL of wash buffer to each assay tube with brief vortexing to resuspend the beads. All reagents were from BD Biosciences. Finally, we analyzed the data using FCAP array software.

Gene analysis

Next generation sequencing was performed using a panel. The panel included the known 271 PID genes (Supplemental table 1) (Bousfiha et al. 2015). Genomic DNA fragments of patients were enriched for panel sequencing using the Agilent (Santa Clara, CA, USA) ClearSeq inherited disease panel kit. Enriched DNA samples were indexed and sequenced on a HiSeq2000 sequencer (Illumina, San Diego, CA) with 100 cycles of single-end reads, according to the manufacturer's protocol. The mutations were confirmed by Sanger sequencing. The Combined Annotation-Dependent Depletion (CADD) scores of the two mutations were obtained through the website (https://cadd.gs.washington.edu).

Results

Case presentation

A 13-month-old Chinese girl, born to nonconsanguineous Chinese parents, presented with recurrent fever since the age of 3 months with no obvious cause. She had a bilateral lower limb skin rash (Fig. 1a) and elevated levels of C-reactive protein (CRP) (49.98 mg/L) and thrombocytes (454×10^9/L), accompanied with fever. Both the echocardiograms and B-ultrasounds of her joints were normal. No pathogens were detected. The lymph nodes, liver, and spleen were within

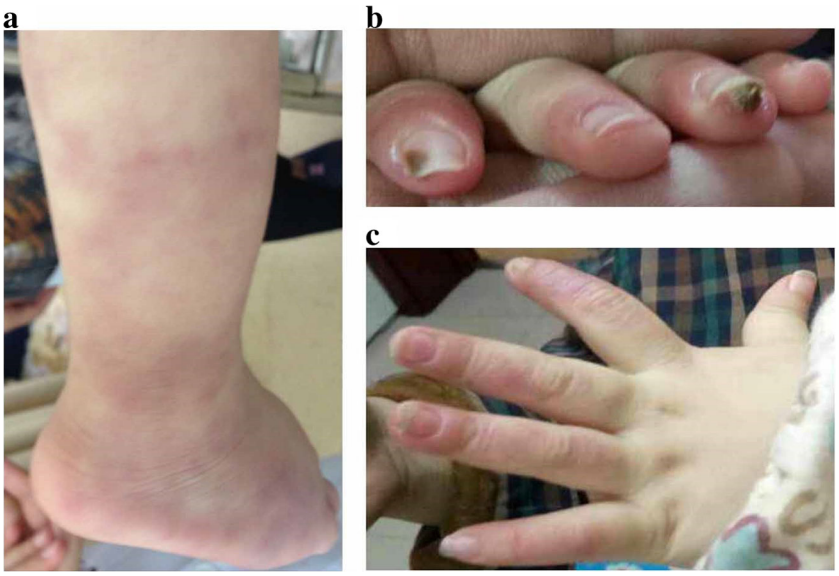

Fig. 1 Clinical manifestations of the patient. **a** Skin rashes on bilateral lower limbs. Fibrous hypertrophy accompanied with inflammatory cells infiltrating showed on biopsy. **b** Digital necrosis of the fingers. **c** Digital necrosis of the fingers improved after tocilizumab treatment

Table 1 Immunological characteristics of the patient

Lymphocyte subpopulation		Reference value (aged 1–4 years)
CD3% (AC/μL)	51.80 (1657.1)	49.70–77.30
CD4% (AC/μL)	30.05 (961.41)	24.42–57.49
CD8% (AC/μL)	18.45 (590.37)	8.44–34.54
CD19% (AC/μL)	26.49 (847.39)	12.36–32.46
CD16/56% (AC/μL)	20.63 (659.94)	3.63–22.40
Immunoglobulin		
IgG (g/L)	10.24	5.52–11.46
IgA (g/L)	0.58	0.06–0.74
IgM (g/L)	0.72	0.6–2.12

normal size. Treatments with antibiotics and intravenous immunoglobulin were ineffective.

