ORIGINAL ARTICLE

An early-onset SLE patient with a novel paternal inherited *BACH2* mutation

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Abstract

BACH2-related immunodeficiency and autoimmunity (BRIDA) is an inborn error of immunity, newly reported in 2017, presenting with symptoms of immunoglobulin deficiency and ongoing colitis. Studies using a mouse model have demonstrated that BACH2 deficiency predisposes individuals to systemic lupus erythematosus (SLE); however, no BACH2 deficiency has been reported in SLE patients. Here we describe a patient with BRIDA presenting with early-onset SLE, juvenile dermatomyositis, and IgA deficiency. Whole exome sequencing analysis of the patient and her parents revealed a novel heterozygous point mutation in *BACH2*, c.G1727T, resulting in substitution of a highly conserved arginine with leucine (R576L), which is predicted to be deleterious, in the patient and her father. Reduced BACH2 expression and deficient transcriptional repression of the BACH2 target, BLIMP1, were detected in PBMCs or lymphoblastoid cell lines of our patient. Notably, extreme reduction of memory B cells was detected in the patient's father, although he had no obvious symptoms. SLE symptoms and recurrent fever were relieved by treatment with prednisone combined with tofacitinib. Thus, we present the second report of BRIDA and demonstrate that *BACH2* may be a monogenic cause of SLE.

Keywords BACH2 deficiency · inborn error of immunity · systemic lupus erythematosus · monogenic lupus

Introduction

BTB and CNC homolog 2 (BACH2) is a critical transcription factor involved in immune responses by controling T and B lymphocyte differentiation and maturation [1]. Preliminary research has shown that BACH2 is required B cell development, and that it can promote class switch recombination (CSR) and somatic hypermutation (SHM) as well as repressing plasma cell differentiation [2–5]. Recent studies have indicated that BACH2 has essential transcriptional activity at super enhancer regions of target genes in T lymphocytes, thereby maintaining T cells in a naïve state, promoting regulatory T cell (Treg) polarization, and mediating immune homeostasis [6–11]. Gene polymorphisms of the human *BACH2* gene are involved in numerous autoimmune and allergic diseases, including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (SLE) [12–14]. Investigation of a mouse model demonstrated that BACH2 deficiency predisposed mice to SLE via T cell-dependent extrafollicular activation of antibody responses [15, 16]. In 2017, Afzali et al. first described patients with BACH2-related immunodeficiency and autoimmunity (BRIDA) resulting from *BACH2* haploinsufficiency; their research demonstrated that all three reported patients had a history of early-onset immunoglobulin deficiency and intestinal inflammation, and developed a common variable immunodeficiency (CVID) phenotype [17].

Here, we report a fourth patient with a novel paternally inherited *BACH2* mutation (reported as P10 in a large early-onset SLE cohort with mutation site and clinical phenotype by our group members [18]); however, unlike the previously reported BRIDA cases, the patient described here had

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early-onset SLE, juvenile dermatomyositis (JDM), and IgA deficiency.

Materials and Methods

Patient

An 8-year-old girl was enrolled in our study. She presented with symptoms of a dry mouth and polydipsia at the age of 2 years, and was diagnosed with SLE and lupus nephritis when she was 4 years old at the Children's Hospital of Chongqing Medical University. Her unusual symptoms and poor response to therapy prompted collection of relevant clinical data, family history, and blood samples during hospital treatment. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee at Children's Hospital of Chongqing Medical University.

Genetic Analysis

Genomic DNA was extracted from EDTA-anticoagulated blood samples collected from the patient and her parents. Whole exome sequencing was performed by MyGenostics (Beijing, China). A mutation in *BACH2* was confirmed by Sanger sequencing, using the following primers: F: 5'-ACC ACTTGCCAGGAAGGATG-3', R: 5'-ATGGGGAAGGCA GTCTCTCT-3'.

Conservation and Pathogenicity Analysis of the Mutated Amino Acid

Conservation analysis of the mutated amino acid was performed using T-Coffee (https://www.ebi.ac.uk/Tools/msa/tcoffee/). Pathogenicity was analyzed as previously described [19]. No structural information or three-dimensional structure was available for BACH2. Analysis of the functional effects of the mutation was performed using annotations from the Uniprot-database and predictions generated using Reprof software. The protein structure was displayed by PyMOL.

Construction of Plasmids and Western Blotting

The cDNA sequence encoding *BACH2* was purchased from Youbao Biotechnology (Changsha, China). The c.G2362A point mutation of *BACH2* was generated by PCR mutagenesis, subcloned into the 7.1-pCMV-3xFlag vector, and confirmed by Sanger sequencing. Cells (HEK293T) were transfected and harvested after 24 h. Antibodies used for western blot analysis were against BACH2 (clone 16B10B53, BioLegend), Flag (DYKDDDDK, Proteintech), β-actin (Proteintech), and GAPDH (Proteintech).

Flow Cytometry and Antibodies

Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-anticoagulated blood samples. The following antibodies were purchased from BioLegend and used to detect distinct lymphocyte subsets: antihuman CD3, CD4, CD8, CD16, CD19, CD21, CD24, CD25, CD27, CD38, CD45, CD45RA, CD45RO, CD56, CD127, TCRαβ, TCRγδ, IgD, IgA, IgG, IgM, CXCR5, PD-1, and ICOS.

Lymphoblastoid Cell Lines

Lymphoblastoid cell lines (LCLs) were generated by Epstein-Barr Virus (EBV) transformation of B-lymphocytes among PBMCs from the patient and three healthy controls (HCs) [20]. Following stimulation with F(ab')2 (anti-human IgG & IgM, Jackson Immuno, 10 μg/ml) for the indicated length of time, RNA was extracted from patient and HC LCLs using a RNeasy Micro kit (QIAGEN). RNA was reverse transcribed to complementary DNA (cDNA) using PrimeScript RT Master Mix (Takara), following the manufacturer's instructions. *PRDM1* mRNA levels were determined by quantitative RT-PCR using TB Green Premix Ex Taq II (Takara). *PRDM1* primers were as follows: F: 5'-TCC AGCACTGTGAGGTTTCA-3', R: 5'-TCAAACTCAGCC TCTGTCCA-3'.

Somatic Hypermutation

RNA samples from the patient and five age-matched HCs were converted to cDNA and *IgA* and *IgG* transcripts amplified from cDNA, as described previously [21]. *IGHV1-69* genes were amplified using *IGHV3* or *IGHV4* primers (primer sequences refer to previously published article [21]) and all PCR products cloned into the 7.1-pCMV-3xFlag vector. Sequencing was performed by Sangon Biotech (Shanghai, China). Obtained sequences were analyzed by comparison with the IMGT database (http://www.imgt.org/).

