Clinical Implications of a New DDX58 Pathogenic Variant That Causes Lupus Nephritis due to RIG-I Hyperactivation

Jiahui Peng,1,2 Yusha Wang,1,3 Xu Han,3 Changming Zhang,2 Xiang Chen,1 Ying Jin,2 Zhaohui Yang,3 Yu An,2 Jiahui Zhang,3 Zhengzhao Liu,2 Yinhua Chen,2 Erzhi Gao,2 Yangyang Zhang,2 Feng Xu,2 Chunxia Zheng,2 Qing Zhou,1,3 and Zhihong Liu1,2

1 Liangzhu Laboratory, Zhejiang University Medical Center, Hangzhou, People’s Republic of China
2 National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, People’s Republic of China
3 Life Sciences Institute, Zhejiang University, Hangzhou, People’s Republic of China

ABSTRACT

Background Lupus nephritis (LN) is one of the most severe complications of systemic lupus erythematosus, with heterogeneous phenotypes and different responses to therapy. Identifying genetic causes of LN can facilitate more individual treatment strategies.

Methods We performed whole-exome sequencing in a cohort of Chinese patients with LN and identified variants of a disease-causing gene. Extensive biochemical, immunologic, and functional analyses assessed the effect of the variant on type I IFN signaling. We further investigated the effectiveness of targeted therapy using single-cell RNA sequencing.

Results We identified a novel DDX58 pathogenic variant, R109C, in five unrelated families with LN. The DDX58 R109C variant is a gain-of-function mutation, elevating type I IFN signaling due to reduced autoinhibition, which leads to RIG-I hyperactivation, increased RIG-I K63 ubiquitination, and MAVS aggregation. Transcriptome analysis revealed an increased IFN signature in patient monocytes. Initiation of JAK inhibitor therapy (baricitinib 2 mg/d) effectively suppressed the IFN signal in one patient.

Conclusions A novel DDX58 R109C variant that can cause LN connects IFNopathy and LN, suggesting targeted therapy on the basis of pathogenicity.

JASN 34: ——–, 2022. doi: https://doi.org/10.1681/ASN.2022040477

Received April 22, 2022. Accepted September 29, 2022.
J.P., Y.W., X.H., and C.Z. contributed equally to this work.

Correspondence: Prof. Zhihong Liu and Prof. Qing Zhou, Zhejiang University, Yuhangtang Road No. 866, Hangzhou 310058 Zhejiang, People’s Republic of China. Email: liuzhihong@zju.edu.cn or zhouq2@zju.edu.cn

Copyright © 2022 by the American Society of Nephrology

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with a complex etiology and diverse clinical manifestations. It can affect the skin, joints, kidneys, and neurologic and hematologic systems. Lupus nephritis (LN) is one of the most severe complications and may result in irreversible organ damage and death. Current diagnosis for LN is primarily on the basis of identification of clinical and pathologic manifestations, and management is largely limited to empirical treatment with immunosuppressants. Identifying genetic causes of LN may tailor diagnosis and lead to targeted therapy for patients. Until now, more than 30 genes have been reported to cause diseases manifesting as SLE or SLE-like phenotype.1 In particular, patients carrying pathogenic variants in C1R,
Lupus nephritis (LN) is the major cause of death among systemic lupus erythematosus patients, with heterogeneous phenotypes and different responses to therapy. Identifying genetic causes and finding potential therapeutic targets of LN is a major unmet clinical need. We identified a novel DDX58 pathogenic variant, R109C, that leads to RIG-I hyperactivation and type I IFN signaling upregulation by disrupting RIG-I autoinhibition, causing LN, which may respond to a JAK inhibitor. Genetic testing of families with multiple cases of LN that identifies this variant may lead to targeted therapy.

Significance Statement

Methods

Patients
All patients who met the diagnostic criteria for SLE with biopsy-proven LN were evaluated from the Nanjing Glomerulonephritis Registry at Jinling Hospital. All patients enrolled in the study were evaluated under a protocol approved by the Institutional Review Boards. All of the patients and family members provided written informed consent, including consent to publish.

Whole-Exome Sequencing and Sanger Sequencing
One microgram of peripheral blood DNA was used for whole-exome sequencing (WES; BGI, Shenzhen, PR China). Candidate variants were filtered by removing those with high frequency, which presented in the gnomAD, Kaviar, and dbSNP databases and an in-house database. Variants were further filtered by dominant inheritance as previously described.12

Sanger sequencing was used to confirm variants identified by WES as previously described.12

Single-Cell RNA Sequencing
Single-cell capture (8000–10,000 cells) and cDNA preparation were performed on a Chromium machine (10X Genomics, Pleasanton, CA). The barcoded cDNA was then amplified by PCR. The library construction, sequencing, and data analysis were performed as previously described.12

Cell Preparation, Culture, and Treatment
PBMCs were separated by lymphocyte separation medium (50494; MP Biomedicals, Irvine, CA) from peripheral blood. The human embryonic kidney (HEK) 293T cell line was from the American Type Culture Collection. PBMCs and HEK293T cells were grown in RPMI 1640 (C11875500BT; Gibco, Grand Island, NY) or DMEM (C11995000BT; Gibco) supplemented with 10% FBS (NFBS-2500A; Noverse).

Poly(I:C) (P1530; Sigma–Aldrich, St. Louis, MO; 5 μg/ml) was used to stimulate HEK293T cells for the indicated amount of time.

Transfection
Transient transfections of plasmids in HEK293T cells were performed using Lipofectamine 2000 (11668019; Invitrogen, Waltham, MA) according to the manufacturer’s instructions. Cells were analyzed 18–24 hours after transfection.

Western Blotting and Immunoprecipitation
HEK293T cells were harvested and lysed in 1% Triton X-100 lysis buffer (20 mM Tris-HCl, pH 7.5, 1% Triton X-100, 150 mM NaCl, 10% glycerol, and 2 mM EDTA). All buffers used throughout processing contained protease and phosphatase inhibitor mixture (78442; Thermo Fisher Scientific, Waltham, MA). Proteins were denatured and separated on SDS-PAGE and then transferred to polyvinylidene difluoride membranes. After blocking with 5% (wt/vol) BSA, the membrane was stained with the corresponding primary and secondary antibodies. Specific bands were
analyzed using an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

For immunoprecipitation, cell lysates were mixed with anti-Myc magnetic beads (B26302; Bimake, Houston, TX) at 4°C overnight. Then, immunocomplexes were washed five times using lysis buffer and subjected to Western blotting.

