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Genetic heterogeneity of pediatric systemic lupus erythematosus with lymphoproliferation

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Abstract

Systemic lupus erythematosus (SLE) is a chronic, rare autoimmune disease. In recent years, multiple monogenic diseases with early onset autoimmunity and lymphoproliferation have been identified, such as autoimmune lymphoproliferative syndrome, rat sarcoma (RAS)-associated autoimmune leukoproliferative disease, signal transducer and activator of transcription 3 gain-of-function syndrome and interleukin-2 receptor α deficiency. Therefore, we performed whole-exome sequencing in children with SLE with lymphoproliferation to identify genes associated with these conditions.

We enrolled 7 patients with SLE with lymphoproliferation from different families. Demographic data, clinical manifestations, laboratory and histopathologic findings, treatment, and outcome were documented. Whole-exome sequencing was performed in 7 patients and their families. Suspected variants were confirmed by Sanger sequencing. Protein levels were detected in patients with gene mutations by western blot.

Four patients were male, and 3 were female. No consanguinity was reported within the 7 families. The average age at onset was 5.0 years (range: 1.2–10.0 years). The most common features were renal (7/7 patients) and hematologic (6/7 patients) involvement and recurrent fever (6/7 patients), while only 2 patients presented with skin involvement. Antinuclear antibodies at a titer of ≥1:320 were positive in all patients. All patients fulfilled four 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria for the classification of SLE. We identified a somatic activating *NRAS* variant (c.38 A>G, p.G13C) in peripheral venous blood from 4 patients, at levels ranging from 8.8% to 42.8% in variant tissues that were absent from their parents. B cell lymphoma (BCL)-2-interacting mediator of cell death levels in peripheral blood mononuclear cells from 4 patients were markedly reduced, whereas those in the control were normal. Another 2 mutations, c.559C>T (p.Q187X) in the *TNFAIP3* gene and c.3061G>A (p.E1021K) in the *PIK3CD* gene were detected in 2 patients.

The SLE is a novel phenotype of somatic mutations in the *NRAS* gene and germline mutations in the *PI3CKD* gene. These genes, *NRAS*, *TNFAIP3*, and *PIK3CD*, should be considered candidates for children with SLE with lymphoproliferation. If patients with SLE and lymphoproliferation present with renal and hematologic involvement and recurrent fever, they need gene testing, especially male patients.

Abbreviations: ACR = American College of Rheumatology, ALPS = autoimmune lymphoproliferative syndrome, ALT = alanine aminotransferase, ANA = antinuclear antibody, BIM = mediator of cell death, EDTA = ethylenediaminetetraacetic acid, EULAR = European League Against Rheumatism, IL2RA = interleukin-2 receptor α, MAS = macrophage activation syndrome, OMIM = Online Mendelian Inheritance in Man, PBMCs = peripheral blood mononuclear cells, PBS = phosphate-buffered saline, RALD = RAS-associated autoimmune leukoproliferative disease, SLE = systemic lupus erythematosus, STAT3 = signal transducer and activator of transcription 3, WES = whole-exome sequencing, αβ-DNT = $CD3^+TCR\alpha\beta^+CD4^-CD8^-$.

Keywords: lymphoproliferation, *NRAS* gene mutation, somatic mutation, systemic lupus erythematosus

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The data sets are not publicly available but are available from the first author on reasonable request.

Ethics board approval and consent was obtained for this work from the Ethics Committee at the Children's Hospital of Fudan University, Shanghai, China (ekyy-2011-48).

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1. Introduction

Autoimmune and immunodeficiency diseases are outcomes of a dysfunctional immune system and represent 2 sides of the same coin.[1] Multiple single-gene defects have been identified, resulting in rare diseases with features of both autoimmunity and immunodeficiency.[2–5] Systemic lupus erythematosus (SLE; Online Mendelian Inheritance in Man [OMIM] 152700) is a prototype autoimmune disease with a strong genetic component characterized by differences in autoantibody profile, serum cytokines, and multisystem involvement commonly affecting the skin, renal, musculoskeletal, and hematopoietic systems.[6] Early onset, familial, and/or syndromic SLE may reveal monogenic pathologies.[7] Autoimmune lymphoproliferative syndrome (ALPS; OMIM 601859), a disease of lymphocyte homeostasis caused by dysfunction of the Fas Cell Surface Death Receptor (FAS)-mediated apoptotic pathway caused by defective lymphocyte homeostasis, is characterized by lymphadenopathy, hepatomegaly, splenomegaly, and autoimmune disease.[2] Rat sarcoma (RAS)-associated autoimmune leukoproliferative disease (RALD; OMIM 614470) also presents as autoimmunity, lymphadenopathy, and/or splenomegaly.[8] At the molecular level, RALD is defined by somatic mutations of either the *NRAS* or *KRAS* gene in a subset of hematopoietic cells.[3,9] Signal transducer and activator of transcription 3 (STAT3) gain-of-function syndrome (OMIM 615952) is a new clinical entity characterized by early onset poly-autoimmunity, lymphoproliferation, and growth failure.[4] Cell-surface interleukin-2 receptor α (IL2RA, CD25) expression is critical for maintaining immune function and homeostasis. Human IL2RA null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity (IL2RA deficiency; OMIM 606367).[5] Therefore, we performed whole-exome sequencing (WES) in children with SLE with lymphoproliferation to identify genes associated with these conditions.