Confocal Microscopy

PBMCs were isolated from EDTA-anticoagulated blood samples and stained with antibodies against CD3 (AF647, BioLegend) and IgD (AF488, BioLegend), then plated on Poly-L-Lysine-coated slides and incubated on ice for 45 min. After fixation and permeabilization, PBMCs were stained with anti-APRIL (Sacha-2, Enzo Life Sciences), Rat IgG (H+L) cross-adsorbed secondary antibody (AF546, Invitrogen), and 4',6-diamidino-2-phenylindole (DAPI, Beyotime Biotechnology); controls were stained with secondary

antibody and DAPI. Samples were analyzed using a Nikon A1Rsi confocal microscope. Mean fluorescence intensity of APRIL was determined using NIS-Elements AR 3.2 software.

Enzyme-linked Immunosorbent Assay (ELISA)

Patient and five age-matched HCs' plasma samples were collected and centrifuged at 12000 × g for 5 min. Plasma BAFF was detected using a Human BAFF ELISA kit (Ruixin Biotech), following the manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean ± SD and were analyzed two-tailed, unpaired Student's t test. All statistical tests were performed in GraphPad Prism 6 software (GraphPad Software, Inc., San Diego, CA). *P < 0.05, **P < 0.01, ***P < 0.001.

Results

A Patient Presenting with Early-onset SLE, Multiple Autoimmune Diseases, and Recurrent Infections

The patient is an 8-year-old girl, and her clinical course is illustrated in Figure 1A. She presented with symptoms of dry mouth and polydipsia at 2 years old and received only traditional Chinese medicine treatment at that time. No other family members had similar symptoms.

When she was 4 years old, the patient presented at our hospital with recurrent fever, mouth dryness, polydipsia, lymph node enlargement, and pneumonia. She was then diagnosed with SLE and lupus nephritis, based on characteristic renal symptoms and serological parameters, including antinuclear antibody titer (1:1000) and positivity for anti-SSA/anti-Sm antibodies. She was presumed to also have a fungal infection, based on detection of β-D-Glucan and *Aspergillus* antigen. Since the onset of SLE, her 24 h urine protein increased until treatment with tofacitinib (Figure 1C). The patient was positive for antinuclear, anti-SSA/anti-Sm antibodies and remained positive at the time of writing. Complement C3 and C4 were both reduced or toward the minimum of the normal range and did not return to normal until recently (Figure 1C). Patient lymphocytes were consistently decreased throughout the course of the disease (Figure 1D).

Notably, she had consistently reduced serum IgA levels, except during the acute phase of SLE, while IgM remained high (Figure 1B). Analysis of patient immunoglobulin subtypes indicated that IgA and IgG2 levels were reduced, while those of IgG3 were markedly elevated (Table 1).

The patient was suspected to have Sjögren's syndrome due to the persistent dry mouth symptoms since 2 years old, but Sjögren's syndrome was not diagnosed due to lack of salivary gland biopsy.

At 6 years old, the patient developed Gottron's papules, elevated serum creatine kinase (3876 U/L), positivity for anti-Zo-β and anti-Ro-52 autoimmune antibodies, and right thigh muscle inflammation (Figure 1E) and was, therefore, diagnosed with JDM.

When diagnosed with SLE, the patient was administered prednisone with hydroxychloroquine and immunosuppressive therapy, but her symptoms did not improve, and she was subsequently hospitalized several times. Next, she was treated with prednisone and belimumab; however, this was also ineffective. She is currently receiving prednisone combined with tofacitinib treatment, which has relieved her recurrent fever, and her urine protein is also now negative. Nevertheless, she has persistent recurrent upper respiratory infections. The treatments undergone by the patient throughout this study are detailed in Figure 1A.
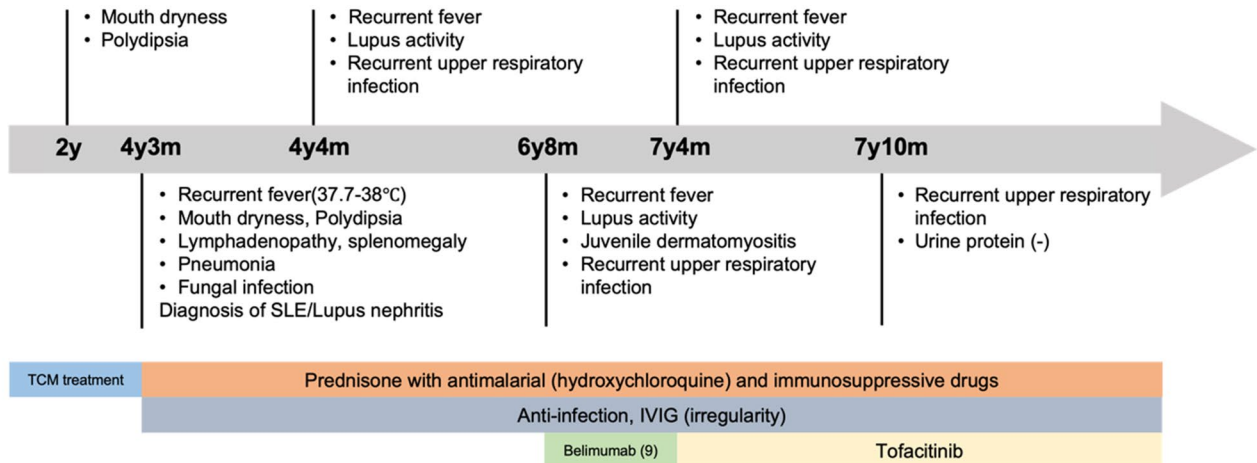
Detection of a Novel Heterozygous Nonsynonymous Mutation in *BACH2* that Reduces Protein Expression

