



## FULL LENGTH ARTICLE

# Novel biallelic *TRNT1* mutations lead to atypical SIFD and multiple immune defects

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**Abstract** Mutations in the gene encoding transfer RNA (tRNA) nucleotidyltransferase, CCA-adding 1 (*TRNT1*), an enzyme essential for the synthesis of the 3'-terminal CCA sequence in tRNA molecules, are associated with a rare syndrome of congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers, and developmental delay (SIFD). Clinical manifestations and immunological phenotypes were assessed in a Chinese patient with novel compound heterozygous mutations in *TRNT1*. The patient required multiple hospitalizations starting at the age of 2 years for recurrent fevers without an infective cause. During the febrile episode, the patient was found to have microcytic hypochromic anemia, B cell lymphopenia, and

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hypogammaglobulinemia. Targeted gene sequencing identified novel compound heterozygous mutations in the *TRNT1* gene (c.525delT, p.Leu176X; c.938T>C, p.Leu313Ser). Immunophenotyping revealed increased CD8<sup>+</sup> T cells, CD4<sup>+</sup> terminally differentiated effector memory helper T lymphocytes (CD4 TEMRA), and CD4<sup>+</sup> effector memory lymphocytes (CD4 EM). Analysis of CD4<sup>+</sup> T subsets identified decreased T follicular helper cells (Tfh) with a biased phenotype to Th2-like cells. The patient also showed a lower percentage of switched memory B (smB) cells. Additionally, defects in the cytotoxicity of the patient's NK and  $\gamma\delta$ T cells were shown by CD107a expression. In conclusion, *TRNT1* mutations may lead to multiple immune abnormality especially humoral and cytotoxicity defects, which indicate that SIFD is not only suffered 'Predominantly antibody deficiencies' in IUIS classification system, and further studies are needed to understand the pathogenesis of immunodeficiency in these patients. Copyright © 2020, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Biallelic loss-of-function mutations in the gene encoding transfer RNA (tRNA) nucleotidyltransferase, CCA-adding 1 (*TRNT1*), cause a rare syndrome of sideroblastic anemia with B cell immunodeficiency, periodic fevers, and developmental delay (SIFD).<sup>1,2</sup> Patients with mutations in *TRNT1* may also present with retinitis pigmentosa, cataracts, sensorineural hearing loss, seizures, cardiomyopathy, hepatosplenomegaly, and brittle hair. SIFD is a severe multi-organ syndrome with life-threatening complications, and many SIFD patients die in the first decade of life.<sup>2,3</sup> Atypical SIFD without sideroblastic anemia or periodic fevers has also been reported, representing a mild phenotype of *TRNT1* deficiency.<sup>4,5</sup>

*TRNT1* is a nucleotidyltransferase involved in tRNA processing. This enzyme is responsible for adding the CCA trinucleotide to the 3' end of all precursor tRNAs, and is required for both mitochondrial and cytoplasmic translation.<sup>6</sup> Disease-causing mutations lead to a reduction in CCA-adding activity, defective mitochondrial translation, and impaired clearance of tRNAs with backbone damage.<sup>7</sup> The loss of *TRNT1*-dependent quality control mechanisms leads to an impaired intracellular stress response.<sup>6</sup>

To date, about 30 patients with *TRNT1* deficiency have been described in the literature, with significant heterogeneity in the clinical phenotype and underlying immunological defects. Here, we analyzed the clinical and immunologic features of a Chinese patient with novel compound heterozygous mutations in *TRNT1*, extending the previously described SIFD phenotypes.

## Materials and methods

### Patient

The patient enrolled in this study was a 5-year-old girl born to a nonconsanguineous family. Clinical data and blood were collected when she first visited the Children's Hospital of Chongqing Medical University in July 2017. After a 2 year follow-up period, the patient was reassessed. 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

Whole blood samples were sent to Kangso Medical Inspection (Beijing, China) and subjected to targeted gene sequencing, including 230 primary immunodeficiency (PID) genes. Genomic DNA (gDNA) was extracted from whole blood using the QIAamp DNA Mini Kit (Qiagen GmbH, Germany). Total RNA was isolated from whole blood using the Total RNA Miniprep Kit (Axygen, China) and subjected to reverse transcription-polymerase chain reaction (RT-PCR) using the EvoScript Universal cDNA Master (Roche Diagnostics GmbH, Germany) according to the manufacturers' instructions. Mutations in the *TRNT1* gene were verified by Sanger sequencing.

### Structural analysis of *TRNT1*

The crystal structure of *TRNT1* (1OU5 from the protein data bank) was used as the template, which was determined by X-ray diffraction at a resolution of 3.4 Å.<sup>8</sup> The structural impact of mutant Leu313Ser was analyzed by Swiss-PdbViewer. The residue 313 and certain nearby residues within 3 Å were illustrated. For clear demonstration of inter-residue relationship, some residues were highlighted in the indicated colors with the computed hydrogen bonds being labeled.