2. Method

The study was approved by the Ethics Committee at the Children's Hospital of Fudan University, Shanghai, China. All the patients' parents provided written informed consent for enrollment in this study.

2.1. Patients

In total, 7 Chinese SLE children from 7 unrelated families were enrolled in this study. All patients fulfilled four 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria for the classification of SLE.[10] Demographic data, clinical manifestations, laboratory and histopathologic findings, treatment, and outcome were documented. All patients were admitted to or followed up at our center (Children's Hospital of Fudan University) between 2011 and 2019. The deadline date of follow-up was August 2019.

2.2. DNA sequencing

Genomic DNA was extracted and purified from peripheral leukocytes in whole-blood samples by a DNA isolation kit (Qiagen, Hilden, Germany). WES and bioinformatic analysis were performed in patient families as previously described.[11] Only genes listed in OMIM (https://www.omim.org/) were considered candidate causative genes. Variants identified by WES were confirmed by Sanger sequencing.

2.3. Peripheral blood mononuclear cell isolation and cell culture

Peripheral venous blood was drawn from one healthy volunteer and 4 patients with *NRAS* mutations. The ethylenediaminetetraacetic acid-anticoagulated blood was diluted with an equal volume of phosphate-buffered saline (PBS), pH 7.4. The diluted blood was carefully added to the top of the Ficoll-Paque PLUS (GE Healthcare, Shanghai, China) and centrifuged at 2000 rpm for 10 minutes at room temperature. The top layer containing plasma was removed, and the remaining blood was diluted with an equal volume of PBS. After being washed twice in PBS, peripheral blood mononuclear cells (PBMCs) were cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS) at a density of 1×10^6 cells/mL. After incubation in a 24-well plate at 37°C in 5% CO_2 for 24 hours, the cells were harvested for subsequent experiments.

2.4. Western blot analysis

Total and nuclear proteins were extracted using a protein extraction kit (Beyotime, Shanghai, China) following the manufacturer's instructions. Equal amounts of cytoplasmic or nuclear extracts were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresisand transferred to 0.45 μm PVDF membranes (Millipore, MA, USA). Blots were probed with primary antibodies against BCL-2-interacting mediator of cell death (BIM) and β-actin (Cell Signaling Technology, Beverly, MA). Primary antibodies were detected with horseradish peroxidase-conjugated secondary antibody. Visualization was conducted using an Enhanced chemiluminescence (ECL) peroxidase substrate.

3. Results

3.1. Clinical data

3.1.1. Clinical characteristics. All 7 patients were Chinese. Four children were male, and 3 were female. No consanguinity was reported within the 7 families. The average age at onset was 5.0 years (range from 1.2 to 10.0 years). The most common features were renal (proteinuria and/or hematuria; 7/7 patients) and hematologic (cytopenia; 6/7 patients) involvement and recurrent fever (6/7 patients), while only 2 patients presented with skin involvement. Antinuclear antibodies at a titer of ≥1:320 were positive in all patients. They fulfilled 2019 EULAR/ACR criteria for the classification of SLE.[10] All patients had hepatomegaly and/or splenomegaly and/or lymphadenectasis. Bone marrow aspiration in all patients showed no malignant cells or nonspecific changes. Cervical lymph node biopsy also revealed no malignant cells or nonspecific changes (lymphocyte proliferation) in patients 2 and 5. Liver biopsy revealed fibrosis in patient 7. Patient 1 had macrophage activation syndrome (MAS) before admission to our center, and P3 presented with MAS after disease flare up, both characterized by cytopenia, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, and increased levels of alanine aminotransferase, glutamic-oxalacetic transaminase, and lactate dehydrogenase. The clinical and laboratory characteristics of the patients are summarized in Tables 1 and 2.

3.2. Immunologic features

The immunologic characteristics of the patients are listed in Table 3. The B lymphocyte subgroups were elevated in patients 1,4 and 5, normal in patients 2 and 3, and decreased in patients 6

Table 1

Clinical features of 7 systemic lupus erythematosus patients with lymphoproliferation.