At the age of 13 months, the patient came to our hospital. She presented with digital necrosis of the fingers (Fig. 1b). Biopsy of skin rashes showed fibrous hypertrophy accompanied with infiltrating inflammatory cells (identified as lymphocytes). She also had growth retardation and mild anemia. Bone marrow aspiration showed hyperactive myeloproliferation, with inverted ratio of granulocytes/erythrocytes. A history of recurrent respiratory tract infections was elicited. Based on her clinical manifestations and history, a diagnosis of vasculitis was suspected, while autoimmune markers and CT angiography (CTA) of the chest and abdomen were unrevealing. Her immunoglobulin (Ig) levels and lymphocyte subsets were normal (Table 1). Serum tumor markers and whole body bone scan were both normal. We excluded infectious diseases, malignancies and autoimmune diseases. Serum interleukin (IL)-6 and IL-8 levels were elevated, particularly when she had a fever (Fig. 2a, Table. 2), while expression levels of tumor necrosis factor (TNF)-α did not rise. Considering her clinical manifestations and elevated levels of inflammatory markers, autoinflammatory diseases were suspected. Therefore, we performed gene sequencing. Simultaneously, she was treated with prednisone (2 mg/kg), with good response evidenced by reduction of CRP levels.

Gene analysis

Gene sequencing revealed that she had novel compound heterozygous mutations in *CECR1* gene (c.1211T>C, p.Phe404Ser from her father; c.1114 G>A, p.Val372Met from her mother) (Fig. 3). The two amino acids are both located in the catalytic domain (Fig. 4) of ADA2 protein. Further analysis indicated that the F404S mutation would change the polar contact to P401, probably resulting in structure changes in this helix (Fig. 4b). Furthermore, compared to WT V372, the distance between M372 and V151/T152 was much closer. In this

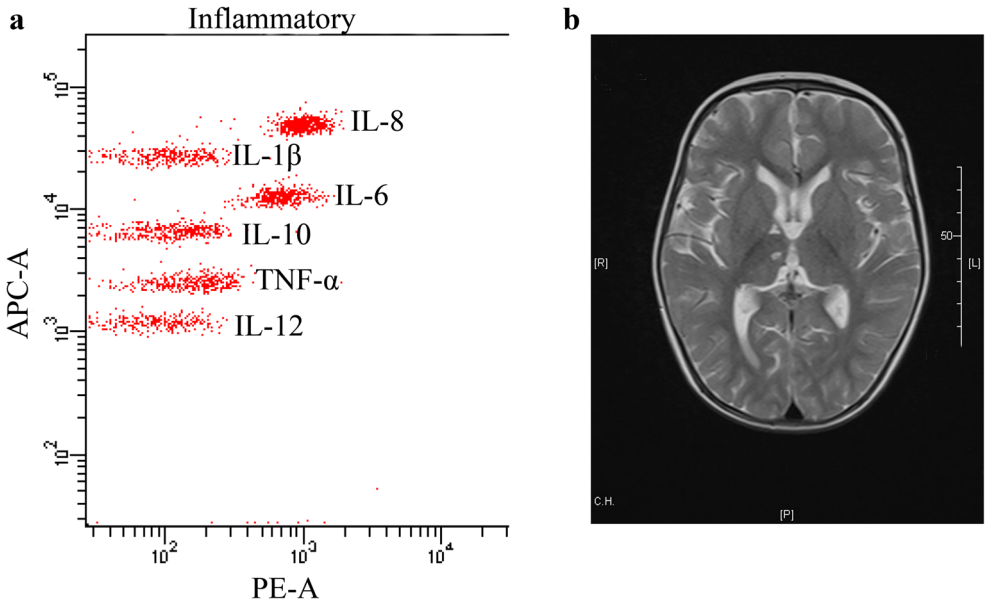

Fig. 2 The results of cytokine expression and cerebral CT. **a** The expression of interleukin-6 (IL-6) and interleukin-8 (IL-8) both increased with cytometric bead array (CBA) by flow cytometry. **b** Low density umbra in the right thalamus seen on cerebral computed tomography (CT)

Table 2 Cytokine expression in the patient

Cytokine		Reference value
IL-8 (pg/mL)	135.25	≤5 pg/mL
IL-1β (pg/mL)	3.58	≤36 pg/mL
IL-6 (pg/mL)	120.85	≤5 pg/mL
IL-10 (pg/mL)	1.87	≤18 pg/mL
TNF-α (pg/mL)	2.79	≤22 pg/mL
IL-12 (pg/mL)	2.03	≤6 pg/mL

way, new contacts would form among these residues and influence the protein structure (Fig. 4c). CADD scores of the c.1211T>C and c.1114 G>A mutations were 24.3 and 26.9, respectively. These variants were not found in databases, including Bravo, gnomAD, and ExAC, and were also not found in whole exome sequencing data of 13,568 in-house patients from our hospital.