### Immunophenotyping

Immunophenotyping of lymphocyte subpopulations was performed with the following antibodies: CD3-PerCP (clone: HIT3a, BioLegend), CD4-FITC (clone: RPA-T4, BioLegend), CD8-BV510 (clone: RPA-T8, BioLegend), CD45RA-PE-Cy7 (clone: HI100, BioLegend), CD27-APC (clone: M-T271, BioLegend), TCR  $\alpha\beta$ -PE (clone: IP2b, BioLegend), TCR  $\gamma\delta$ -BV421 (clone: B1, BioLegend), CD19-APC (clone: HIB19, BioLegend), CD27-V450 (clone: M-T271, BioLegend), IgD-AF488 (clone: IA6-2, BioLegend), CD24-PE (clone: ML5,

BioLegend), and CD38-PerCP (clone: HIT2, BioLegend). The samples were acquired on a FACSCanto II flow cytometer (BD Biosciences), and the data were analyzed using FlowJo. All reference values were obtained from our recent study on peripheral lymphocyte phenotyping.<sup>9</sup>

### Flow cytometric analysis of CD4<sup>+</sup> T cell and B cell subsets

Circulating follicular helper T (cTfh) cells, circulating follicular regulatory T (Tfr) cells, Th1 cells, Th2 cells, Th17 cells, and subsets of Tfh cells were quantified in separate experiments in 50  $\mu$ L whole blood. The whole blood was stained with CD3-PerCP (clone: HIT3a, BioLegend), CD4-PE-Cy7 (clone: RPA-T4, BioLegend), CD45RO-APC (clone: UCHL1, BD Biosciences), CD45RA-FITC (clone: HI100, BD Biosciences), CXCR5-BV421 (clone: J25ID4, BioLegend), CD25-APC (clone: MT271, BioLegend), CD127-PE (clone: A019D5, BioLegend), CXCR3-APC (clone: 1C6, BD Biosciences), and CCR6-PE (clone: G034E3, BioLegend) for 30 min at 4 °C. To analyze T regulatory cells (Treg), PBMCs were stained with CD4-PE-Cy7 (clone: RPA-T4, BioLegend), CD25-BV421 (clone: BC96, BioLegend), and CD45RA-FITC (clone: HI100, BD Biosciences), and then fixed and permeabilized using the eBioscience Intracellular Fixation and Permeabilization kit (ThermoFisher Scientific) and stained with FOXP3-PE (clone: PCH101, eBioscience/ThermoFisher Scientific), CD152-APC (clone: BNI3, BD Biosciences) and Helios-PerCP-cy5.5 (clone: 22F6, BioLegend). For characterization of circulating B cell subsets, PBMCs were stained with CD19-PerCP-Cy5.5 (clone: SJ25C1, BioLegend), CD27-PE-Cy7 (clone: MT271, BioLegend), and IgM-APC (clone: G20-127, BD Biosciences). The samples were acquired on a FACSCanto II flow cytometer, and the data were analyzed using FlowJo.

### Proliferation of T cells and B cells

PBMCs were incubated with 1.25  $\mu$ L/mL carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen/ThermoFisher Scientific) at 37 °C. After 10 min, the cells were washed twice with 5 mL of 4 °C Roswell Park Memorial Institute (RPMI) medium containing 10% fetal bovine serum (FBS). Cells were then resuspended in 600  $\mu$ L RPMI/10% FBS and seeded in 96-well plates with 5  $\mu$ g/mL phytohemagglutinin (PHA), 10  $\mu$ g/mL lectin from pokeweed mitogen (PWM), and the same volume of RPMI for 72 h. After staining with CD3-PerCP (clone: HIT3a, BioLegend), CD4-PE-Cy7 (clone: RPA-T4, BioLegend), CD8-PE (clone: RPA-T8, BioLegend), and CD19-APC (clone: HIB19, BioLegend) antibodies, cells were analyzed on a FACSCanto II flow cytometer.

### Quantification of T cell receptor excision circles

Nest and real-time quantitative PCR (RT-qPCR) for detecting T cell receptor excision circles (TRECs) and CD3 was performed as described previously.<sup>10,11</sup> The RT-qPCR reactions were performed in a final volume of 20  $\mu$ L containing 2X SuperReal PreMix (Tiangen Biotech, Beijing, China), 6  $\mu$ M TREC or CD3 forward and reverse primers (Sangon Biotech, Shanghai, China), 4  $\mu$ M probes (Sangon

Biotech, Shanghai, China), and 5  $\mu$ L DNA. Samples were heated at 95 °C for 5 min and then subjected to 40 cycles of 95 °C for 15 s, 58 °C for 30 s, and 68 °C for 30 s (CFX96 Real-Time System, Bio-Rad). The copy numbers of TRECs and CD3 were calculated relative to standard curves generated from serially diluted plasmids.

### CDR3 spectratyping

Briefly, each T cell receptor (TCR) V $\beta$  fragment was amplified with one of 23 V $\beta$ -specific primers and a 5' FAM-labeled C $\beta$  primer.<sup>12,13</sup> The cycling conditions were 45 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min. The PCR product was sent to the Sangon Biotech Company (Shanghai, China) for sequencing. The obtained data was then analyzed using Gene Mapper V3.5, and a scoring system was used to evaluate the TCR V $\beta$  diversity, in which a score of less than 4 indicated a skewed subfamily.

### NK and $\gamma\delta$ T cell degranulation assay

To evaluate NK and  $\gamma\delta$ T cell degranulation,  $1 \times 10^6$  PBMCs were mixed with  $2 \times 10^5$  target cells (K562 cells) in 400  $\mu$ L RPMI/10% FBS and seeded in 96-well plates for 4 h at 37 °C. Thereafter, the cells were stained with CD3-PerCP (clone: HIT3a, BioLegend), TCR  $\gamma\delta$ -BV421 (clone: B1, BioLegend), CD56-PE (clone: MY31, BD Biosciences), and CD107a APC (clone: H4A3, BD Biosciences) for 30 min. CD107a positivity was measured on a FACSCanto II flow cytometer.