				Affected system or symptom			Lymphoproliferation				
Case	G	Age at onset, yr	Initial symptoms	Hematologic	Renal	Others	Hepatomegaly	Splenomegaly	Lymphadenectasis	Biopsy	Family history
1	M	1.5	Epistaxis	Thrombocytopenia Hypoleucocytosis Anemia	Proteinuria Hematuria	Recurrent fever	Y	N	N	N	N
2	M	4.0	Cervical lymphadenopathy	Thrombocytopenia Anemia	Proteinuria	Recurrent fever	N	N	Neck	Nonspecific changes	N
3	M	3.0	Armpit Lymphadenectasis Skin rash	Thrombocytopenia Anemia	Proteinuria	Recurrent fever	Y	Y	Neck, armpit, groin	N	N
4	F	1.2	Hepatosplenomegaly	Thrombocytopenia Anemia	Proteinuria	Recurrent fever	Y	Y	N	N	N
5	F	8.5	Purpura in lower limbs	N	Proteinuria Hematuria	N	N	N	Neck	Nonspecific changes	N
6	M	10.0	Splenomegaly	Hypoleucocytosis	Proteinuria Hematuria	Recurrent fever	N	Y	Neck	N	N
7	F	7.0	Hepatosplenomegaly	Thrombocytopenia Anemia	Proteinuria Hematuria	Fever Rash	Y	Y	N	Liver fibrosis	Y

G = gender, N = no, Y = yes.

and 7. However, the immunoglobulin (Ig)G level was elevated in all patients. Increased IgA levels in patients 1 to 3 and 6 and normal IgA levels in patients 4, 5, and 7 were detected. IgM levels were elevated in patients 1, 6, and 7 and normal in patients 2 to 5. Except for the normal IgE level in patient 3, an increased IgE level was detected in the other patients. We observed that the proportion of double-negative T cells (DNT) ($CD3^+CD4^-CD8^-$) was increased in patient 7, while it was normal in others. The proportion of $CD3^+CD4^+CD8^-$ T cells was reduced in patients 1 and 4 to 6 and normal in patients 2, 3, and 7. The proportion of $CD3^+CD4^-CD8^+$ T cells was elevated in patients 3, 6, and 7 and normal in patients 1, 2, 4, and 5. Reduced numbers of natural killer cells ($CD3^-CD16^+CD56^+$) in patients 2–6, and normal numbers of natural killer cells ($CD3^-CD16^+CD56^+$) in patients 1 and 7 were detected. Obvious monocytosis was found in almost all patients by routine blood examination.

3.3. Therapy and follow-up

The mean follow-up time was 4.5 years (range from 1.6 to 7.7 years). All patients were treated with hydroxychloroquine, glucocorticoid, and immunosuppressive agents, including cyclosporine, mycophenolatemofetil, tacrolimus, and cyclophosphamide (Fig. 1). Methylprednisolone pulse therapy was given to patients 1 (at the local hospital), 4 and 5 at the beginning because of severe conditions. Mycophenolatemofetil was switched to sirolimus in patient 6, and etanercept was added to patient 7 after molecular diagnosis. Patient 1 was treated with cyclosporine at the beginning, and patient 3 was replaced with cyclosporine, both because of being complicated by MAS. The disease flared up again during tapering of glucocorticoids and reduction in the dose of immunosuppressive agents in patients 1, 3, 4, and 7.

Table 2

Laboratory finding of 7 patients.

	Autoantibody			Level of complement			Anti-phospholipid antibody						
Case	ANA	Anti-dsDNA	Others (positive)	C3, g/L	C4, g/L	CH50, IU/L	Lupus anticoagulant	Anticardiolipin antibody	Anti-β2 glycoprotein I	Direct Coombs test	Gene	Nucleotide change	Amino acid change
1	P	N	–	0.72	0.11	30	N	P	P	P	NRAS	NM_002524 c.38G>A	p.G13D
2	P	P	SSA, p-ANCA MPO	0.29	0.04	10	N	N	N	P	NRAS	NM_002524 c.38G>A	p.G13D
3	P	P	U1-RNP, Anti-Sm	0.51	0.0	22	N	P	N	P	NRAS	NM_002524 c.38G>A	p.G13D
4	P	P	U1-RNP, Anti-Sm p-ANCA	0.77	0.01	22	N	N	N	N	NRAS	NM_002524 c.38G>A	p.G13D
5	P	P	Anti-nucleosome Anti-ribonucleoprotein	0.44	0.02	5	N	N	N	N	No	–	–
6	P	N	p-ANCA	0.11	0.05	3	N	N	N	N	PIK3CD	NM_005026 c.3061G>A	p.E1021K
7	P	N	SSA, p-ANCA MPO	0.22	0.06	10	N	N	N	P	TNFAIP3	NM_006290 c.559C>T	p.Q187X

ANA = antinuclear antibody, N = negative, P = positive, RS = reference sequence.