Native PAGE
TSDG buffer-lysed protein (10 mM Tris-HCl, pH 7.2, 10 mM NaCl, 1.1 mM MgCl₂, 0.1 mM EDTA, 1 mM dithiothreitol, 2 mM ATP, and 10% glycerol) was separated on native PAGE (3%–12%; BN1001BOX; Invitrogen) and immunoblotted as indicated.

Semi-Denaturing Detergent Agarose Gel Electrophoresis
Cellular lysate in hypotonic buffer (10 mM Tris-Cl, pH 7.5, 10 mM KCl, 0.5 mM EGTA, and 1.5 mM MgCl₂) was obtained and centrifuged at 1000×g for 5 minutes. The supernatant was isolated and further centrifuged at 10,000×g for 10 minutes. The supernatant and pellet were then separated. Crude mitochondria in the pellet isolated from HEK293T cells were resuspended in 1× sample buffer (0.5× TBE, 10% glycerol, 2% SDS, and 0.0025% bromophenol blue) and loaded onto a vertical 1.5% agarose gel. After electrophoresis in the running buffer (1× TBE and 0.1% SDS) for 30 minutes with a constant voltage of 100 V at 4°C, Western blotting was performed.

Antibodies and Plasmids
The commercial antibodies were as follows: (1) Cell Signaling Technology (Danvers, MA): p65 (8242S), p-p65 (3033S), TBK1 (3504S), p-TBK1 (5483S), STAT1 (14994), p-STAT1 (9167S), STAT2 (72604), p-STAT2 (4441S), IRF3 (11904), p-IRF3 (37829), IFIT3 (87781S), GAPDH (5174), and HA (3724); and (2) Proteintech (Chicago, IL): β-actin (66009-1-Ig), Flag (20543-1-AP), and Myc (60003-2-Ig).

Tag-tagged RIG-I was made by cloning the corresponding human cDNA into different carrier vectors. Mutant plasmids were generated by site-directed mutagenesis. The plasmids for MAVS and K63-ub were kindly provided by Dr. Pinglong Xu. All plasmids were confirmed by DNA sequencing.

RNA Extraction and Quantitative PCR
Total RNA was extracted from cells with RNeasy Mini kit (74104; Qiagen, Hilden, Germany). cDNA was reverse transcribed (R333; Vazyme, Nanjing, PR China). Quantitative PCR (qPCR) was performed with 2× Universal SYBR Green Fast qPCR Mix (RK21203; Abclonal, Wuhan, PR China). The reactions were run on a LightCycler 480 II (Roche, Basel, Switzerland). Relative mRNA expression levels were normalized to ACTB and analyzed by the 2−ΔΔCt method. The following primers were used in human cells: ACTB-F: CGAGGCCCAGACAGCAAGAGAG; ACTB-R: CCGTTGGCCCT TAGGGTTCAAG; IFIT1-F: GGCTTCTGACTGTTGGAGGAAA; IFIT1-R: ATCCAGAGGCTAGGCGAGATC; IFIH4L-F: TGCACTAGGAGGACGTTG; IFIH4L-R: TCATTGCAGGACACAGTAC; iISG15-F: CTCCTGAGCATCTGGTGAGGAAA; iISG15-R: AAGGTCAGGCAGACAGCTG; RSAD2-F: CCGTCTGGAAAGATGGCTCC; RSAD2-R: CGGTCTTGAGGAAATGGCTCC; IFI27-F: CGTCTGCCATTACGAGGCAAGAT; IFI27-R: ACCCAATGGGAGCCCGATGAA.

RNA Sequencing
One microgram of RNA was used for library preparation. The libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) and sequenced on an Illumina Novaseq (San Diego, CA). A set of 150-bp paired-end reads was generated and mapped to the human reference genome (GRCh38) using STAR v2.7.10. featureCounts was used to count the reads numbers mapped to each gene. Differential expression analysis was performed using the DESeq2 package for R.

Calculation of IFN Score
We selected 28 IFN response genes as previously described.14 Z scores of each 28 genes were calculated with the mean and standard deviation of controls. Then, the IFN score was calculated by summing all 28 z scores for each sample. The 28 genes were as follows: CXCL10, DDX60, EPSTI1, GBP1, HERC5, HERC6, IFI27, IFI44, IFI44L, IFI6, IFIT1, IFIT2, IFIT3, IFIT5, IISG15, LAMP3, LY6E, MX1, OAS1, OAS2, OAS3, OASL, RSAD2, RNP4, SIGLEC1, SOCS1, SPATS2L, and USP18.

Luciferase Reporter Gene Assay
HEK293T cells were transfected with indicated Myc-tagged RIG-I or mutant plasmids with IFN-stimulated response element (ISRE), IFN-β, NF-κB, or luciferase construct for 48 hours using Lipofectamine 2000, and luciferase reporter gene assays were performed using a Dual Luciferase Reporter Assay System (E1910; Promega, Madison, WI) on the basis of the protocol provided by the manufacturer.

Cytometric Bead Array Assay
The concentrations of cytokines in serum were measured by cytometric bead array (CBA; BD Biosciences, Franklin Lakes, NJ). All data were analyzed by FCAPArray v3 software (BD Biosciences).

Statistical Analyses
Data are expressed as mean±SD, and statistical evaluation was performed using a two-tailed t test with GraphPad
**RESULTS**

**Identification of a Novel DDX58 Mutation in Patients with LN**

The proband (P1) of the first family (Figure 1A) was a 37-year-old woman who initially had a fever accompanied by respiratory infections at the age of 12 years. She then gradually developed acute nephritis syndrome with proteinuria, hematuria, hypoalbuminemia, and elevated serum creatinine. She had a history of spontaneous abortions twice and lacunar cerebral infarction once. Renal biopsy revealed class IV+V LN (Figure 1B). Laboratory testing showed hemolytic anemia, positive ANA, anti-dsDNA and anticardiolipin antibodies, and decreased C3. She was subsequently diagnosed with SLE and LN according to the European Alliance of Associations for Rheumatology/American College of Rheumatology classification criteria. Additionally, her family history was positive for a paternal grandmother of the type I IFN pathway, was detected in patients with the DDX58 R109C variant.