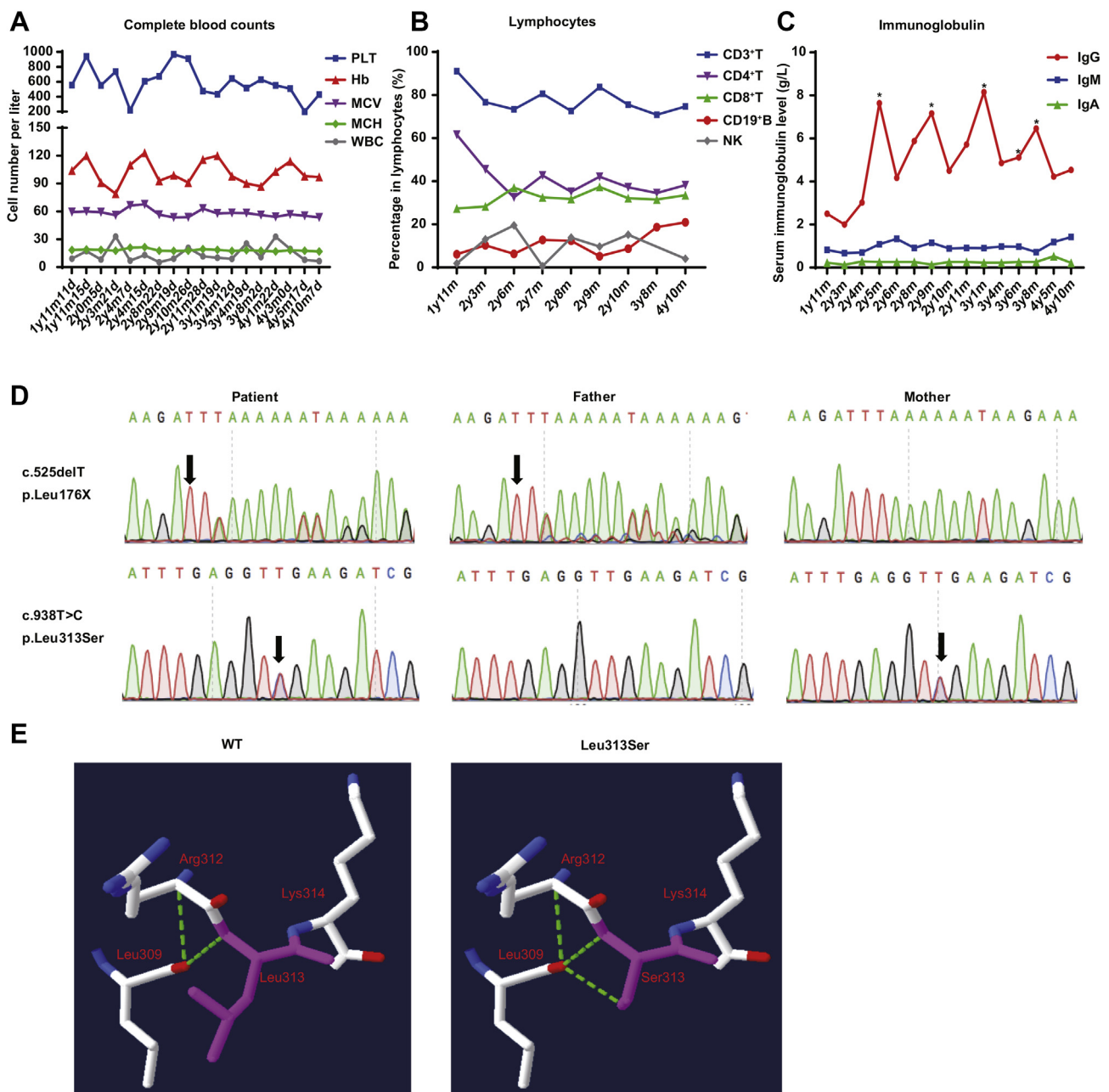
### Statistical analysis

Data were analyzed using an unpaired two-tailed Student t test. All statistical analyses were conducted in GraphPad Prism 7 software (GraphPad Software, Inc., San Diego, CA).  $P \leq 0.05$  was considered to indicate a significant difference.

## Results

### Clinical characteristics

The patient first presented with fever at 7 months, and the initial examination indicated cytomegalovirus (CMV) infection. She required multiple hospitalizations beginning at the age of 2 for recurrent fevers and elevated inflammatory markers, without an infective cause. The frequency and duration of febrile episodes were remarkably consistent, occurring at predictable 1 month intervals, with resolution within 5 days apparently uninfluenced by antibiotics. However, the interval between the attacks increased over time. During a febrile episode, she also developed a subcutaneous mass in the right shoulder and left waist. Acute cellulitis was diagnosed and successfully treated with antibiotics. Meanwhile, microcytic hypochromic anemia was detected, but microscopic investigation of the peripheral blood and bone marrow showed no signs of sideroblastic anemia. Immune deficiency was suspected, and the patient was found to have B cell lymphopenia and hypogammaglobulinemia. There were no



**Figure 1** Clinical and genetic characterization of the patient. **(A)** The complete blood counts showed increased platelets (blue), fluctuations in white blood cell numbers (gray) and hemoglobin levels (red), and low mean corpuscular volume (MCV; purple) and mean corpuscular hemoglobin (MCH; green). The normal reference ranges are indicated as following: WBC 4–12\*10<sup>9</sup>/L; PLT 100–380\*10<sup>9</sup>/L; Hb 110–150 g/L; MCV 80–100 fL; MCH 26–32 pg. **(B)** B cell numbers were initially low, but gradually normalized (red). The normal reference ranges are indicated as following: CD3<sup>+</sup>T 55–78%; CD4<sup>+</sup>T 27–53%; CD8<sup>+</sup>T 19–34%; CD19<sup>+</sup>B 10–31%; NK 4–26%. **(C)** Low levels of IgG (red) were observed. \* indicates a measurement taken after immunoglobulin supplementation therapy. The normal reference ranges are indicated as following: IgG 5.3–21.9 g/L; IgM 0.5–2.3 g/L; IgA 0.6–3.5 g/L. **(D)** Sanger sequencing confirmed compound heterozygous mutations in *TRNT1* in the patient. The black arrows indicate the locations of mutations. The reference transcript sequence of *TRNT1* is NM\_182916. **(E)** The structural impact of mutant Leu313Ser was analyzed on the basis of template of 1OU5 from PDB by Swiss-PdbViewer. Carbon atoms in white, oxygen in red and nitrogen in blue. Hydrogen bonds are shown as dotted lines. Residues Leu313/Ser313 are highlighted in purple.