The early-onset of SLE, lupus nephritis, multiple autoimmune diseases, recurrent infections, fungal infection, and poor therapeutic response of our patient prompted us to consider that genetic factors may have contributed to disease pathogenesis. Therefore, we conducted whole exome sequencing of whole blood samples from the patient and her parents.
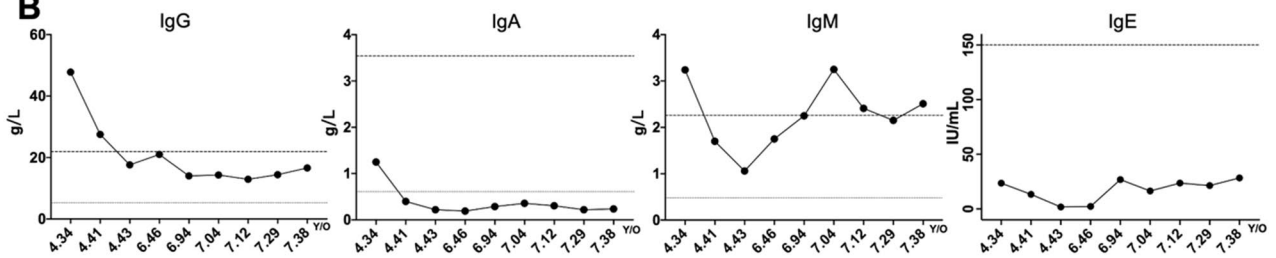
We detected a novel heterozygous nonsynonymous mutation in *BACH2*, c.G1727T, encoding an arginine to leucine (R576L) substitution in the patient (II1) and her father (I1). The mutation was confirmed by Sanger sequencing (Figure 2A). Interestingly, although the R576L mutation was inherited from the father, he had no obvious clinical symptoms except for facial skin photosensitivity. The mutated amino acid is located between the BTB/POZ and bZIP domains of BACH2 (Figure 2C); highly conserved, according to multiple sequence alignment analysis (Figure 2D); and predicted to be deleterious (Figure 2E). The allele frequency of R576L is 0.000003976 in total and it is 0 in East Asian population (gnomAD database). Protein structure prediction showed that the mutant residue is smaller and more hydrophobic than the WT residue. Further, arginine is positively charged, while leucine is neutral, hence the mutation may lead to loss of interactions with other molecules or residues (Figure 2E).

Next, we generated plasmids expressing WT and c.G2362A point mutated *BACH2* sequences. HEK293T cells were transfected with Flag-tagged WT or Flag-tagged mutant BACH2 protein-expressing plasmids, and reduced
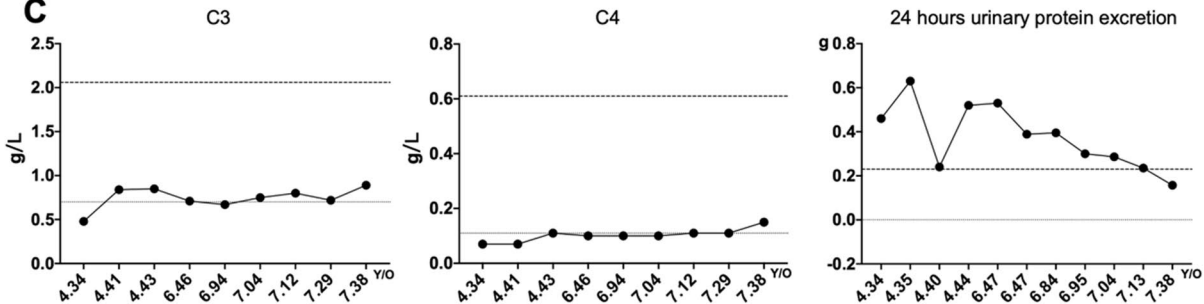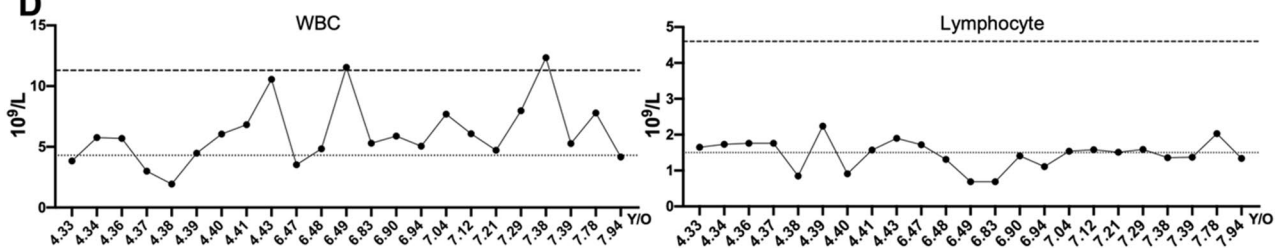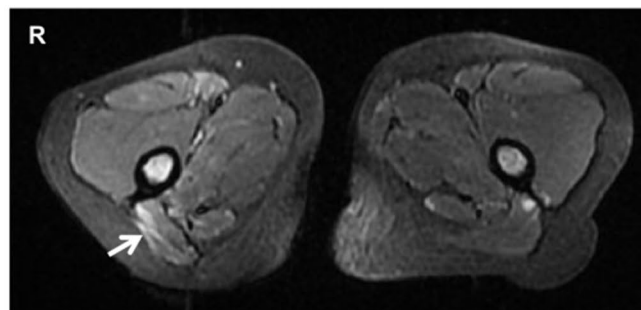
A

- Mouth dryness
- Polydipsia

- Recurrent fever
- Lupus activity
- Recurrent upper respiratory infection

- Recurrent fever
- Lupus activity
- Recurrent upper respiratory infection

| 2y | 4y3m | 4y4m | 6y8m | 7y4m | 7y10m |

- Recurrent fever(37.7-38℃)
- Mouth dryness, Polydipsia
- Lymphadenopathy, splenomegaly
- Pneumonia
- Fungal infection
Diagnosis of SLE/Lupus nephritis

- Recurrent fever
- Lupus activity
- Juvenile dermatomyositis
- Recurrent upper respiratory infection

- Recurrent upper respiratory infection
- Urine protein (-)

TCM treatment | Prednisone with antimalarial (hydroxychloroquine) and immunosuppressive drugs
Anti-infection, IVIG (irregularity)
Belimumab (9) | Tofacitinib

B

IgG   IgA   IgM   IgE

C

C3   C4   24 hours urinary protein excretion

D

WBC   Lymphocyte

E

◄**Fig. 1** Clinical course and manifestations of the patient. (**A**) Clinical course of the patient. (**B**) Serum immunoglobulin subtypes throughout the course of the disease. (**C**) Complement C3 and C4, 24 h urine protein throughout the course of the disease. (**D**) WBC and lymphocytes count throughout the course of the disease. (**E**) Magnetic resonance imaging of right thigh. Y, year; m, month; -, negative; Y/O, years old; WBC, White blood cells

expression of BACH2 was observed in cells expressing the mutant isoform (Figure 3A). We next evaluated BACH2 expression by western blotting and found that it was decreased in PBMC samples from the patient's father (I1) and greatly reduced in the patient (II1) (Figure 3B).

BACH2 Deficiency Leads to Deficient Transcriptional Repression and SHM

Due to the limited amount of primary patient sample, we established patient LCLs by EBV transformation of PBMCs. *PRDM1*, which encodes BLIMP1, is a target of BACH2-mediated transcriptional suppression. We found that stimulation of patient-derived LCLs with Fab2 led to significantly higher *PRDM1* mRNA levels at all time points compared with those in HC LCL controls, suggesting deficient BACH2 transcription repression in patient-derived cells (Figure 3C).