Table 3

Routine evaluation of immunologic function in 7 systemic lupus erythematosus patients with lymphoproliferation.

Lymphocyte subgroup	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Normal range
$CD4^+/CD8^+$	0.98	1.04	0.85	0.45	0.85	0.3	0.84	
$CD16^+CD56^+$	108.1	332.1	127.0	1110.4	53.9	355.4	114.9	
$CD16^+CD56^+$, %	14.71	11.4	3.0	21.9	2.2	10.0	16.3	11–23
$CD19^+$	284.4	429.1	632.0	1428.9	871.6	41.4	71.3	
$CD19^+$, %	38.7	14.4	15.0	28.2	34.9	1.2	10.1	14–21
$CD3^+$	339.3	2087.3	3385.0	2330.6	1555.5	3096.8	502.7	
$CD3^+$, %	46.2	71.7	81.0	45.9	62.3	86.8	71.4	64–73
$CD4^+$	163.0	1011.3	1490.0	670.3	666.8	705.5	224.7	
$CD4^+$, %	22.2	34.7	35.0	13.2	26.7	19.8	31.9	29–36
$CD8^+$	166.3	973.1	1744.0	1498.1	788.2	2356.3	267.2	
$CD8^+$, %	22.6	33.4	42.0	29.5	31.6	66.0	38.0	24–34
DNT, %	1.4	3.6	4.0	3.2	4.3	1.0	4.2	0.37–4.16
Monocyte, %	16.7	11.6	12.6	9.8	2.4	8.8	21.6	3–8
Immunoglobulin								
IgG, g/L	21.4	25.7	26.4	32.8	15.8	37.9	21.6	5.0–12.7
IgA, g/L	1.5	2.8	2.8	1.0	1.8	2.3	1.5	0.3–1.9
IgM, g/L	3.6	2.4	1.2	2.5	1.2	3.1	9.3	0.7–2.0
IgE, kU/L	265.1	147.38	14.5	288.0	802.7	972.4	207.8	<100

DNT = double-negative T cells ($CD3^+CD4^-CD8^-$), Ig = immunoglobulin.

3.4. Whole-exome sequencing

An average of 11.6 Gb of raw sequence data was generated with 92.68 × depth of exome target regions for each individual as paired-end 150 base pair reads. A total of 91.4% of the raw date sequencing quality was above Q30. The coverage of at least 10 × and 20 × of the target regions was 99.62% and 97.6%, respectively. We identified a heterozygous c.38 A>G mutation (p.G13C) in the *NRAS* gene in peripheral venous blood from patients 1 to 4. Neither parent harbored a mutation in the *NRAS* gene, suggesting that the patient harbored a de novo germline or somatic mutation (Fig. 2). Another 2 heterozygous mutations, c.559C>T (p.Q187X) in the *TNFAIP3* gene and c.3061G>A (p.E1021K) in the *PIK3CD* gene were detected in 2 patients. The former is from her father, and the latter is absent from their parents. No mutations were detected in P5, and no mutations in other genes associated with primary immunodeficiencies and monogenic SLE were identified in all patients.