**R109C Mutation Constitutively Activates IFN Signaling**

To further investigate the potential effect of the DDX58 R109C variant on type I IFN signaling, we transfected constructs of wild-type (WT) or R109C mutant RIG-I into HEK293T cells. Consistent with the results of PBMCs, RIG-I-R109C–overexpressing cells showed higher activation in type I IFN and NF-κB signaling pathways than RIG-I-WT–transfected cells detected by RNA sequencing (Figure 3A). The 28-gene IFN score analysis confirmed the activation of type I IFN signaling (Figure 3B), and the gene set enrichment analysis related the activation of JAK-STAT and NF-κB signaling to the R109C variant (Figure 3C).

Transcriptional activity of the promoter of IFN-β, ISRE, and NF-κB was assessed by luciferase reporter gene assay. Significantly higher luciferase activity was detected in RIG-I-R109C–overexpressing cells than RIG-I-WT–transfected cells at the basal level, which was similar to that observed for the other four previously reported pathogenic variants (Supplemental Figure 2, A and B).9–11 Strikingly, luciferase activity in RIG-I-R109C–overexpressing cells upon no stimulation was even higher than RIG-I-WT–transfected cells stimulated with poly(I:C), which could model the actions of extracellular dsRNA. After binding to viral dsRNA, RIG-I triggers the activation of type I IFN and NF-κB pathways, which could be reflected by the phosphorylation of TBK1, IRF3, and p65. Elevated phosphorylation was observed in these proteins in RIG-I-R109C–overexpressing cells, even without poly(I:C) stimulation, whereas RIG-I-WT–transfected cells presented similar levels of phosphorylation to WT cells transfected with vector (Figure 3E). The phosphorylated IRF3 is prone to be dimerized, which is essential for its nuclear translocation and the transcription
of type I IFN. The secreted type I IFN binds to the IFNAR on the surface of adjacent cells, which leads to the activation of JAK1 and TYK2 and subsequent phosphorylation of STAT1 and STAT2. In the RIG-I-R109C–overexpressing cells, we observed increased dimerization of IRF3 shown by native PAGE, increased phosphorylation of STAT1 and STAT2, and elevated protein level of ISG-IFIT3 upon poly(I:C) stimulation or not (Figure 3E). Increased expression of
Table 1. Clinical characteristics of patients with the DDX58 R109C variant

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age at Onset (yr)</th>
<th>Disease Course (yr)</th>
<th>Primary Diagnosis</th>
<th>Kidney Presentation</th>
<th>Extrarenal Presentations</th>
<th>Renal Biopsy</th>
<th>Positive Immunologic Features</th>
<th>SMS-Related Characteristics</th>
<th>Treatment</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Woman</td>
<td>12</td>
<td>25</td>
<td>SLE, LN, anticardiolipin antibody syndrome</td>
<td>Acute nephritis syndrome</td>
<td>Hemolytic anemia, abortion, stroke</td>
<td>LN-IV+V</td>
<td>ANA, ds-DNA, anticardiolipin, anti-Sm, SS-A, nRNP-Sm, C3</td>
<td>No</td>
<td>Glucocorticoids, CTX, AZA, MMF, hydroxychloroquine</td>
<td>Partial remission, normal renal function</td>
</tr>
<tr>
<td>P5</td>
<td>Man</td>
<td>51</td>
<td>1.42</td>
<td>SLE, LN</td>
<td>RPGN</td>
<td>Anemia, pleural effusion</td>
<td>LN-IV</td>
<td>ANA, nRNP-Sm, RF, c-ANCA, PR3-ANCA, C3 and C4</td>
<td>No</td>
<td>Glucocorticoids, MMF, RRT</td>
<td>Loss of follow-up</td>
</tr>
<tr>
<td>P6</td>
<td>Man</td>
<td>15</td>
<td>6.42</td>
<td>Psoriasis, LN</td>
<td>Malignant hypertension, nephrotic range proteinuria, hematuria</td>
<td>Psoriatic skin rash, anemia, epileptic seizure</td>
<td>LN-III</td>
<td>Anticardiolipin</td>
<td>Psoriasis</td>
<td>ACEI/ARB</td>
<td>Died, ESKD</td>
</tr>
<tr>
<td>P7</td>
<td>Woman</td>
<td>28</td>
<td>13.17</td>
<td>SLE, LN</td>
<td>Nephrotic syndrome</td>
<td>Malar rash, oral ulcers, arthritis, pancytopenia, pleural effusion</td>
<td>LN-IV</td>
<td>ANA, ds-DNA, nRNP-Sm, C3</td>
<td>No</td>
<td>Glucocorticoids, CTX, MMF, tacrolimus</td>
<td>Partial remission, CKD stage 3</td>
</tr>
<tr>
<td>P8</td>
<td>Man</td>
<td>45</td>
<td>14.33</td>
<td>SLE, LN</td>
<td>Proteinuria, hematuria, AKI</td>
<td>Rash, oral ulcers, arthritis, thrombocytopenia, hemolytic anemia</td>
<td>LN-III</td>
<td>ANA, ds-DNA, anticardiolipin, C3 and C4</td>
<td>No</td>
<td>Glucocorticoids, MMF</td>
<td>Complete remission</td>
</tr>
</tbody>
</table>

ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; AZA, azathioprine; RPGN, rapidly progressive GN.
ISGs was also confirmed by qPCR assay with overexpressing RIG-I-R109C than RIG-I-WT upon poly(I:C) stimulation or not (Figure 3F). Taken together, these data suggest that the DDX58 R109C variant constitutively activates type I IFN signaling in vitro.