Treatment and outcome

Considering her clinical manifestations and high IL-6 levels, we started anti-IL-6 therapy (tocilizumab) based on the previous studies (Van Eyck et al. 2014; Hashem et al. 2017a, b); simultaneously, we gradually reduced steroid dosages after obtaining ethics committee approval and informed consent. Tocilizumab treatment led to improvement in clinical manifestations (Fig. 1c) and normalization of IL-6 and IL-8 levels, with no further activity of disease for 5 months.

At the age of 8 months and 1 year, she suddenly developed blurred vision in the right eye and unsteady gait accompanied with head-tilt to the left. Cerebral computed tomography (CT) showed a low density umbra in the right thalamus (Fig. 2b). Meanwhile, ocular examination revealed right central retinal artery occlusion. She had suffered a cerebral infarction, despite tocilizumab therapy. The expression of IL-6 and IL-8 levels rose again. The repeated immunoglobulin and lymphocyte subsets remained within normal values. She responded poorly to tocilizumab treatment, and conventional anticoagulant therapy with dextran, *Salvia miltiorrhiza* and aspirin. Finally, she underwent hematopoietic stem cell transplantation (HSCT) at the age of 2 years (in another hospital), experiencing substantial improvement. She currently remains in remission.

Discussion

DADA2, as a novel monogenic disease first described in 2014 (Zhou et al. 2014; Navon Elkan et al. 2014), is characterized by a wide spectrum of phenotypes ranging from limited