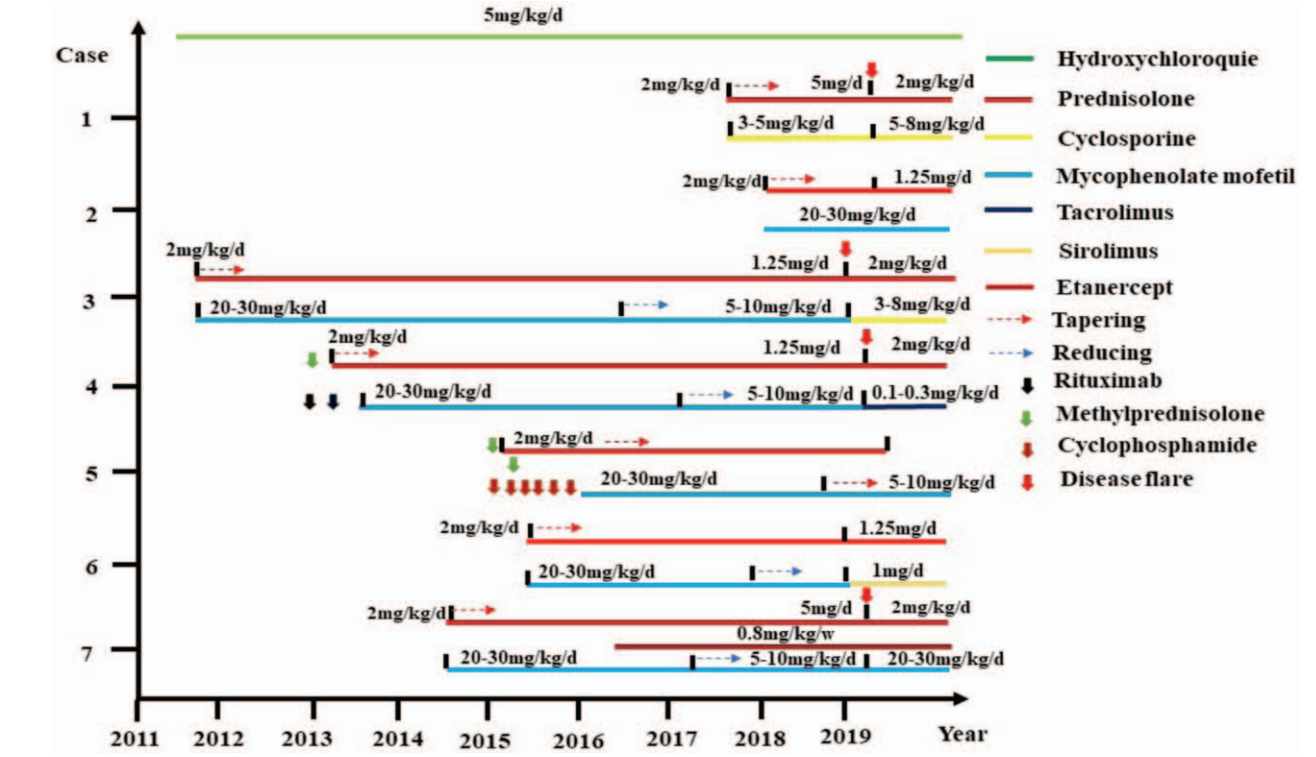


Figure 1. Therapies for all patients.