Since BACH2 is a critical regulator of CSR and SHM, we also analyzed SHM levels in *IGHV* genes encoding IgA and IgG [2, 3]. SHM frequencies of IgA in the patient were significantly decreased relative to those in HC samples, and those of IgG also exhibited a downward trend, but the difference was not statistically significant (Figure 3D). Tracking patient immunoglobulin levels revealed that serum IgM remained consistently increased, or at the maximum of the normal range, while IgA was decreased (Figure 1B). Although the patient had a normal memory B cell (MBC) percentage, the IgA$^+$ MBC proportion was extremely low (Figure 4A). Together, these data indicate that IgA CSR may have been impaired in the patient.

Abnormal Distributions of Distinct Lymphocyte Subsets in the Patient and her Father

Surprisingly, although there were no obvious clinical symptoms, the MBC proportion was extremely low (0.9%) in the patient's father (I1); within these few MBCs, the proportions of IgA$^+$ and IgG$^+$ MBCs were comparable (Figure 4A). We also measured serum immunoglobulins in the father and found that they were all at normal levels (Table 1).

BACH2 promotes Treg polarization and plays an indispensable role in Treg homeostasis maintenance and function [7, 10, 11]. We found significantly lower percentages of CD4$^+$CD25^{hi}CD127^{lo} Tregs in the patient and her father, relative to those in age-matched healthy controls (Figure 4B).

We next explored the percentages of Th1, Th2, and Th17 cells in the patient, according to the CCR6 and CXCR3 surface markers; the results showed they were biased toward the Th1 phenotype subset (Figure 4C).

Peripheral blood lymphocyte subsets were also analyzed (Table 2). Samples from the patient's father showed slightly decreased proportions of T cells, B cells, and CD8$^+$ and CD4$^+$ T cells, while proportions of CD4$^+$ and CD8$^+$ terminally differentiated effector memory T cells were clearly higher. Notably, we analyzed his MBC proportion again (2 months apart), and found that it was still 0.89%, while that of naïve B cells was markedly higher. In the patient, the proportion of T cells was higher, and that of B cells was slightly decreased, whereas the proportion and number of natural killer (NK) cells were markedly reduced. Further, the percentage of CD8$^+$ T cells in the patient was much higher than the maximum reference value, but the absolute number was normal, consistent with lymphopenia. CD4$^+$ naïve T cells were significantly decreased, while the percentage and absolute number of TCRαβ$^+$ double-negative T cells were markedly increased. Among B cell subsets in the patient, MBC number was normal, that of transitional B cells was decreased, and the number of plasmablasts was significantly raised.

Autoimmunity Pathogenesis in the Patient

Frequencies of CD21$^-$ or CD21^{low} B cells are increased in multiple autoimmune diseases [22]. Therefore, we analyzed the frequencies of CD21^{low} B cells, CD21^{low} CD27$^-$ B cells, CD21^{low} CD38$^-$ B cells, and CD21^{low} IgD$^+$ B cells and found that all were markedly increased in the patient, relative to those in HC samples (Figure 5A).
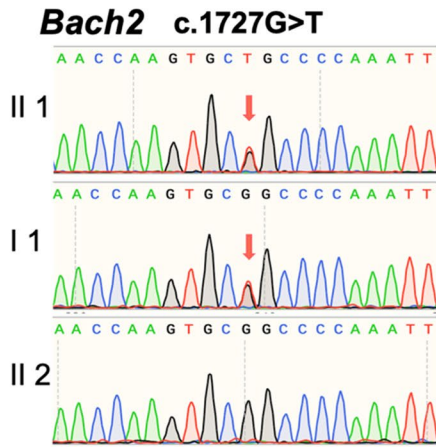
BACH2 deficiency is reported to cause CD4$^+$ T cells to overexpress ICOS and differentiate into extrafollicular helper T cells, predisposing individuals to SLE [15]. Here,

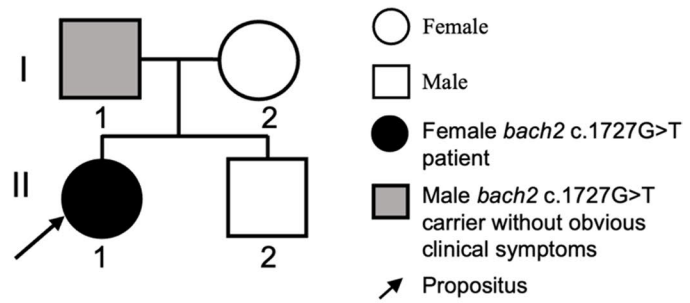**Table 1** Immunoglobulin subtypes of the patient (II1) and her father (I1)

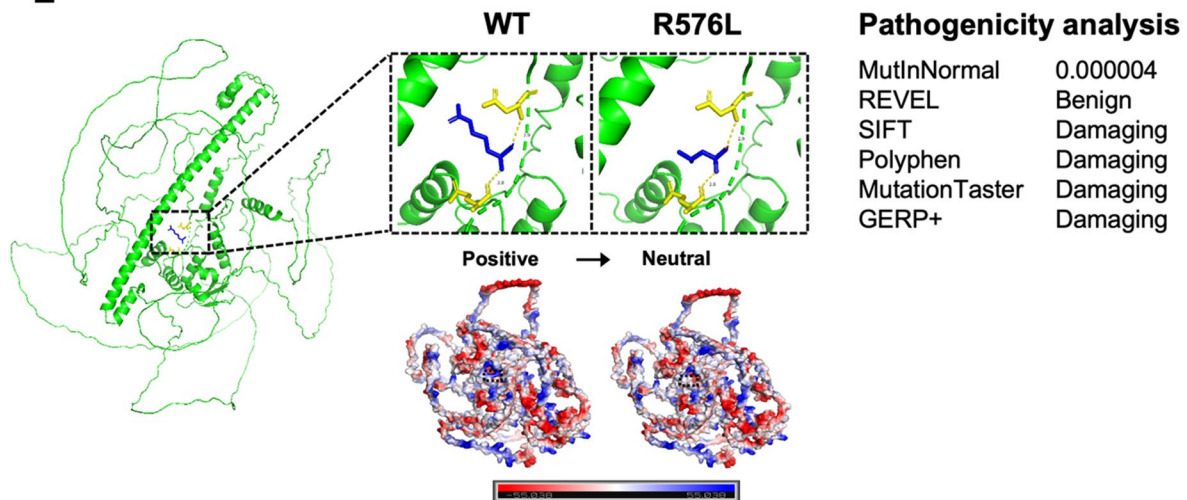| Parameters	I1		II1	
	Result	Reference	Result	Reference
IgG (g/L)	10.4	5.28-21.9	-	-
IgG1(mg/L)	-	-	10799	2420-10740
IgG2(mg/L)	-	-	623.8	710-5110
IgG3(mg/L)	-	-	1334.6	30-910
IgG4(mg/L)	-	-	66	10-1200
IgD(ug/ml)	-	-	20.65	
IgM(g/L)	1.55	0.48-2.26	2.28	0.48-2.26
IgE(IU/ml)	94	0-165	31.32	0-150
IgA(g/L)	2.8	0.44-4.41	0.35	0.61-3.45