### R109C Mutation Disrupts RIG-I Autoinhibition

Next, we investigated the molecular mechanism underlying the R109C mutation-mediated activation of RIG-I. In the absence of viral dsRNA, caspase activation and recruitment domains (CARDs) of RIG-I are masked by the intramolecular interaction with DEAD helicase domain, which leads RIG-I to a signaling repressed state. After Carboxy-terminal domain recognizing and binding to viral dsRNA in the cytosol, RIG-I undergoes a conformational change, releasing CARDs from the auto-repressed state. The R109 residue is located in the interface between CARD2 domain and DEAD helicase domain.

Structural modeling predicts that R109 in CARD2 domain may interact with C520, M521, and E531 in DEAD helicase domain without dsRNA binding (Figure 4A). The substitution of Arg to Cys at residue 109 disrupts three polar bonds within the R109 residue (Figure 4A), which suggests that the R109C mutation may affect intramolecular interaction of CARDs and DEAD helicase domain. We constructed RIG-I fragments lacking the two N-terminal CARDs (DCARD) and WT/R109C mutated N-terminal CARDs (Figure 4B). By co-immunoprecipitation analysis, we observed that R109C mutated CARDs co-precipitated less DCARD fragments than WT CARDs, suggesting a weaker interaction between R109C mutated CARDs and DEAD helicase domain, and this defective binding was unique to R109C variant, which wasn’t observed for the other four previously reported pathogenic variants (Figure 4C). By attenuating the CARDs and DEAD helicase domain interaction, the R109C mutation may lead to the exposure of CARDs and release from autoinhibition state, which results in RIG-I signaling activation.

Free CARDs are further activated by the K63-linked ubiquitination at K172, which contributes to effective RIG-I oligomerization and interaction with the adaptor

### Table 2. Renal biopsies of patients with the DDX58 R109C variant

<table>
<thead>
<tr>
<th>Imaging Method</th>
<th>P1</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light microscopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. glomeruli</td>
<td>23</td>
<td>34</td>
<td>12</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>No. cellular/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibrocellular crescents</td>
<td>11</td>
<td>30</td>
<td>2</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Pattern of GN</td>
<td>DPGN</td>
<td>MPGN</td>
<td>FPGN</td>
<td>MPGN</td>
<td>FPGN</td>
</tr>
<tr>
<td>Acute tubular injury</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tubular atrophy and</td>
<td>—</td>
<td>—</td>
<td>Severe</td>
<td>Moderate</td>
<td>—</td>
</tr>
<tr>
<td>interstitial fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>Moderate</td>
<td>Severe</td>
<td>Severe</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>Collapsing glomerulopathy</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thrombotic microangiopathy</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>2+MES/CW</td>
<td>2+MES/CW</td>
<td>—</td>
<td>2+MES/CW/BC/TBM/BV</td>
<td>2+MES/CW</td>
</tr>
<tr>
<td>IgA</td>
<td>1+MES/CW</td>
<td>1+MES/CW</td>
<td>2+MES/CW</td>
<td>1+MES/CW</td>
<td>1+MES/CW</td>
</tr>
<tr>
<td>IgM</td>
<td>1+MES/CW</td>
<td>2+MES/CW</td>
<td>1+MES/CW</td>
<td>1+MES/CW</td>
<td>1+MES/CW</td>
</tr>
<tr>
<td>C3</td>
<td>2+MES/CW/TBM</td>
<td>2+MES/CW/TBM</td>
<td>2+MES/CW/TBM/BC</td>
<td>3+MES/CW/TBM/BV</td>
<td>2+MES/CW/BV</td>
</tr>
<tr>
<td>C1q</td>
<td>2+MES/CW</td>
<td>2+MES/CW</td>
<td>—</td>
<td>traces</td>
<td>1+MES/CW/BV</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electron-dense deposits</td>
<td>Mesangial,</td>
<td>Mesangial,</td>
<td>Mesangial,</td>
<td>Mesangial,</td>
<td>Mesangial,</td>
</tr>
<tr>
<td></td>
<td>subendothelial,</td>
<td>subendothelial,</td>
<td>subendothelial,</td>
<td>subendothelial,</td>
<td>subendothelial,</td>
</tr>
<tr>
<td></td>
<td>subepithelial</td>
<td>subepithelial</td>
<td>subepithelial</td>
<td>subepithelial</td>
<td>subepithelial</td>
</tr>
<tr>
<td>Podocyte foot process</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>Focal</td>
</tr>
<tr>
<td>effacement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubuloreticular inclusions</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Renal biopsy diagnosis</td>
<td>LN-IV+V</td>
<td>LN-IV</td>
<td>LN-III</td>
<td>LN-IV</td>
<td>LN-III</td>
</tr>
<tr>
<td>AI, activity index</td>
<td>14</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>CI, chronicity index</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

AI, activity index; BC, Bowman’s capsule; BV, blood vessels; CI, chronicity index; CW, glomerular capillary wall; DPGN, diffuse proliferative (mesangial and endocapillary) GN; FPGN, focal proliferative GN; MES, mesangial; MPGN, membranoproliferative GN; TBM, tubular basement membrane.
Figure 2. Hyperactivation of type I IFN and NF-κB signaling in patients with the DDX58 R109C variant. (A) RNA sequencing analysis of type I IFN and NF-κB pathways in P1’s, P3’s, and P4’s PBMCs compared with three unaffected controls (C1–C3). Analysis of each sample was performed in duplicate. (B) Quantification of 28-gene IFN score of RNA sequencing data from (A). Data are presented as the mean±SD; ****P<0.0001, t test. (C) qPCR analysis of the expression of the IFN-stimulated genes in PBMCs from P1 compared with three unaffected controls. Data are presented as the mean±SD; n=3 independent experiments; ***P<0.001, ****P<0.0001, t test. (D) Marker-based annotation on UMAP plot of single-cell RNA sequencing data from two unaffected controls (C1 and C2) and patient P1. (E) Visualization of upregulated IFN-stimulated and inflammatory genes among all
protein MAVS on mitochondria. Because K63-linked ubiquitination is essential for RIG-I activation, we performed immunoprecipitation experiments to assess the ubiquitination level of RIG-I and found the RIG-I R109C K63-linked ubiquitination was significantly increased (Figure 4D).