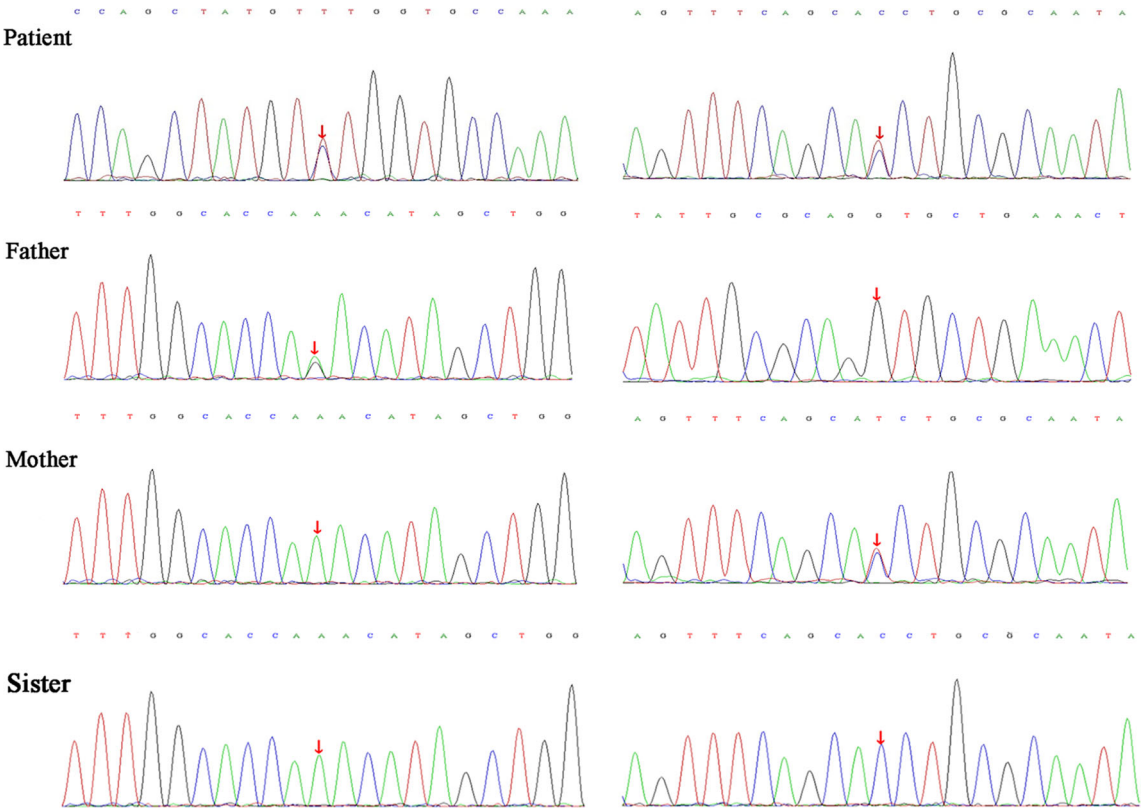

Fig. 3 Gene sequencing in *CECR1* gene. The patient had compound heterozygous mutations in the *CECR1* gene (c.1211T>C, from her father; c.1114 G>A, from her mother). The gene analysis of her sister was normal

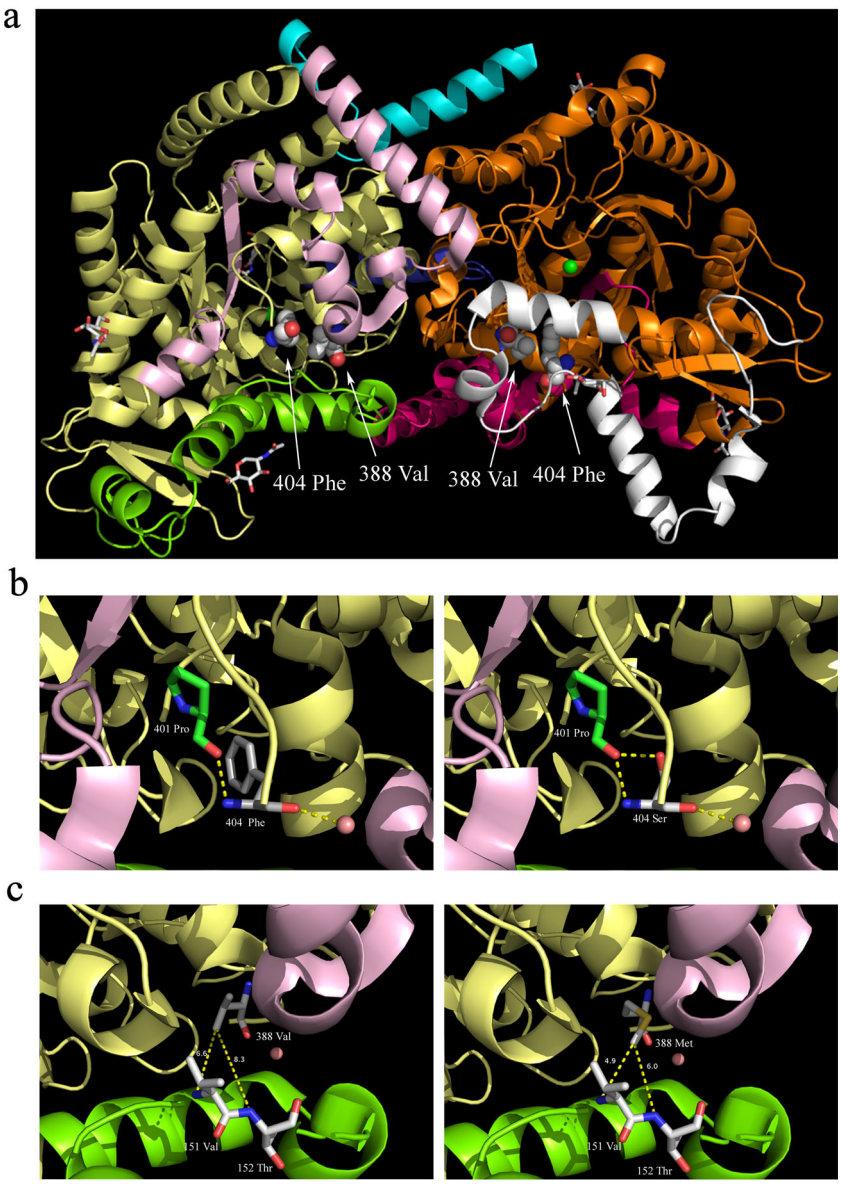

Fig. 4 The schematic model of WT and mutated ADA2 protein. **a** Localization of the mutational amino acid, shown as a sphere, in the 3D model of an ADA2 dimer. **b** The polar contacts of WT F404 (left) and mutated S404 (right) to the P401 in the helix. **c** The distance of WT V388 (left)/mutated M388 (right) to the V151 and T152. The signal peptide, dimerization, and PRB and catalytic domains are shown in cyan, pink, green and yellow in one subunit and in blue, magenta, white, and orange in the second subunit, respectively. Small unique elements are painted in orange. Catalytic Zn^{2+} are shown as green spheres. Asparagine-linked N-acetyl glucosamine is shown with sticks. WT wild type, PRB putative receptor-binding domain