**Table 1** Immunophenotyping of peripheral blood lymphocyte subsets.

	2017.07(2 y 10 m)				2019.07(4 y 10 m)			
	Percentage	Reference	numbers/ $\mu$ l	Reference	Percentage	Reference	numbers/ $\mu$ l	Reference
T cells	83.7 $\uparrow$	53.4–72.0	4124.4 $\uparrow$	1775.5–3953.0	74.7	59.5–75.6	2548.0	1480.3–2847.3
CD8 $^{+}$ T cells	30.3	16.3–30.0	1843.8 $\uparrow$	531.2–1520.7	33.5 $\uparrow$	19.7–32.0	1142.7 $\uparrow$	552.6–1127.3
CD8 naïve	45.1	38.2–86.2	831.6	294.6–971.3	40.9	38.0–79.1	467.4	293.4–768.4
CD8 TEMRA	24.5	0.5–24.5	451.7 $\uparrow$	3.1–294.0	28.6 $\uparrow$	1.3–22.9	326.8 $\uparrow$	9.1–209.8
CD8 CM	26.4	6.7–34.1	486.8 $\uparrow$	54.2–379.4	25.4	11.9–36.9	290.2	79.6–350.4
CD8 EM	4.05	0.6–12.0	74.7	4.0–163.0	5.0	1.1–14.5	57.1	7.9–104.2
CD4 $^{+}$ T cells	58.1 $\uparrow$	26.2–45.5	2074.5	948.2–2476.5	36.2	28.5–41.1	1233.7	767.3–1592.5
CD4 naïve	49.0	46.4–81.2	1016.5	529.7–1836.6	44.7	40.8–72.7	551.5	338.7–1037.0
CD4 TEMRA	1.5 $\uparrow$	0.0–0.5	30.7 $\uparrow$	0.0–12.3	2.4 $\uparrow$	0.0–1.5	29.9 $\uparrow$	0.0–16.7
CD4 CM	39.1	17.1–47.6	811.1 $\uparrow$	220.4–799.7	41.8	21.7–52.7	515.7	232.1–600.9
CD4 EM	10.4 $\uparrow$	0.9–5.2	215.8 $\uparrow$	14.2–93.5	11.1 $\uparrow$	1.9–9.2	136.9 $\uparrow$	20.5–96.8
TCR $\alpha\beta$ $^{+}$ DNT	1.3	0.57–2.4	54.3	16.1–57.6	1.3	0.2–2.4	33.4	3.8–49.5
$\gamma\delta$ T cells	18.5 $\uparrow$	5.1–17.6	763.0 $\uparrow$	127.7–520.1	19.4	7.0–19.6	494.3 $\uparrow$	133.7–427.8
B cells	5.2 $\downarrow$	13.9–30.5	253.9 $\downarrow$	537.1–1464.4	21.0	10.5–21.8	714.7	303.5–777.3
Memory B	12.3	3.6–18.6	31.2 $\downarrow$	33.1–145.9	6.0 $\downarrow$	8.6–20.2	43.0	37.7–114.8
Naïve B	70.6	59.6–85.3	179.3 $\downarrow$	371.5–1196.7	85.9 $\uparrow$	52.0–75.8	614.0 $\uparrow$	171.5–469.3
Transitional B	28.9 $\uparrow$	4.7–15.7	73.4	33.5–180.8	9.4	3.4–11.2	67.1 $\uparrow$	14.4–59.6
Plasmablasts B	8.2	0.6–10.3	20.7	4.0–87.6	2.5	0.8–9.8	17.9	3.9–39.8
NK cells	9.7	6.5–22.2	477.2	241.1–977.9	4.1 $\downarrow$	7.8–21.1	138.8 $\downarrow$	227.5–667.8
CD4/CD8	1.1	1.1–2.5	—	—	1.1	1.0–2.1	—	—

TEMRA: terminally differentiated effector memory helper T lymphocytes; CM: central memory; EM: effector memory.

Naïve, CD45RA $^{+}$ CD27 $^{+}$ ; TEMRA, CD45RA $^{+}$ CD27 $^{-}$ ; CM, CD45RA $^{-}$ CD27 $^{+}$ ; EM, CD45RA $^{-}$ CD27 $^{-}$ ; TCR $\alpha\beta$  $^{+}$ DNT, CD3 $^{+}$ TCR $\alpha\beta$  $^{+}$ CD4 $^{-}$ CD8 $^{-}$ ; Memory B cells, CD19 $^{+}$ CD27 $^{+}$ IgD $^{-}$ ; naïve B cells, CD19 $^{+}$ CD27 $^{-}$ IgD $^{+}$ ; transitional B cells, CD19 $^{+}$ CD24 $^{++}$ CD38 $^{++}$ ; plasmablasts, CD19 $^{+}$ CD24 $^{-}$ CD38 $^{++}$ .