The tetramer of RIG-I CARDs functions as a nucleus for the induction of MAVS filament formation. Filamentous MAVS then serves as a signaling platform for the recruitment and activation of TBK1, IRF3, and other downstream signaling, leading to the transcriptional activation of type I IFN. The co-immunoprecipitation experiment showed that the interaction occurred between RIG-I-R109Cs but not between RIG-I-WTs without poly(I:C) stimulation (Figure 4E), indicating that the R109C mutation promotes spontaneous RIG-I oligomerization. In addition, RIG-I R109C could co-precipitate more MAVS (Figure 4F), and the induction of RIG-I R109C triggered MAVS aggregation more efficiently compared with RIG-I WT (Figure 4G).

These data suggest that the R109C mutation activates RIG-I-MAVS-mediated IFN signaling by disrupting the autorepressed conformation of RIG-I.

Potential Therapeutic Implication with JAK Inhibitor Baricitinib

When P1 was diagnosed with SLE and LN at the age of 12 years, she received intravenous methylprednisolone (MP) and cyclophosphamide (CTX) as induction therapy, and then switched to oral glucocorticoids and azathioprine for maintenance therapy. At the age of 28 years, she was given intravenous MP and CTX again as disease relapsed. After partial remission, she switched to MP 8 mg plus mycophenolate mofetil (MMF) 1.25 g and hydroxychloroquine 400 mg per day for about 6 years, but the titer of antibodies remained high, and complement levels were still low.

The strong IFN signature in patients with the DDX58 R109C variant suggests that JAK inhibitor therapy could be beneficial, as it has been used in the treatment of other IFNopathies. Subsequently, baricitinib treatment was initiated in patient P1. Baricitinib is an oral selective JAK1 and JAK2 inhibitor via STAT1 and STAT2 pathways, which may affect the release of several proinflammatory cytokines, including type I IFN, IL-6, and others.

Before the targeted treatment, P1 stopped MMF for 5 weeks, and then began to receive oral baricitinib for 6 months. The dose of baricitinib was 2 mg once daily as recommended by the package insert. After baricitinib treatment for 6 months, the disease was stable, with the titer of antibody (ANA) decreasing to 1:512 and an elevated complement level. However, due to irreversible organ damage, renal disorder was not alleviated. No adverse effects or serious adverse events were observed (Supplemental Table 2).

To observe further the effectiveness of baricitinib treatment, we performed scRNAseq on the PBMCs of P1. Before baricitinib treatment, the patient exhibited enhanced gene expression in the type I IFN pathway such as IRF1 and IFI30 and proinflammatory cytokines such as IL1B and TNF compared with controls (Figures 2D and 5A). The difference in the gene expression was more significant in monocytes (Figure 5, A and B). After baricitinib treatment, we observed proinflammatory cytokines and ISG expression could be effectively suppressed, especially in CD14+ monocytes (Figure 5, A and B). Consistent with scRNAseq results, CBA analysis of serum from P1 showed a significantly decreased concentration of proinflammatory cytokines such as IL-6, IL-8, and IL-1β and chemokine CXCL10 after baricitinib treatment (Figure 5C).

The suppression of type I IFN signature in P1 with JAK inhibitor therapy provided the clinical implication that inhibiting IFN/JAK/STAT pathway through baricitinib may be a potential treatment strategy for patients with LN caused by the DDX58 R109C heterozygous variant.

DISCUSSION

In summary, we identified a novel heterozygous gain-of-function mutation, R109C, in the DDX58 gene in five unrelated families with LN. The RIG-I R109C mutation constitutively activated the type I IFN pathway due to impaired autoinhibition and increased K63 ubiquitination and MAVS aggregation. Furthermore, in one of the DDX58 R109C variant-driven LN patients, initial experience with the JAK inhibitor baricitinib has been positive, with the effective suppression of the type I IFN signature (Figure 6).

DDX58 variants have been associated with an elevated IFN response in SMS patients. In this study, our patients also presented with a strong IFN signature but manifested as systemic inflammation and autoimmune disease, which were characterized strikingly by SLE and LN. This indicates that DDX58-mediated disorders may have phenotypic heterogeneity. This study demonstrates a new manifestation of DDX58-mediated disease with a distinct pathophysiology and genetic underpinning from SMS. SMS DDX58 variants (C268F and E373A), occurring
Figure 3. R109C mutation constitutively activates the IFN pathway in HEK293T cells. (A) RNA sequencing analysis of type I IFN and NF-κB pathways in HEK293T cells overexpressing with or without RIG-I-WT or R109C for 24 hours. Analysis of each sample was performed in triplicate. (B) Quantification of 28-gene IFN score of RNA sequencing data from (A). Data are presented as the mean±SD; ****P<0.0001, t test. (C) The gene set enrichment analysis plot of differential expression gene sets of RNA sequencing data from (A) enriched on JAK-STAT and NF-κB signaling pathways in RIG-I-WT and R109C overexpressing cells. (D) Luciferase reporter gene assay with IFN-β, ISRE, and NF-κB in HEK293T cells overexpressing with or without RIG-I-WT or R109C upon 5 μg/ml poly(I:C) stimulation for 12 hours or not. Data are presented as the mean±SD; n=3 independent experiments; ***P<0.001, t test.
in the HEL-1 domain (Supplemental Figure 2A), impair ATPase activity, preventing ATP hydrolysis, and recognizing self-RNA constitutively.22,23 The other two SMS DDX58 variants (E510V and Q517H) happened in the HEL-2i domain (Supplemental Figure 2A), weakening the RNA proofreading capabilities, failing to distinguish self from nonself RNA, and inducing hyperactivation of RIG-I.24 However, the R109C variant causing LN, located in the CARD2 domain (Supplemental Figure 2A), leads to defective CARDs binding and loss of autoinhibition of RIG-I. The different mechanisms result in various activation of RIG-I and a distinct pattern of upregulation of downstream IFN signaling, as a consequence, presenting with different phenotypes. However, identification of additional

Figure 3. (Continued) (E) Western blotting analysis of the activation of type I IFN and NF-κB signaling by using the indicated antibodies and IRF3 dimer detected by native PAGE in HEK293T cells overexpressing with or without RIG-I-WT or R109C for 24 hours upon 5 μg/ml poly(I:C) stimulation for 12 hours or not. (F) qPCR analysis of the expression of the IFN-stimulated genes in HEK293T cells treated as in (E). Data are presented as the mean±SD; n=3 independent experiments; **P<0.01, ***P<0.001, t test. NES, normalized enrichment score.