cutaneous manifestations to severe vasculitis and strokes (Van Eyck Jr et al. 2015; Meyts and Aksentijevich 2018), possibly influenced by specific mutations or environmental factors. In the present study, we identified a Chinese patient with two novel *CECR1* mutations (c.1211T>C, p.Phe404Ser and c.1114 G>A, p.Val372Met). She presented at a very early age (from 3 months old) with recurrent fever, skin rashes, digital necrosis, and cerebral strokes. Overall, her symptoms were consistent with DADA2. However, both levels of immunoglobulin (including IgA, IgG, and IgM) and lymphocyte subsets were within normal ranges. Hypogammaglobulinemia, as previously reported in some patients (Zhou et al. 2014; Gonzalez Santiago et al. 2015), was not present in our patient, possibly influenced by her mutations, onset age, race, and disease course. Her clinical symptoms were possibly relevant to the background of persistent inflammation, with elevated levels of IL-6, IL-8, CRP, and platelets.

Genetic analysis confirmed that she had two novel *CECR1* gene mutations (c.1211T>C, p.Phe404Ser and c.1114 G>A, p.Val372Met). As the previous report (Zhou et al. 2014; Navon Elkan et al. 2014) described, the *CECR1* gene, located on chromosome 22q11, includes ten exons, encoding the protein ADA2 composed by four domains: a signal sequence, a dimerization domain, a putative receptor-binding domain, and a catalytic domain. F404 and V372 amino acids are both located in the catalytic domain of ADA2 protein. Further analysis indicated that the two mutations might result in structural changes and may influence protein structure. Regrettably, plasma ADA2 activity was not tested in the patient to show if the two mutations were pathogenic, a major defect of our study. Nevertheless, combining the clinical symptoms, structure analysis, and CADD scores, the two mutations are predicted to be pathogenic.

Several studies suggested that anti-TNF-α and anti-IL-6 could contribute to clinical improvement. Anti-IL-6 therapy with tocilizumab could control recurrent fever and inflammations (Van Eyck et al. 2014). However, our patient suffered a stroke even though she received tocilizumab therapy as with DADA2 patients reported earlier (Nanthapisal et al. 2016; Meyts and Aksentijevich 2018).

DADA2 patients frequently develop hematological manifestations, including anemia, cytopenia, and lymphopenia (Hashem et al. 2017a, b; Michniacki et al. 2018). HSCT may control the vasculopathy, the hematological phenotype and immunodeficiency (Zhou et al. 2014; Bucciol et al. 2017; Meyts and Aksentijevich 2018). Importantly, 14 DADA2 patients with HSCT were cured of their hematological features, immunodeficiencies and vasculopathies (Hashem et al. 2017a, b). HSCT might be the most suitable for DADA2 patients with hematological and/or immunological dysfunction. Finally, our patient underwent HSCT, experiencing improvement of inflammation and clinical manifestations. Only a few Chinese DADA2 patients have been reported, and every report of successful or unsuccessful HSCT in this devastating disease counts. Our study suggested that, for DADA2 patients who were unresponsive to reagent treatments, HSCT should be considered. Because persistent inflammation might increase the risk of complications in these patients, controlling inflammation before HSCT appears necessary. It is important, therefore, to choose the most suitable therapeutic strategies, regarding the wide spectrum and severity of phenotypes of patients with ADA2 deficiency.

In conclusion, we reported the clinical characteristics, immunological features, treatments and successful HSCT in a 13-month-old Chinese patient with DADA2 harboring novel *CECR1* gene mutations. Our study provides more evidence for clinical manifestations and therapeutic strategies for DADA2.

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Compliance with ethical standards

The study was approved by the Ethics Committee of the Children's Hospital of Fudan University. The patient's parents gave written informed consent.

Conflict of interest The authors declare that they have no conflict of interest.

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