-, not detected

◄**Fig. 2** Detection of a novel heterozygous nonsynonymous mutation in *BACH2*. (**A**) Sanger sequencing of the BACH2 gene in the patient and her family members. (**B**) Family pedigree. (**C**) Schematic diagram of BACH2 protein. Red, the novel mutation of our patient. Black, reported mutations of *BACH2*. BTB, broad complex–tamtrack–bric-a-brac region. POZ, Pox virus and Zinc finger domain. bZIP, the basic and leucine zipper domain. NES, nuclear export signal. (**D**) Amino acids sequence of BACH2 in different species. Some highly conserved sites are indicated. (**E**) Ribbon representations and residue charge of BACH2. The pathogenicity analysis is listed on the right. Blue, the 576 amino acid. For the residue charge, blue, positive; white, neutral; red, negative

we analyzed the mean fluorescence intensity of ICOS on CD4$^+$ T cells by flow cytometry and found that it was significantly elevated in the CD4$^+$ T cell and T follicular helper cell (Tfh) populations (Figure 5B). Notably, the proportion of Tfh was much higher than that in HCs, and further

analysis of Tfh subsets revealed that they were biased toward a Th1-like phenotype (Figure 5B).

The B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) system is an important modulator of autoimmunity and SLE pathogenesis [23]. Therefore, we analyzed BAFF and APRIL secretion. By using anti-APRIL (Sacha-2), which binds to amino acids 105–250 and 16–108 of the APRIL protein, we found that expression and secretion of APRIL in patient PBMCs were significantly higher than those in normal controls, while samples from the father showed intermediate levels between those of the patient and HCs (Figure 5C). Serum BAFF levels were detected by ELISA, and found them slightly higher in both the patient and her father than in normal controls, while there was no significant difference between the two family members (Figure 5D).
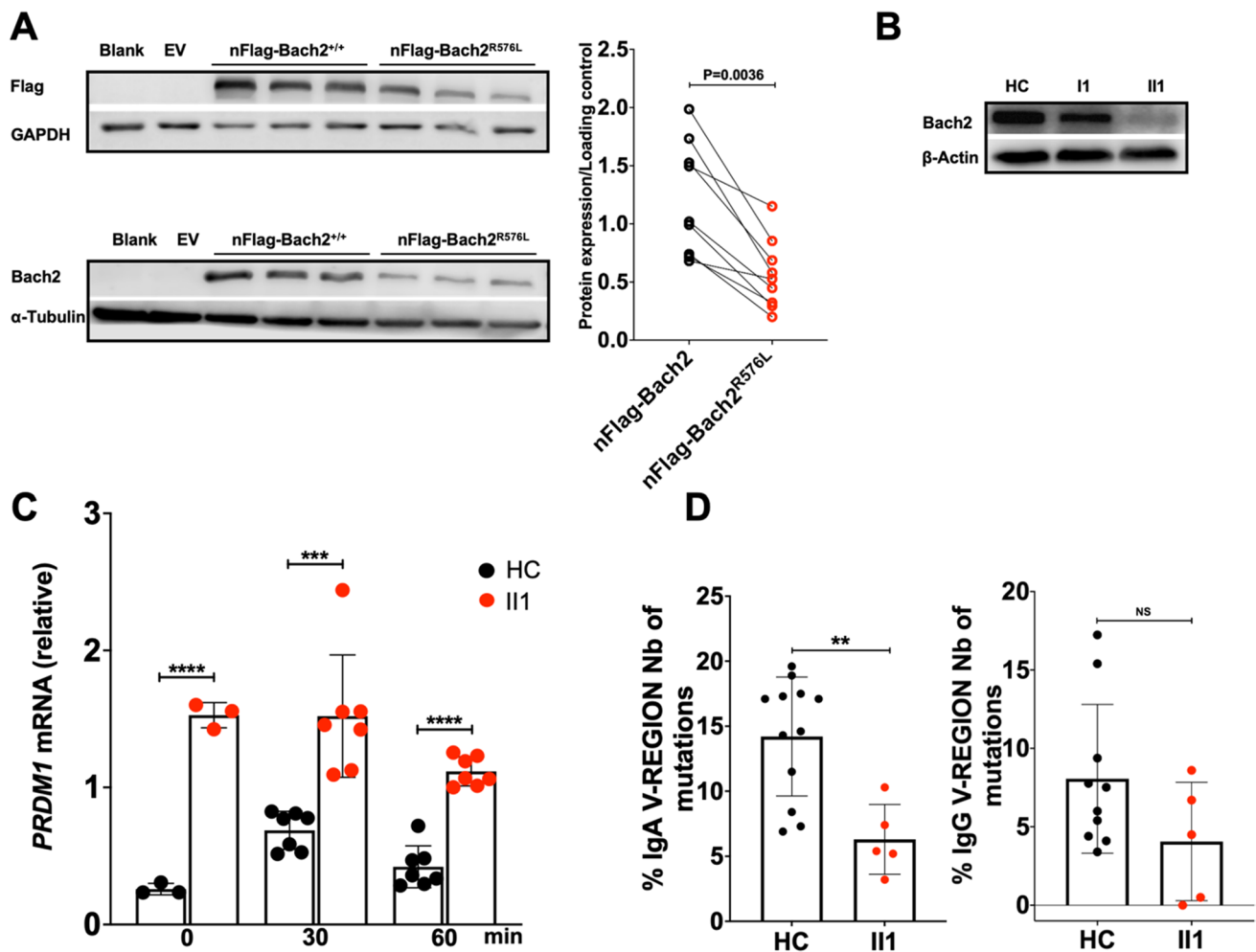