Figure 4. R109C mutation activates IFN signaling by disrupting the autorepressed conformation of RIG-I. (A) Molecular dynamics snapshot of intramolecular interaction in the CARD2 (brown): DEAD helicase (green) interface of duck RIG-I WT and R109C. The corresponding residues in human RIG-I are shown in parentheses. The polar bonds are shown by yellow lines. Models were generated using Pymol v2.3.5 and Protein Data Bank accession 4a2w. (B) Schematic of human RIG-I full length domains and the truncated fragments (CARDs and ΔCARD) constructed in this study. (C) Co-precipitation of Myc-tagged WT ΔCARD or mutated ΔCARD with Flag-tagged WT CARDs or R109C mutated CARDs, respectively, in HEK293T cells overexpressing with indicated plasmids for 24 hours. Immunoprecipitation was carried out with anti-Myc beads, and the precipitates were analyzed using anti-Flag antibody. (D) K63-linked ubiquitination of RIG-I-WT or R109C detected by SDS-PAGE. HEK293T cells were transfected with Myc-tagged RIG-I-WT or R109C, together with HA-tagged K63-Ub for 24 hours. Cell lysates were immunoprecipitated with anti-Myc beads, followed by immunoblotting analysis with the indicated antibodies. (E) Co-precipitation of Flag-tagged RIG-I-WT or R109C with Myc-tagged RIG-I-WT or R109C, respectively, in HEK293T cells overexpressing with indicated plasmids for 24 hours, with or without 5 μg/ml poly(I:C) stimulation for 12 hours. Immunoprecipitation was carried out with anti-Myc beads, and the precipitates were analyzed using anti-Flag antibody. (F) Co-precipitation of Myc-tagged RIG-I-WT or R109C with Flag-tagged MAVS in HEK293T cells overexpressing with indicated plasmids for 24 hours. Immunoprecipitation was carried out with anti-Myc beads, and the precipitates were analyzed using anti-Flag antibody. (G) MAVS aggregates formation detected by SDD-AGE in HEK293T cells overexpressing with RIG-I-WT or R109C for 24 hours. Ub, ubiquitination.
individuals with the DDX58 variant would help to define this condition further. More interestingly, in the PBMCs of our patient, scRNAseq revealed activation of the IFN signaling pathway was most significant in monocytes, highlighting the fundamental role of monocyte activation in the pathogenicity of patients with DDX58 R109C variant-driven LN.

Reduced penetrance can be seen in DDX58-associated diseases, as the patients AP1 in family 4 and AP2 in family 5 with the DDX58 R109C variant are asymptomatic. It is possible that other factors, such as modifier genes, may also contribute to disease expression. Reduced penetrance is commonly seen in dominant inherited diseases, which has been reported in individuals with the IFIH1 variant, for example.

The IFIH1 gene encodes the dsRNA helicase enzyme, melanoma differentiation-associated protein 5 (MDA5), which has similar functions to RIG-I as a dsRNA sensor. Gain-of-function variants in both MDA5 and RIG-I lead activation of the IFN signaling pathway. Heterozygous variants in IFIH1 have been reported in Aicardi–Goutières syndrome, SMS,

Figure 5. Treatment with baricitinib suppresses type I IFN signature. (A) Visualization of expression of IRF1, IFI30, IL1B, and TNF on UMAP plot from P1 before (n=8596 cells) and after baricitinib treatment for 3 months (n=5933 cells) and two unaffected controls (C1 [n=11,116 cells] and C2 [n=13,925 cells]). Colored dots indicate single cells, and cells with high expression level are highlighted in red. (B) Violin plots showing the expression of IFN-stimulated genes in the CD14⁺ and CD16⁺ monocytes of P1 before and after baricitinib treatment for 3 months compared with two unaffected controls (C1 and C2). (C) CBA analysis of serum proinflammatory cytokines and chemokines (IL-6, IL-8, IL-1β, and CXCL10) levels in P1 before and after baricitinib treatment for 3 months compared with six unaffected controls.
and SLE. Intriguingly, overlapping phenotypes of SLE and SMS were identified in one patient with the IFI1H1 R822Q variant. The disease spectrum of IFI1H1 variants overlaps with those reported in this study. Given this, it appears that DDX58- and IFI1H1-driven diseases are part of the same spectrum of errors in dsRNA sensor activation.

LN has been considered as a prototype of type I IFN-related kidney disease. It is supported by the fact that increased expression of ISGs in PBMCs is detectable in 50%–80% of patients with SLE, and high circulating type I IFN levels are associated with disease activity and flares in patients with LN. The common lesions of type I IFN-mediated kidney disease appear to be inflammatory-proliferative nephritis, collapsing glomerulopathy, and thrombotic microangiopathy. In this study, all the patients demonstrated the patterns of proliferative lesion; none of them showed collapsing glomerulopathy or thrombotic microangiopathy (Table 2). It is possible that type I IFN contributes to glomerular injury in LN through damaging resident kidney cells directly, inducing the production and deposition of autoantibodies, and recruiting the inflammatory cells. From this perspective, when screening

Figure 6. Schematic model of RIG-I R109C mutation leading to spontaneous activation of RIG-I and upregulation of type I IFN signaling and then causing LN.
the clinical phenotypes of enough individuals who carried the other \textit{DDX58} variants and presented with elevated levels of type I IFN, it is therefore possible that LN or LN-like patients would be identified. Notably, another interesting finding was that four of the patients had tubuloreticular inclusion in the glomerular endothelial cells under electron microscopy (Figure 1B), which was referred as a lesion of IFN footprints. Our study revealed that the \textit{DDX58} R109C variant results in elevated type I IFN signaling, and the strong IFN signature was detected in our patients. Thus, it may be that this kind of ultrastructural feature is related to type I IFN, but this needs further validation.