The age-matched reference values were obtained from one previous study (Ding et al,2018) focusing on peripheral lymphocyte phenotyping in healthy Chinese children.

manifestations of developmental delay. Complete blood counts revealed fluctuating levels of hemoglobin (range, 79–123 g/L) and white blood cell counts (range, 5.4–32.9 $\times$ 10<sup>9</sup>/L), as well as elevated platelet counts (range, 202–967 $\times$ 10<sup>9</sup>/L) (Fig. 1A). The cytokine detections showed increased levels of surface Interleukin 2 receptor (range, 754–1755 U/mL), Interleukin 6 (range, 18.1–176 pg/mL), Interleukin 8 (range, 75.1–337 pg/mL) and TNF $\alpha$  (range, 8.61–25.8 pg/mL) during febrile episodes. Immunological analyses revealed decreased numbers of B cells and reduced IgA and IgG levels, but normal IgM levels (Fig. 1B, C).

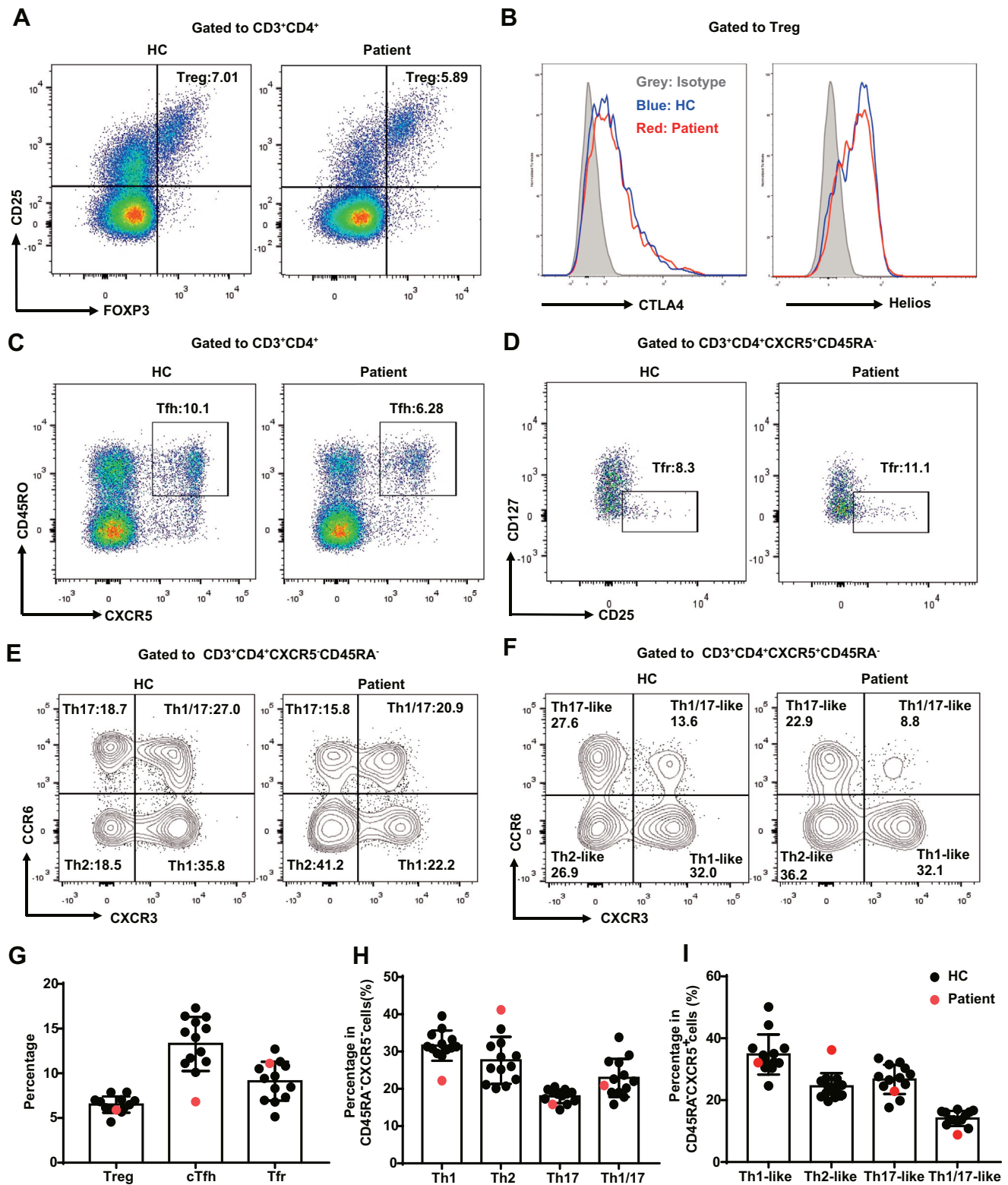
### Novel compound heterozygous mutations in TRNT1

Whole exome sequencing identified novel compound heterozygous mutations in the *TRNT1* gene: a deletion in exon 5 (c.525delT) leading to a premature stop codon (p.Leu176X), and a missense mutation in exon 7 (c.938T>C, p.Leu313Ser) (Fig. 1D). The Leu313Ser mutation has not been reported in the Exome Aggregation Consortium (ExAC) or 1000 Genomes databases, and was predicted to cause disease by several algorithms [Scale Invariant Feature Transform (SIFT), Polyphen2, and Mutation Taster]. Structural analysis showed that replacing the hydrophobic Leu313 with a hydrophilic serine promotes the formation of hydrogen bonds with Leu309, it may affect the interaction between neighboring helices for Leu313 is located at the