Fig. 3 R576L mutation reduces BACH2 expression and leads to deficient transcriptional repression and SHM. (**A**) BACH2 expression in WT/ R576L mutant plasmids transfected HEK293T cells. Images were cropped for displaying, original images were shown in additional file. EV, empty vector. (**B**) BACH2 expression in the PBMCs of patient and HC. Images were cropped for displaying, original images were shown in additional file. (**C**) *PRDM1* mRNA expression in LCLs of patient and HCs stimulated by 10 µg/ml F(ab')2 for indicated time points. (**D**) Percentages of IgA and IgG somatic hypermutation in the patient. I1, the patient's father. II1, the patient
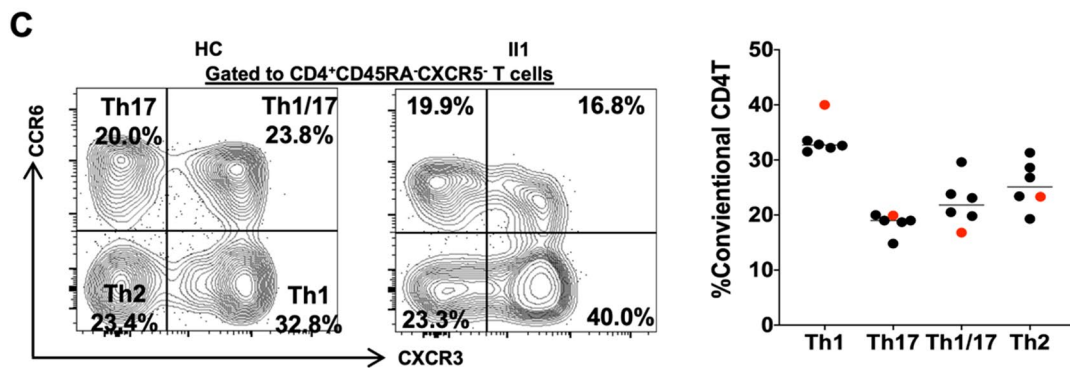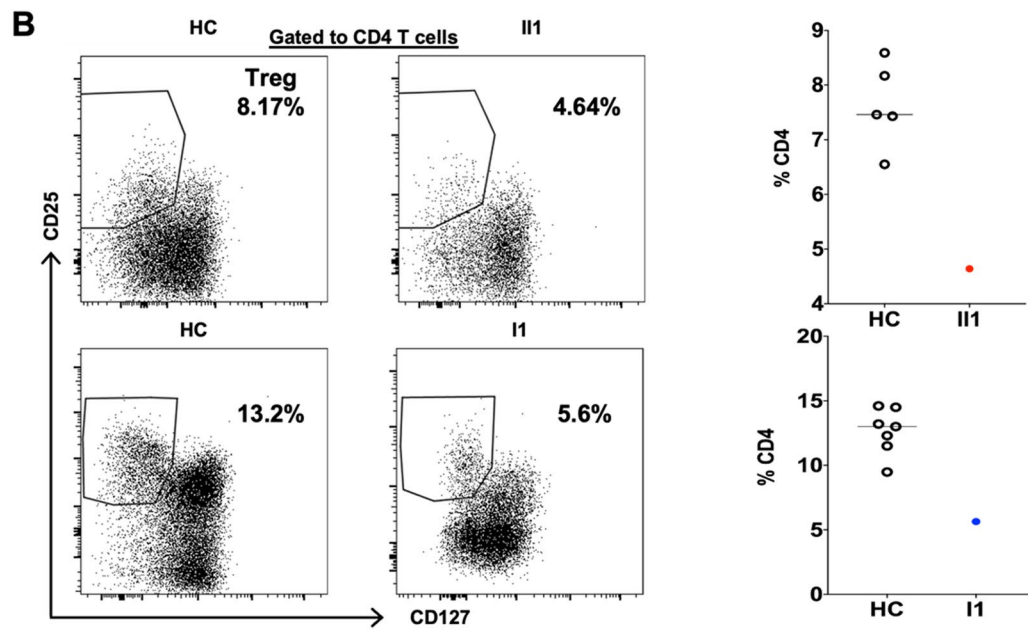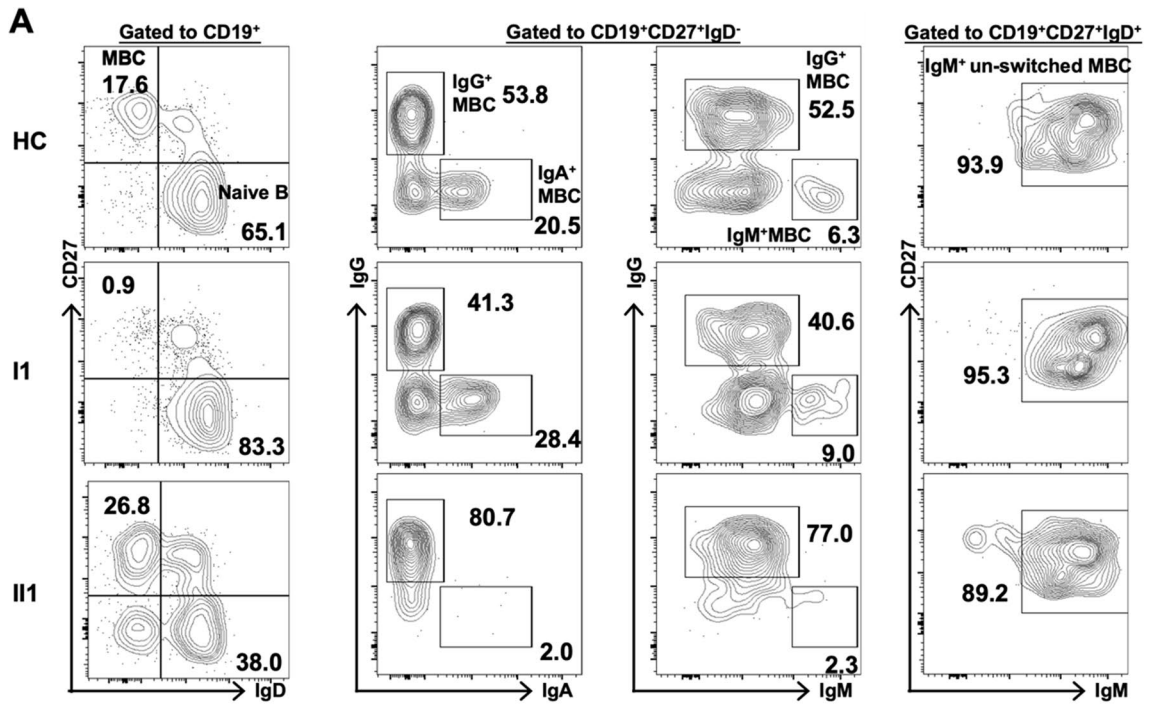
◄**Fig. 4** Abnormal distributions of memory B cells, Tregs and CD4 subpopulation. (**A**) Frequencies of IgG$^+$, IgA$^+$ and IgM$^+$ MBCs in the patient, her father and an adult HC. (**B**) Proportion of Treg in the patient and her father. Five age-matched HCs are included in II1. Seven age-matched HCs are included in I1. (**C**) Subpopulations of CD4$^+$ T cells in the patient. Five age-matched HCs are included. I1, the patient's father. II1, the patient

Discussion

BRIDA was first reported by Afzali et al. in 2017 in three patients presenting with defects in lymphocyte maturation, resulting in immunoglobulin deficiency and ongoing colitis [17]. Here, we describe a fourth patient with BRIDA caused by BACH2 haploinsufficiency, who manifested primarily with multisystem autoimmune disease (early-onset SLE and JDM), accompanied by a sustained reduction in serum IgA, without symptoms of intestinal inflammation. Overall, the three previously reported patients developed a CVID phenotype, while our patient mainly displayed autoimmune symptoms. The novel R576L BACH2 mutation detected in our patient was inherited from her father; however, surprisingly, he had no obvious clinical symptoms, despite having an extremely low proportion of MBCs. Thus, regular follow-up of the father is required. The clinical phenotype of the two family members is not fully penetrant. This could be either due to different degrees of tolerance to self-antigens or because of environmental factors that we are not aware of. Further, despite the high level of serum BAFF in the patient, conventional SLE treatment, with or without belimumab, was ineffective. Nevertheless, the patient's symptoms improved significantly on subsequent treatment with prednisone combined with tofacitinib.