JAK inhibitors have been approved as a therapeutic option for patients with immune-mediated inflammatory diseases, such as rheumatoid arthritis, psoriatic arthritis, and SLE.\textsuperscript{34} In this study, one of the patients with the \textit{DDX58} variant received JAK inhibitor treatment and showed a certain response, with decreased activation of IFN signaling pathway. This is the initial experience of selected treatment with a JAK inhibitor on the basis of the \textit{DDX58} variant in a LN patient. It not only confirmed the pathogenic effect of the \textit{DDX58} mutation in the disease, but also shed light on more precisely targeted therapy for patients with the \textit{DDX58} variant. Apart from that, extended treatment observations in more patients with the \textit{DDX58} variant are needed to prove the clinical significance further.

SLE is a complex disorder, with extensive heterogeneous phenotypes. Current treatment for SLE does not adequately control disease activity and tissue damage, especially to the kidney, and is associated with significant side effects. Identifying genetic causes and finding potential therapeutic targets of LN is a major unmet clinical need. Additionally, dissecting the pathogenic genetic variants has a profound significance to define subsets of LN better and to shed light on the pathogenesis. This study provided the first example of how LN patients might be treated with targeted therapy when a genetic diagnosis is indicated.

**REFERENCES**


**ACKNOWLEDGMENTS**

We would like to thank the patients, their families, and the unaffected controls for their contributions to this study. We thank Natalie T. Deutch from the National Human Genome Research Institute for providing valuable advice and editing the manuscript. All samples were from Renal Biobank of National Clinical Research Center of Kidney Diseases, Jiangsu Biobank of Clinical Resources.

**AUTHOR CONTRIBUTIONS**

Y. An, Y. Chen, E. Gao, Zhengzhao Liu, Y. Jin, F. Xu, C. Zhang, Y. Zhang, and C. Zheng were responsible for resources; X. Chen and X. Han were responsible for software; X. Han, J. Peng, and Y. Wang were responsible for data curation; X. Han, J. Peng, Y. Wang, Z. Yang, and J. Zhang were responsible for the formal analysis; Zhihong Liu, J. Peng, Y. Wang, C. Zhang, and Q. Zhou were responsible for conceptualization and project administration; Zhihong Liu, Y. Wang, C. Zhang, and Q. Zhou were responsible for funding acquisition; Zhihong Liu and Q. Zhou were responsible for the investigation and supervision and reviewed and edited the manuscript; and J. Peng and Y. Wang wrote the original draft of the manuscript.

**DATA SHARING STATEMENT**

All data associated with this study are present in the paper or the Supplemental Material. Primary data of DNA-based and RNA-based assays can be accessed by contacting the corresponding authors (Q. Zhou or Zhihong Liu).

**SUPPLEMENTAL MATERIAL**

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2022040477/-/DCSupplemental.

Supplemental Appendix 1. Clinical synopsis of patients with the \textit{DDX58} R109C variant.

Supplemental Table 1. Clinical comparison of individuals with \textit{DDX58}-associated Singleton–Merten syndrome.

Supplemental Table 2. Changes of clinical parameters before and after treatment with baricitinib.

Supplemental Figure 1. Hyperactivation of type I IFN and NF-\kappaB signaling in patients with the \textit{DDX58} R109C variant.

Supplemental Figure 2. Functional comparison of R109C variant with previously established pathogenic variants in \textit{DDX58}.

**DISCLOSURES**

All authors have nothing to disclose.
34. Ding X, Ren Y, He X: IFN-I mediates lupus nephritis from the beginning to renal fibrosis. Front Immunol 12: 676082, 2021
SUPPLEMENTAL MATERIALS

Table of Contents:

Supplemental Appendix 1. Clinical synopsis of patients with the DDX58 R109C variant.

Supplemental Table 1. Clinical comparison of individuals with DDX58-associated Singleton-Merten syndrome.

Supplemental Table 2. Changes of clinical parameters before and after treatment with baricitinib.

Supplemental Figure 1. Hyperactivation of type I IFN and NF-κB signaling in patients with the DDX58 R109C variant.

Supplemental Figure 2. Functional comparison of R109C variant with previously established pathogenic variants in DDX58.
Supplemental Appendix 1. Clinical synopsis of patients with the DDX58 R109C variant

The proband (P5) of second family was a man who initially presented with recurrent edema in lower limbs and face, chest pain occasionally, and foamy urine at 51-year-old. The symptoms gradually worsened, and he had poor appetite, general fatigue, oliguria, and serous effusion. Laboratory testing indicated proteinuria (3+), hematuria (3+), hypoalbuminemia, elevated serum creatinine, and moderate anemia. He was positive for ANA, anti-RNP, and rheumatoid factor. Complements C3 and C4 levels were decreased. Renal biopsy showed class IV LN. His serum creatinine gradually declined with glucocorticoids and mycophenolate mofetil treatment.

The proband (P6) of third family was a 20-year-old boy, with a history of psoriasis for 5 years. He presented with hypertension, proteinuria, hematuria, hypoalbuminemia, elevated serum creatinine, and anemia. ANA, anti-dsDNA, anti-Sm, anti-RNP antibodies were negative. Renal biopsy showed class III LN. During hospitalization, he had an epileptic seizure. His serum creatinine gradually elevated and the patient died soon.

The proband (P7) of fourth family was a female. She initially presented with malar rash and photosensitivity at 28-year-old. Seven months later, she suffered from oral ulcers, arthralgia, alopecia, and developed with pancytopenia, positive ANA and anti-dsDNA antibody, and decreased complement C3 level. Urinalysis revealed moderate proteinuria and microscopic hematuria, and renal biopsy showed class IV LN. CT scan revealed a small bilateral pleural effusion. Based on these findings, SLE was diagnosed. P7’ mother was suspicious of SLE.