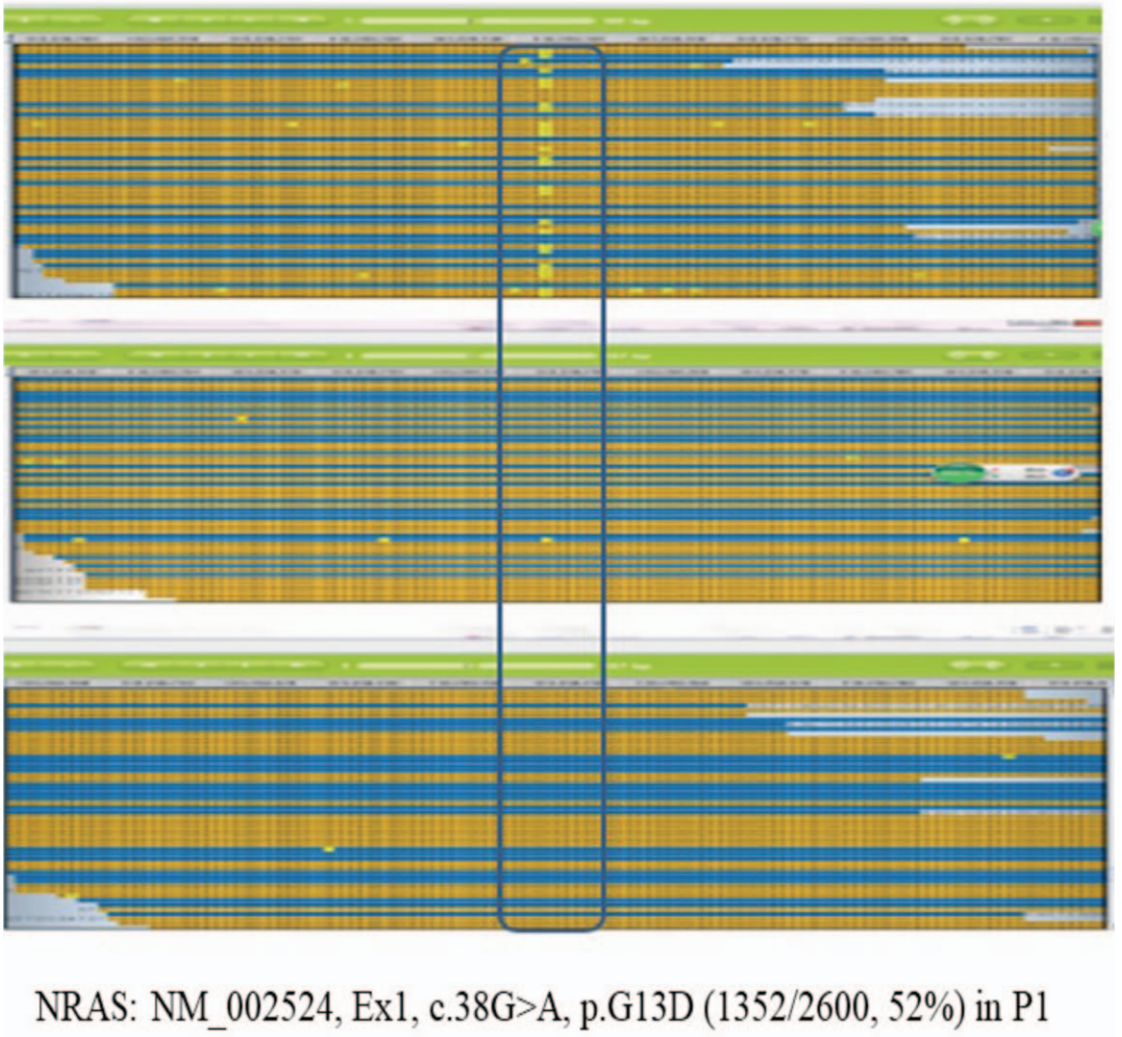


Figure 2. A heterozygous mutation in the NRAS gene (c.38G>A, p.G13D, 52%) was detected in monocytes collected from peripheral blood of patient 1. No mutation was identified in his parents.

3.5. Sanger sequencing

All mutations were confirmed by Sanger sequencing in 7 families (Fig. 3). Using DNA extracted from somatic cells (nails and buccal mucosa) in patients with *NRAS* mutations, *NRAS* exon 1 was amplified by PCR, and then the products were cloned. Mutated alleles were observed less frequently in the buccal mucosa and nails (42.8% and 8.8%, respectively) than in the blood (52.0%) in patient 1 (Fig. 3A). Similar results were found in the other 3 patients (Fig. 3B). The exon, including 559C>T in the *TNFAIP3* gene, was screened by Sanger sequencing in patient 7's grandparents. The mutation was not identified in her grandparents. All mutations were checked in mutation databases for human populations, such as ExAC Browser (http://exac.broadinstitute.org/), 1000 Genomes (http://www.internationalgenome.org/), and HGMD (http://www.hgmd.cf.ac.uk/ac/index.php). They were all found in the above mutation databases.

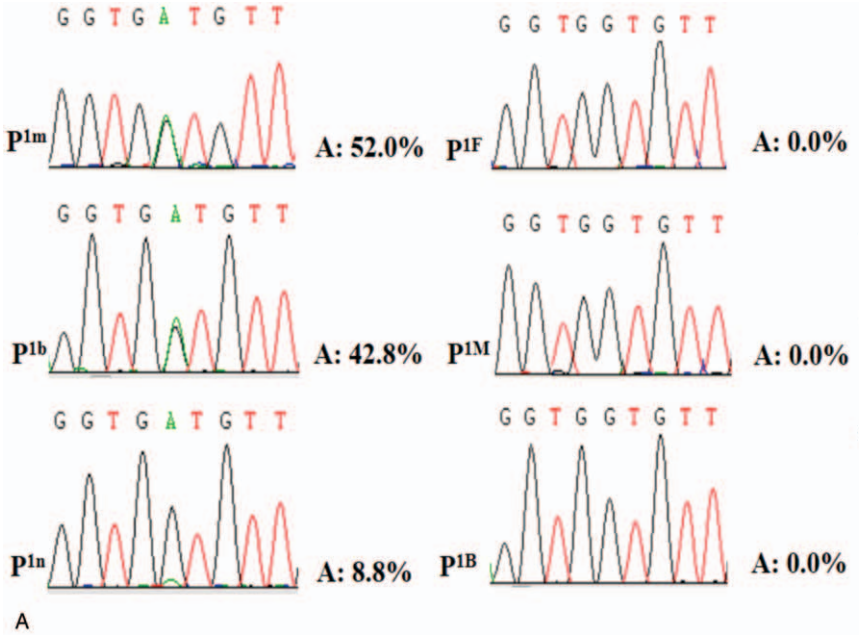
3.6. Levels of BIM in PBMCs from patients

Gain-of-function *NRAS* mutations hyperactivate the RAS/RAF/ERK pathway, which in turn negatively regulates BIM expression in patients with *NRAS* mutations. Western blot analysis showed that BIM levels in PBMCs from 4 patients were markedly reduced, whereas those in the control were normal (Fig. 4).