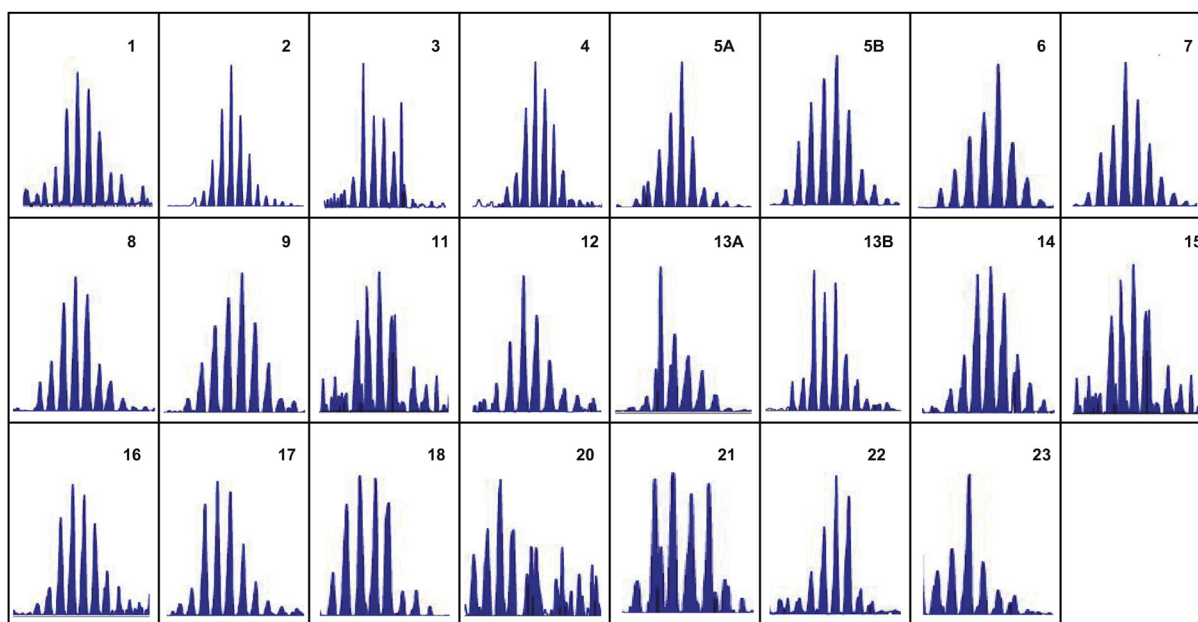
end of helix (Fig. 1E). The patient also had a heterozygous mutation in the beta-thalassemia gene (NM\_000518.4: c.-78A>G). Both of the patients' parents were heterozygous for *TRNT1* gene mutations, and mutations were confirmed by Sanger sequencing (Fig. 1D).

### Skewed peripheral T cell subsets

Immunophenotyping of peripheral blood lymphocyte subsets at two time-points (the age of 2 y 10 m and 4 y 10 m) revealed increased percentage and numbers of CD8 $^{+}$  T cells, CD4 $^{+}$  terminally differentiated effector memory helper T lymphocytes (CD4 $^{+}$  TEMRA), and CD4 $^{+}$  effector memory lymphocytes (CD4 EM) (Table 1). The frequency of Treg was normal, as was the expression of CTLA4 and Helios (Fig. 2A, B, G). Analysis of CD4 $^{+}$  cells showed reduced numbers of cTfh, but the frequency of Tfr was comparable to that of healthy controls (Fig. 2C, D, G). When non-cTfh memory and cTfh subsets were analyzed, Th2 and Th2-like cells were increased, while Th1 and Th1/17-like cells were reduced and Th17-phenotype cells were unaffected (Fig. 2E, F, H, I). The patient showed a normal polyclonal TCR repertoire (Fig. 3), and the TREC count was slightly elevated at 438 copies/10<sup>5</sup> cells (normal range, 291–429 copies/10<sup>5</sup> cells). Additionally, T cell proliferation was normal as the percentages of proliferating CD4 $^{+}$  T and CD8 $^{+}$  T cells after stimulation by PHA were 30.0 and 58.9% in the patient (Fig. 4).



**Figure 2** Skewed peripheral T cell subsets in a patient with *TRNT1* deficiency. (A) and (B) Compared with healthy controls, the patients showed normal percentages of Treg and expression of CTLA4 and Helios. (C) and (D) The percentage of cTfh cells decreased while the Tfr cell frequency remained normal. (E) The non-cTfh memory cells ( $CD3^+CD4^+CXCR5^+CD45RA^-$ ) were skewed to a Th2 phenotype. (F) The cTfh cells ( $CD3^+CD4^+CXCR5^+CD45RA^-$ ) were skewed to a Th2-like phenotype, and Th1/17-like cells were reduced. (G)–(I) Percentages of Treg, cTfh, Tfr, non-cTfh memory subsets, and cTfh subsets in the patient and in healthy controls (n = 12).