Monogenic lupus is rare, and only around 30 genes have been reported to cause monogenic SLE and SLE-like phenotypes [24]. Monogenic lupus features childhood disease onset (<5 years old) and/or family history of clinical autoimmune

Table 2 Lymphocyte subpopulations of the patient (II1) and her father (I1)

Parameters	I1		II1	
	Relative (%)	Absolute (cells/ul)	Relative (%)	Absolute(cells/ul)
T cell	45.8 (56.84-75.02)	-	87.21 (59.50-75.56)	1316.87 (1480.28-2847.32)
CD8$^+$T cell	16.76 (21.91-36.80)	-	51.59 (19.70-32.04)	779.00 (552.62-1127.28)
CD8 Naive	47.9 (35.34-72.32)	-	49.7 (38.03-79.08)	387.17 (293.36-768.42)
CD8 TEMRA	38.3 (5.08-31.24)	-	25.6 (1.30-22.85)	199.43 (9.05-209.78)
CD8 CM	9.12 (10.96-31.00)	-	9.26 (11.91-36.87)	72.14 (79.59-350.41)
CD8 EM	4.7 (2.38-15.84)	-	15.40 (1.11-14.51)	119.97 (7.90-104.18)
CD4$^+$T cell	21.8 (22.25-39.00)	-	32.20 (28.49-41.07)	486.22 (767.26-1592.48)
CD4 Naive	50.4 (39.50-66.26)	-	26.30 (40.75-72.70)	127.88 (338.68-1036.97)
CD4 TEMRA	7.23 (0.00-1.54)	-	1.32 (0-1.47)	6.42 (0-16.71)
CD4 CM	33.7 (25.34-49.90)	-	61.10 (21.66-52.74)	297.08 (232.09-600.93)
CD4 EM	8.65 (4.68-15.70)	-	11.30 (1.90-9.20)	54.94 (20.54-96.75)
CD4:CD8	1.30 (0.65-1.65)	-	0.62 (1.02-2.05)	-
TCRαβ$^+$DNT	0.4 (0.61-2.31)	-	13.07 (0.19-2.43)	172.15 (3.77-49.48)
γδ T cell	13.7 (6.55-20.28)	-	4.08 (7.00-19.60)	53.73 (133.70-427.77)
NK cell	-	-	2.56 (7.83-20.99)	38.66 (227.47-667.76)
B cell	6.34 (8.84-17.76)	-	10.21 (10.46-21.77)	154.17 (303.52-777.25)
Memory B	0.89 (7.15-23.10)	-	19.5 (8.61-20.19)	30.06 (37.69-114.81)
Naive B	83.3 (53.78-78.64)	-	37 (52.04-75.78)	57.04 (171.45-469.28)
Transitional B	3.18 (1.38-9.42)	-	2.31 (3.41-11.17)	3.56 (14.36-59.62)
Plasmablasts	0.77 (0.49-7.06)	-	12.2 (0.80-9.75)	18.81 (3.87-39.83)

-, not detected; CD8 Naïve, naïve differentiated cytotoxic T lymphocyte, CD3$^+$CD8$^+$CD45RA$^+$CD27$^+$; CD8 TEMRA, terminally differentiated effector memory cytotoxic T lymphocyte, CD3$^+$CD8$^+$CD45RA$^+$CD27$^-$; CD8 CM central memory cytotoxic T lymphocyte, CD3$^+$CD8$^+$CD45RA$^-$CD27$^+$; CD8 EM, effector memory cytotoxic T lymphocyte, CD3$^+$CD8$^+$CD45RA$^-$CD27$^-$; CD4 naïve, naive differentiated helper T lymphocyte, CD3$^+$CD4$^+$CD45RA$^+$CD27$^+$; CD4 TEMRA, terminally differentiated effector memory helper T lymphocyte , CD3$^+$CD4$^+$CD45RA$^+$CD27$^-$; CD4 CM, central memory helper T lymphocyte, CD3$^+$CD4$^+$CD45RA$^-$CD27$^+$; CD4 EM, effector memory lymphocyte, CD3$^+$CD4$^+$CD45RA$^-$CD27$^-$; TCRαβ$^+$DNT, TCRαβ$^+$ double negative T lymphocytes, CD3$^+$TCRαβ$^+$CD4$^-$CD8$^-$; Memory B cells, CD19$^+$CD27$^+$IgD$^+$; Naive B cells, CD19$^+$CD27$^-$IgD$^+$; Transitional B cells, CD19$^+$CD24^{++}CD38^{++}; Plasmablasts, CD19$^+$CD24$^-$CD38^{++}
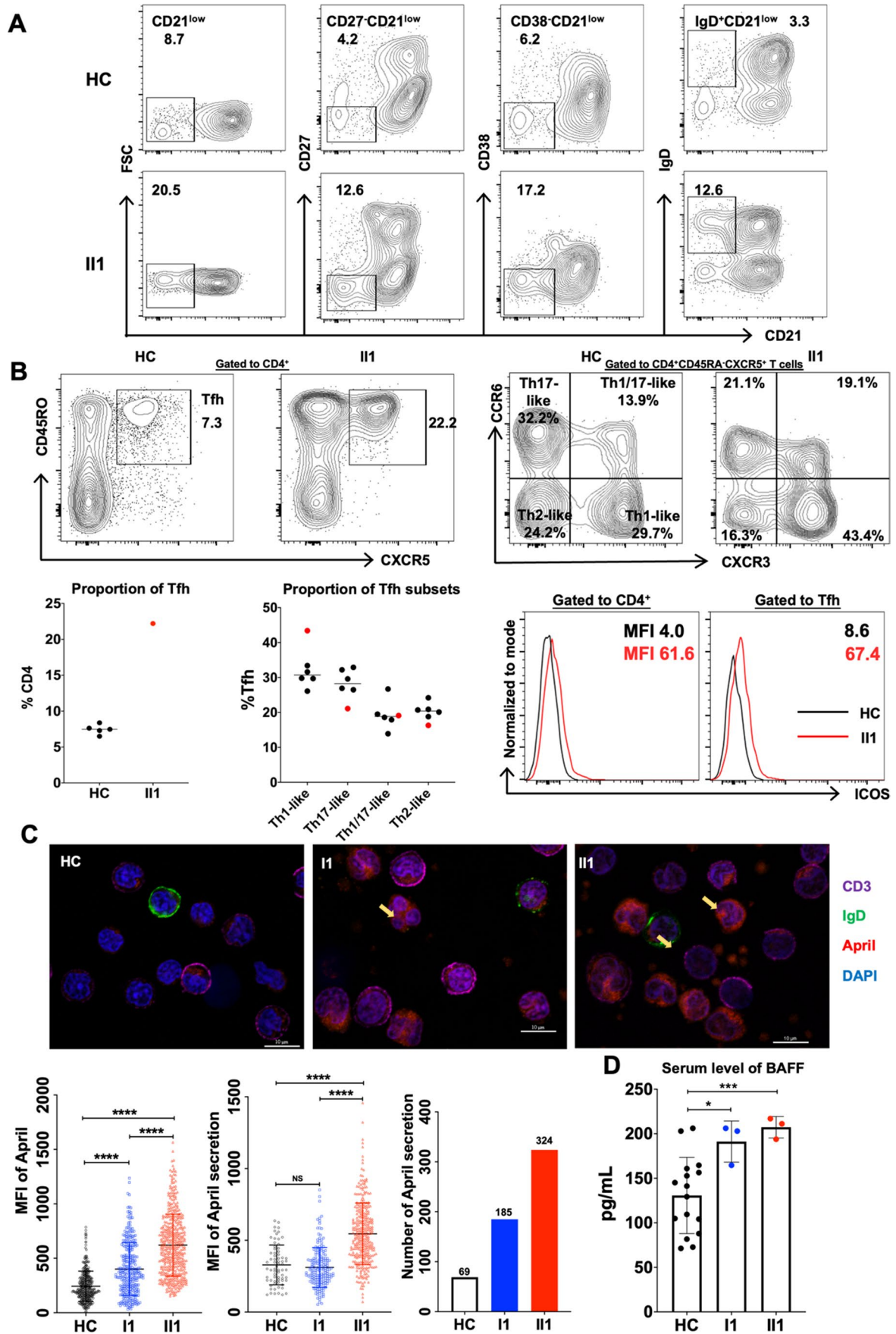
◄**Fig. 5** Proportion of CD21^{low} B cells, expression of ICOS on CD4 T cells and secretion of BAFF/APRIL. (**A**) Subsets of CD21^{low} B cells in II1 an age-matched HC. All of them are gated to CD19$^+$ B cells. (**B**) Proportion of Tfh subsets and MFI of ICOS on CD4 T cells and Tfh in II1 and an age-matched HC. (**C**) Confocal microscopy reveals higher expression and secretion of APRIL in I1 and II1. MFI quantification of APRIL and number of APRIL secretion (bottom). (**D**) BAFF level of I1 and II1 serum. I1, the patient's father. II1, the patient