4. Discussion

Here, we report a cohort of patients with SLE and chronic lymphoproliferation. The clinical and laboratory data in all patients fulfilled four 2019 EULAR/ACR criteria for the classification of SLE.[10] The average age at onset was 5.0 years (range from 1.2 to 10.0 years). The male-to-female ratio was 4:3. In recent years, multiple monogenic causes of early onset autoimmunity and lymphoproliferation have been identified, such as the *FAS*, *CASPAS10*, *NRAS*, *IL2RA*, and *STAT3* genes.[5,12–17] Therefore, we performed WES in our patients. The results of our study showed germline mutations in the *TNFAIP3* and *PIK3CD* genes and somatic mutations in the *NRAS* gene and no mutations in other genes associated with primary immunodeficiencies and monogenic SLE in patients, such as the *FAS*, *CASPAS10*, *IL2RA*, and *STAT3* genes.

WES revealed a heterozygous c.559C>T (p. Q187X) mutation in the *TNFAIP3* gene in patient 7, which is from her father, and not identified in her grandparents. The patient was reported in



Sample	Frequency (%)			
	Patient 1	Patient 2	Patient 3	Patient 4
Peripheral blood	52.0	43.8	48.6	50.0
Buccal mucosa	42.8	23.1	26.3	22.4
Nail	8.8	12.0	10.2	11.7

B

Figure 3. The mutation c.38G>A confirmed by Sanger sequencing. (A) P1m = monocyte of patient 1, P1b = buccal mucosa of patient 1, P1n = nail of patient 1, P1F: father of patient 1, P1M = mother of patient 1, P1B = brother of patient 1. (B) Frequency of the mutated allele (A) varied in different tissues from patients.

our previous study.[18] Recently, heterozygous germline mutations in the *TNFAIP3* gene have been found to cause haploinsufficiency of A20, which displays an early onset autoinflammatory disease mainly characterized by SLE or Behçet-like disease.[19,20] Mutations in the *TNFAIP3* gene have also been reported in children with uncharacterized autoimmune diseases and lymphoproliferation and the autoimmune lymphoproliferative syndrome phenotype.[21,22]

The de novo mutation c.3061G>A (p.E1021K) in the *PIK3CD* gene was detected in patient 6, which was also reported in our previous study.[23] Gain-of-function mutations in the *PIK3CD* gene, encoding PI3K p110δ, were recently associated with a novel combined immune deficiency characterized by recurrent sinopulmonary infections, reduced class-switched memory B cells, lymphadenopathy, $CD4^+$ lymphopenia, CMV and/or EBV viremia and EBV-related lymphoma.[24,25] PI3Kδ contributes to the induction of enhanced SLE memory T-cell survival, and its pathway is frequently activated in SLE patient PBMCs and T cells, more markedly in active disease phases.[26] Additionally, the magnitude of PI3K pathway activation in patients with SLE paralleled activated/memory T-cell accumulation.[26] Therefore, the PI3K pathway may be involved in human SLE.

A heterozygous mutation, c.38 A>G (p.G13C) in the *NRAS* gene was identified in patients 1 to 4. Neither parent harbored a mutation in the *NRAS* gene, suggesting that the patient harbored a de novo germline or somatic mutation. Using DNA extracted from somatic cells (nails and buccal mucosa), *NRAS* exon 1 was amplified by PCR. Then, the products were cloned. Mutated alleles were observed less frequently in the buccal mucosa and nails (42.8% and 8.8%, respectively) than in the blood (52.0%) in patient 1. Similar results were found in the other 3 patients. Consequently, these patients harbored a somatic NRAS mutation. Obvious monocytosis in routine blood examination and elevated IgG levels in serum were found, while the CD3 $^+$TCRαβ$^+$CD4$^-$CD8$^-$ (αβ-DNT) cell count was normal. These 4 patients fulfilled RALD diagnosis based on lymphoproliferation, autoimmune cytopenia, and without a defect in FAS-dependent apoptosis or an increase in peripheral αβ-DNT cells.

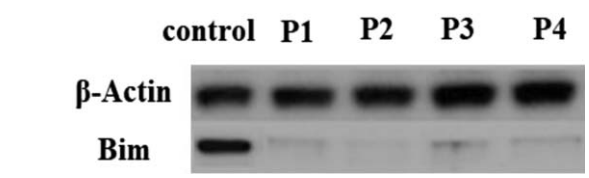


Figure 4. Western blot mediator of cell death (BIM) and β-actin are shown for patients.