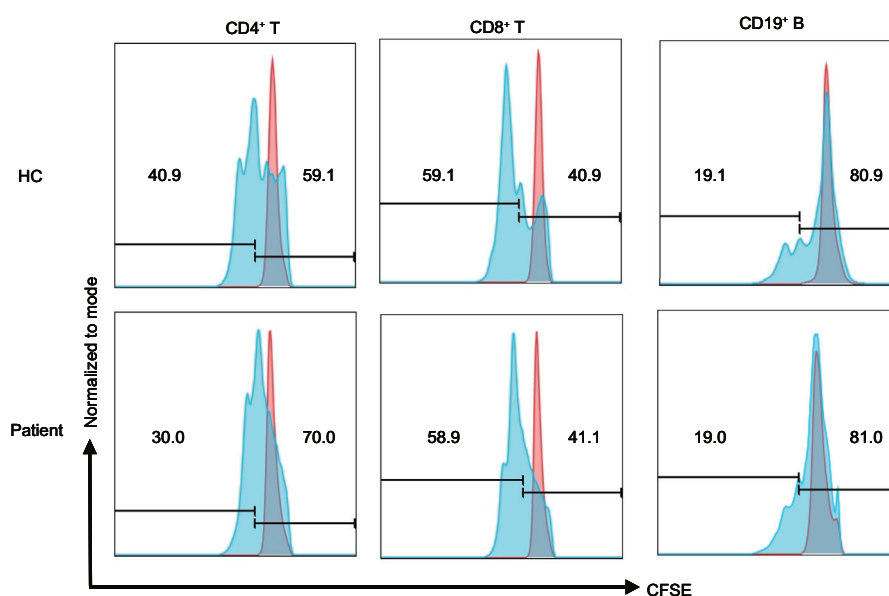


**Figure 3** CDR3 spectratyping revealed a normal polyclonal TCR repertoire. The numbers of V $\beta$ -specific primers were indicated on the right upper corner and a Gaussian distribution of blue peaks indicated polyclone of TCRV $\beta$  subfamilies.

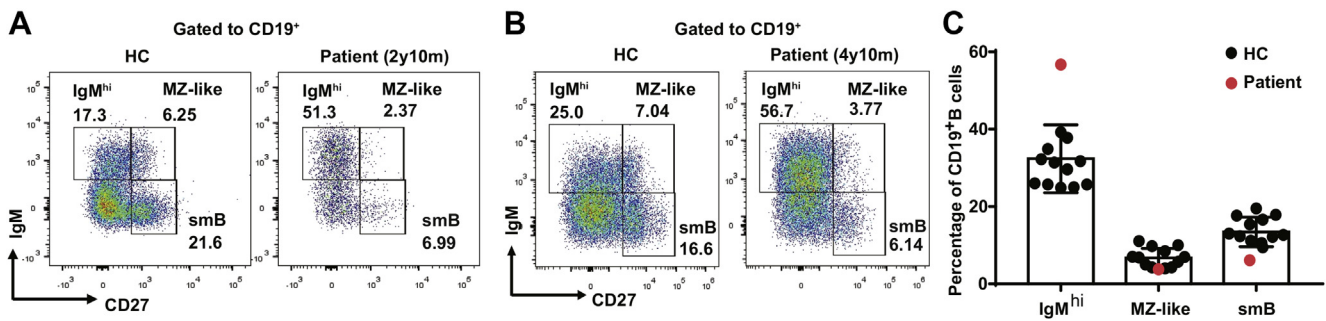
### Abnormal B cell phenotypes

Immunophenotyping of peripheral blood lymphocyte subsets initially revealed decreased numbers of total B cells, and increased numbers of transitional B cells. However, at

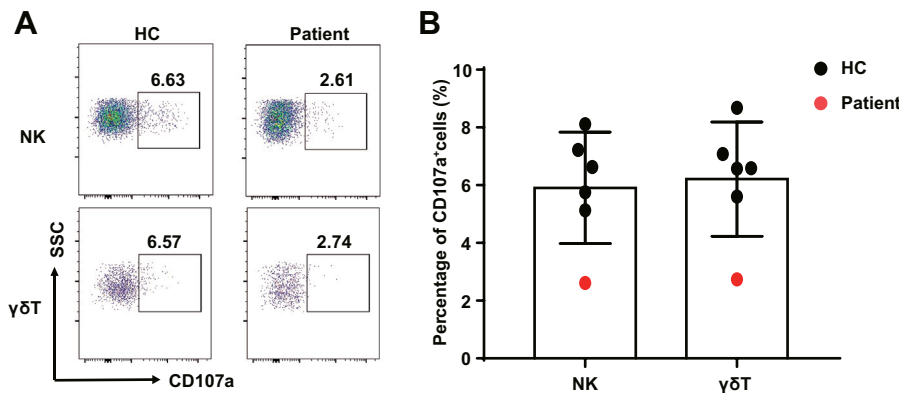
the second blood draw, the patient showed normal total B cells and compromised memory B cells (Table 1). At both time-points, the patient showed a decrease in switched memory B (smB) and increase in IgM<sup>hi</sup> cells, compared with healthy controls (Fig. 5A–C). The percentage of



**Figure 4** The proliferation of CD4<sup>+</sup>T cells (left panel), CD8<sup>+</sup> T cells (middle panel) and CD19<sup>+</sup> B cells (right panel) was normal in patient, compared with age-matched healthy control. Red peak: unstimulated; blue peak: after stimulated.



**Figure 5** Abnormal B cell phenotype of a patient with *TRNT1* deficiency. (A) and (B) B cell subsets were analyzed when the patient was 2 years, 10 months of age, and 2 years later. The patient consistently showed reduced smB and increased IgM<sup>hi</sup> B cells. (C) Percentages of smB and MZ-like B cells in the patient and healthy controls (n = 12).



**Figure 6** When stimulated with K562 cells, the patient's NK and  $\gamma\delta$ T cells showed less CD107a expression than healthy controls. (A) The patient showed decreased percentage of CD107a<sup>+</sup> cells in NK and  $\gamma\delta$ T cells. (B) Percentage of CD107a<sup>+</sup> cells in the patient and healthy controls (n = 5).

proliferating CD19<sup>+</sup> B cells after stimulation by PWM was 19.0 in patient (Fig. 4).

### Cytotoxicity defects in NK and $\gamma\delta$ T cells

Immunophenotyping of peripheral blood lymphocyte subsets showed increased  $\gamma\delta$ T cells and decreased NK cells (Table 1). When co-cultured with K562 cells, NK and  $\gamma\delta$ T cells of patient expressed lower CD107a compared with healthy controls (Fig. 6).