disease, and inborn errors of immunity (also referred to as primary immunodeficiencies) are among the main causes [25]. The typical SLE symptoms in our patient began when she was 4 years old, with lupus nephritis, and she was diagnosed at an early stage of her life. Moreover, BACH2 deficiency predisposes mice to SLE; spontaneous fatal autoimmunity was observed [7], and abundant antidouble-stranded DNA IgM and IgG were detected in BACH2 knockout mouse serum [15]. Thus, *BACH2* may be a novel monogenic lupus-causing gene; however, more cases are required to confirm this hypothesis.

In previously published cases of BRIDA, chronic diarrhea and inflammatory bowel disease were the initial symptoms, and lymphadenopathy and recurrent pulmonary infections were observed in all three patients. Other than colitis and diarrhea, all the remaining symptoms described in the previously reported patients were also identified in our case. Of the three patients in the original publication, two had low levels of all immunoglobulins, and the other had high levels of IgM and IgG, with absence of IgA. Our patient had high IgM levels and IgG in the normal range (except during the acute phase of lupus), while IgG1 and IgG3 were higher, IgG2 lower, and IgA consistently lower in serum. Antinuclear IgG antibodies are considered pathogenic in SLE [26], and serum IgG subclasses vary among autoimmune disorders. Serum IgG1 and/or IgG3 are significantly increased in patients with SLE [27]. Although our patient had three episodes of pneumonia, along with a single mild fungal infection, she was successfully treated with antibiotics, and there was no evidence of chronic lung damage.

BACH2 plays a major role in adaptive immunity regulation and is tightly controlled during lymphocyte differentiation. Consistent with the previous report of BRIDA, our patient and her father had low numbers of Tregs compared with age-matched controls. The findings of decreased serum IgA level, almost no IgA$^+$ MBCs, deficient transcriptional repression of BLIMP1, and restricted IgA CSR in our patient support previous research in BACH2$^{-/-}$ model mice [3], demonstrating that BACH2 inhibits B cell differentiation and promotes CSR by repressing BLIMP1. Further, a recent study showed that BACH2 expression peaks in immature CD27$^+$CD11b$^+$ cells and negatively regulates NK cell terminal maturation [28]; however, while the proportion and number of NK cells were markedly reduced in our patient, the underlying mechanism involved requires further research.

BACH2 is established as required for B cell and memory T cell differentiation [29], which may account for the reduction in MBCs detected in the father of our patient.

Activation of the BAFF/APRIL system, expansion of CD21^{low} B cells, and high expression of ICOS on CD4 T cells and Tfh indicated active autoimmune responses in our patient. BACH2-deficient autoreactive B cells preferentially react at extrafollicular sites, which requires assistance from ICOS^{hi} helper T cells [15]. Tofacitinib is an inhibitor of the enzymes Janus kinase 1 and 3 (JAK1 and JAK 3) and can suppress T cell activation by upregulating TGFβRI expression, ameliorate lupus [30], repress CD4$^+$ T cell polarization to a Th1 phenotype, and inhibit self-tolerance in experimental arthritis [31]. The findings of these studies may explain the poor curative effect of belimumab treatment in our patient as well as the improvement of SLE symptoms in response to tofacitinib combined with prednisone therapy; however, further study on the mechanism involved in tofacitinib treatment of patients with BRIDA-related SLE is required.

In summary, we describe a patient with a novel R576L mutation in BACH2 resulting protein insufficiency and represent the second report of a patient with BRIDA. The patient presented with early-onset SLE, indicating that *BACH2* mutation may be a cause of monogenic lupus. We suggest that genetic factors should be considered when children present with early-onset symptoms or family history of SLE. Tofacitinib combined with prednisone treatment achieved satisfactory therapeutic effects in our patient, and additional study of the underlying mechanism involved is warranted in the future.

Declarations

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