The NRAS is a member of the p21 small GTPase family of proteins that also includes *HRAS* and *KRAS*. Germline RAS mutations are associated with specific developmental disorders, including Noonan (NS; OMIM 613224), Costello (OMIM 218040), and cardiofaciocutaneous syndromes (OMIM 115150).[27,28] Somatic RAS mutations are seen in 30% of all human cancers. A previous study confirmed that the G13D *NRAS* mutation in germline cell causes BIM downregulation and defective intrinsic mitochondrial apoptosis prominently in lymphocytes, leading to RALD and hematopoietic malignancies.[15] However, another study revealed that somatic mosaicism, again for the G13D NRAS mutation, causes BIM downregulation in activated T cells from children's patients, leading to RALD and juvenile myelomonocytic leukemia.[9] Western blot analysis in our study showed that BIM levels in PBMCs from these 4 patients were markedly reduced, whereas those in the control were normal. However, our patients all presented with SLE. Thus, SLE may be a novel phenotype of patients with somatic *NRAS* mutations. Interestingly, both germinal and somatic mutations in *the NRAS* gene might be involved in the pathogenesis of autoimmune diseases.

RASopathies are autosomal dominant neurodevelopmental syndromes resulting from germline mutations in genes that participate in the rat sarcoma/mitogen-activated protein kinases pathway, an important signal transduction pathway through which extracellular ligands stimulate cell proliferation, differentiation, survival, and metabolism.[29,30] The association between RASopathies and autoimmunity has been highlighted by the presence of autoimmune antibodies in 52% of 42 patients with RASopathies, including 39% of 37 NS patients.[31] Of these, 6 patients fulfilled the clinical criteria for autoimmune diseases, including SLE.[31] The prevalences of NS and SLE are approximately 1 per 2000 births and 3.3 to 24 per 100,000 children, respectively.[30,32] The relationship of these 2 rare diseases and the high overall percentage of patients with NS who have autoimmune features suggest that they might be related and that RASopathies must be added to this growing list of monogenic SLE, including *NRAS* gene mutations.

In the typical form of SLE, SLE is considered a disease of women of reproductive age, although males or females of any age can be affected. It is very rare in <5 years. The typical age at diagnosis is between 15 and 45 years. The female to male ratio varies among cohorts but is generally estimated at approximately 9:1 and 4:1 in adult and child onset disease, respectively.[32,33] The more common early manifestations are arthritis, photosensitive rashes, glomerulonephritis, and cytopenias. Of these patients with mutations in our study, the most commonly affected systems or features were renal (6/6 patients) and hematologic (6/6 patients) involvement and recurrent fever (6/6 patients), while only 1 patient presented with skin involvement. The average age of onset was 4 years. Thus, SLE differs from classic SLE presentation by a higher male-to-female ratio of 1:1, a lower rate of skin involvement (1/6 patients), and the occurrence of a lymphoproliferative disorder in some patients. So we think if patients with SLE and lymphoproliferation present with renal and hematologic involvement and recurrent fever, they need gene testing, especially in male patients.

A few previous reports showed that patients with somatic NRAS or KRAS mutations could follow a more benign clinical course requiring minimal medications.[9,30] However, MAS was observed in patient 1 at the beginning and in patient 3 after disease flare, both characterized by fever, multilineagecytopenia, hyperferritinemia, hypertriglyceridemia, and hypofibrinogenemia. Our patients were all treated with oral prednisolone and suppressive agents, and methylprednisolone pulse therapy was given to patients 1, 4, and 5 at the beginning because of severe conditions. Clinical features improved rapidly after treatment. However, the disease flared up again when oral doses of prednisolone were tapered to 1.25 mg per day to 5 mg per day in 3 patients (1, 3, and 4). Therefore, we believe SLE complicated with lymphoproliferative disorder, caused by associated gene mutations, is not a benign disease. In addition, it is not yet clear whether patients with somatic *NRAS* mutations progress to full-blown disease and maintain a stable clinical course. These patients need to be monitored carefully.

No mutations in genes associated with monogenic SLE and primary immunodeficiencies were detected in patient 5. She presented with persistent cervical lymphadenopathy, proteinuria, hematuria, purpura in the lower limbs, and no recurrent fever or hematologic involvement, which are different from those with mutations in this study.

5. Conclusion

Our findings revealed SLE may be a novel phenotype of somatic mutation in the *NRAS* gene and germline mutation in the PI3CKD gene. These genes, *NRAS*, *TNFAIP3*, and *PIK3CD*, should be considered candidates for children SLE with lymphoproliferation. There are some limitations in our study, such as low number of cases, relatively single phenotype, and only 1 healthy control used for western blot analysis. An unbiased genetic screening of larger cohorts of patients with childhood-onset SLE with diverse clinical presentations is needed to better estimate the relations between genotypes and phenotypes of monogenic SLE. In addition, WES is an effective method for identifying clinically significant exonic variants. However, there are some limitations for evolutionary conserved regulator DNA elements in untranslated, intronic, and intergenic regions that may be associated with the disease. Whole-genome sequencing can cover these limitations and also identify small copy number variations and mitochondrial DNA mutations.

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