### Treatment and outcome

The patient received short-term prednisone during febrile episodes. Immunoglobulin supplementation therapy was started since low level IgA and IgG levels were detected and was discontinued after 1 year. The frequency of febrile episodes decreased over time, and the time between attacks increased to 3–4 months. The patient has been asymptomatic for the past several months.

### Discussion

Patients with *TRNT1* deficiency show significant clinical heterogeneity. Two different *TRNT1*-related disease

entities have been reported: a pediatric disorder that starts early in life and features congenital sideroblastic anemia, immunodeficiency, fevers, and developmental delays, and another phenotype that mainly shows ophthalmological features, including adult-onset retinitis pigmentosa or childhood cataracts.<sup>2,4,5</sup> In this paper, we report a patient who presented with a mild phenotype, including recurrent fevers, hypogammaglobulinemia, and microcytic hypochromic anemia. The diagnosis of sideroblastic anemia was ruled out because neither iron deposition nor ringed sideroblasts were detected. The patient was diagnosed with a periodic fever syndrome because the frequency and duration of febrile episodes was remarkably consistent. During the 2 year follow-up, the frequency of febrile episodes decreased over time, and the interval between attacks extended to 3–4 months. Targeted gene sequencing identified novel compound heterozygous mutations in the *TRNT1* gene (c.525delT, p.Leu176X; c.938T>C, p.Leu313Ser). Structural analysis showed that replacing the hydrophobic Leu313 with a hydrophilic Ser may affect the interaction between neighboring helices since abnormal formation of hydrogen bond. Therefore, we suggest the milder phenotype may result from the missense mutation and further research is needed to study the pathogenesis of immunodeficiency in patients with *TRNT1* mutations.



Most patients with TRNT1 deficiency show significant B cell lymphopenia and hypogammaglobulinemia. Progressive B cell immunodeficiency was also noted in a 23-year-old male patient whose total lymphocyte and B cell numbers were in the lower end of the normal range in childhood but dropped significantly after the age of 21.<sup>14</sup> Additional lymphocyte immunophenotyping in previous studies revealed a decreased frequency of Tfh in patients with TRNT1 deficiency, suggesting that the immunodeficiency is not necessarily confined to B cells.<sup>15,16</sup> The patient described here showed normal numbers of Treg and Tfr, as well as normal expression of CTLA4 and Helios. This is consistent with the fact that Tfr arise from Tregs, which are largely unaffected by the mutations reported here. However, cTfh numbers were decreased in the patient compared with healthy controls. Tfh are a distinct subset of T cells with a crucial role in humoral adaptive immunity.<sup>17</sup> Interactions between Tfh and their cognate B cells provide fundamental signals that drive the production of high-affinity antibodies through affinity maturation and class-switch recombination.<sup>18</sup> Circulating follicular helper T cells were recently identified in human subjects as reflective of Tfh cells, decreased numbers of cTfh have been found in patients with several monogenic immunodeficiency disorders associated with humoral deficiency.<sup>19,20</sup> Differentiation of naïve CD4<sup>+</sup> T cells into Tfh is a complex process requiring integration of signals delivered by dendritic cells, B cells, and cytokines, and driven by specific signaling pathways and transcription factors. Therefore, whether the deficiency in cTfh was directly caused by TRNT1 mutation remains unknown. When non-cTfh memory and cTfh subsets were analyzed, Th2 and Th2-like cells were found to be significantly increased in this patient. The skewing of these subsets to a Th2 phenotype is different from the findings of a previous study,<sup>14</sup> emphasizing the immunological heterogeneity of TRNT1 deficiency. The patient was also noted to have decreased switched memory B cells and increased IgM<sup>hi</sup> B cells. It is also worth mentioning that although the patient's total B cell numbers were normal at the most recent analysis, smB cells remained decreased. This decrease in smB cells is consistent with the patient's reduced serum IgG and IgA levels.

NK cell abnormalities in TRNT1-deficient patients have been noted. NK cell phenotyping revealed a significant increase in CD56<sup>bright</sup> cells, which represent a more immature stage of NK differentiation than CD56<sup>dim</sup> cells.<sup>14</sup> Patients with classical NK cell deficiency display defects in both NK cell numbers and function in the peripheral blood. They typically suffer from complicated and disseminated varicella zoster virus, herpes simplex virus, cytomegalovirus, Epstein–Barr virus (EBV), and human papillomavirus infections.<sup>21</sup> Cytomegalovirus infection was observed in our patient when she was 7 months old. When co-cultured with K562 cells, NK and  $\gamma\delta$ T of patient expressed lower CD107a compared with cells from healthy controls, indicating compromised NK and  $\gamma\delta$ T cell function. Additional data is needed to determine whether the patient's NK and  $\gamma\delta$ T cells exhibit defects in cytotoxicity against CMV-infected cells.

## Conclusion

In conclusion, we report a Chinese patient with novel heterozygous mutations in *TRNT1* who presented with recurrent fevers, hypogammaglobulinemia, and microcytic hypochromic anemia, further expanding the clinical spectrum associated with *TRNT1* mutations. Comprehensive immunological assessments illustrated that *TRNT1* mutations may affect multiple lymphocyte subsets. The pathogenesis of immunodeficiency in patients with *TRNT1* mutations remains poorly characterized, and further detailed research is needed.

## Author contributions

All authors contributed to the study conception and design. Lu Yang and Xiuhong Xue designed, conducted, and analyzed immunological experiments; Xuemei Chen, Ting Zeng, and Qin Zhao assisted with basic immunological analysis; Xuemei Tang and Jun Yang managed the patient; Yunfei An and Xiaodong Zhao designed the project and supervised the research. The first draft of the manuscript was written by Lu Yang and revised by Yunfei An, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Conflict of interest

The authors declare no conflict of interest.

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