The proband (P8) of fifth family was a man. He presented with oral ulcers, arthritis, and
pinpoint-like rashes on the abdomen and both lower limbs at 45 years of age. Urinary test showed moderate proteinuria and hematuria. Renal biopsy showed class III LN. ANA, anti-dsDNA and anticardiolipin antibodies were positive. Blood routine examination revealed increased creatinine, thrombocytopenia and anemia with Coombs test (+). And bone marrow biopsy revealed megakaryocyte hypoplasia. P8 was diagnosed as SLE and remission was obtained with glucocorticoids and mycophenolate mofetil treatment. His sister (P9) presented with lupus-like syndrome, with mild hematuria, positive anticardiolipin antibody and decreased complement C3 level. Besides, she had transient ANA positivity and leukopenia. Complete remission was obtained with short-term glucocorticoids therapy.
**Supplemental Table 1. Clinical comparison of individuals with DDX58-associated Singleton-Merten syndrome**

<table>
<thead>
<tr>
<th>No.</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
<th>Family 4</th>
<th>Family 5</th>
<th>DDX58-associated SMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>II-6</td>
<td>III-7</td>
<td>III-1</td>
<td>II-3</td>
<td>IV-4</td>
<td>total n (%)</td>
</tr>
<tr>
<td>Patients</td>
<td>II-6</td>
<td>III-7</td>
<td>III-1</td>
<td>II-3</td>
<td>IV-4</td>
<td>n</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Aortic and valvular calcification</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Psoriasiform rash</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Acro-osteolysis or tuft erosion of distal phalanx</td>
<td>Y</td>
<td>Y</td>
<td>ND</td>
<td>Y</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Delayed secondary dentition</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>N</td>
<td>N</td>
<td>ND</td>
<td>N</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tendon rupture</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Joint subluxation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hyplastic/aplastic toenails</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Dental problems</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Short stature</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

**Abbreviations:** ND, no data; SMS, Singleton-Merten syndrome.
**Supplemental Table 2. Changes of clinical parameters before and after treatment with baricitinib**

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Maintenance therapy*</th>
<th>0 month**</th>
<th>1 month</th>
<th>3 month</th>
<th>6 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.3</td>
<td>3</td>
<td>1.1</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/H)</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Hematologic involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>148</td>
<td>146</td>
<td>143</td>
<td>147</td>
<td>149</td>
</tr>
<tr>
<td>White blood cell (10^9/L)</td>
<td>7.48</td>
<td>5.66</td>
<td>6.44</td>
<td>8.38</td>
<td>7.17</td>
</tr>
<tr>
<td>Platelet (10^9/L)</td>
<td>218</td>
<td>179</td>
<td>183</td>
<td>196</td>
<td>223</td>
</tr>
<tr>
<td>Renal involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine protein (g/24h)</td>
<td>1.28</td>
<td>0.68</td>
<td>0.81</td>
<td>0.66</td>
<td>0.89</td>
</tr>
<tr>
<td>Urinary red blood cell (/ul)</td>
<td>6.7</td>
<td>7.6</td>
<td>1.7</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.65</td>
<td>0.7</td>
<td>0.68</td>
<td>0.68</td>
<td>0.70</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>39.7</td>
<td>38.8</td>
<td>34.7</td>
<td>36.4</td>
<td>38.9</td>
</tr>
<tr>
<td>Immunological disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td>1: 1024</td>
<td>1: 1024</td>
<td>1: 1024</td>
<td>1: 1024</td>
<td>1: 512</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>&lt;1:10</td>
<td>&lt;1:10</td>
<td>&lt;1:10</td>
<td>&lt;1:10</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>C3 (g/L)</td>
<td>0.769</td>
<td>0.6985</td>
<td>0.607</td>
<td>0.6</td>
<td>0.639</td>
</tr>
<tr>
<td>C4 (g/L)</td>
<td>0.126</td>
<td>0.107</td>
<td>0.109</td>
<td>0.1012</td>
<td>0.1100</td>
</tr>
<tr>
<td>IgG (GPL-U/ml)</td>
<td>267.62</td>
<td>241.57</td>
<td>305.86</td>
<td>205.49</td>
<td>243.28</td>
</tr>
<tr>
<td>IgA (APL-U/ml)</td>
<td>17.04</td>
<td>negative</td>
<td>negative</td>
<td>20.09</td>
<td>21.5</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>2.42</td>
<td>2.6</td>
<td>2.41</td>
<td>2.59</td>
<td>2.63</td>
</tr>
<tr>
<td>Anti-β2 glycoprotein I (RU/ml)</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Anti-C1q (U/ml)</td>
<td>4.14</td>
<td>5.57</td>
<td>3.4</td>
<td>6.65</td>
<td>2.69</td>
</tr>
</tbody>
</table>

*: maintenance therapy was including methylprednisolone 8mg per day, mycophenolate mofetil 1.25g per day, hydroxychloroquine 400mg per day and warfarin.

**: mycophenolate mofetil was stopped for 5 weeks at baseline. Methylprednisolone, hydroxychloroquine and warfarin were continued in addition to baricitinib 2mg.
Supplemental Figure 1. Hyperactivation of type I IFN and NF-κB signaling in patients with the **DDX58** R109C variant.

(A) RNA-sequencing analysis of type I IFN and NF-κB pathways in P7’s PBMCs during the active phase of SLE, compared with three unaffected controls (C1 - C3). Analysis of each sample was performed in duplicate.

(B) Quantification of 28-gene IFN score of RNA sequencing data from Supplemental Figure 1A. Data are presented as mean ± SD; **** p<0.0001, Student’s t-test.

(C) qPCR analysis of the expression of the IFN-stimulated genes in PBMCs from P7 during the active phase of SLE, compared with three unaffected controls (C). Data are presented as mean ± SD; n = 3 independent experiments; **** p<0.0001, Student’s t-test.

Supplemental Figure 2. Functional comparison of R109C variant with previously established pathogenic variants in **DDX58**.

(A) Schematic representation of disease-causing variants relative to human RIG-I protein. Red indicates the novel mutation reported in this study. CARD, caspase activation and recruitment domain; CTD, carboxy-terminal domain; HEL, helicase domain; P, pincer.

(B) Luciferase reporter gene assay with IFNβ, ISRE, NF-κB in HEK293T cells overexpressing with or without RIG-I-WT or mutant. Data are presented as mean ± SD; n = 3 independent experiments; ISRE, IFN-stimulated response element.
Supplemental Figure 2

A

RIG-I

c.325C>T p.Arg109Cys
c.1529A>T p.Glu510Val
c.1551G>C p.Gln517His

c.803G>T p.Cys268Phe
c.1118A>C p.Glu373Ala

N CARD1 CARD2 HEL-1 HEL-2i HEL-2 P CTD C

B

B

IFNβ

Rel. Luc. Activity

0 5 10 15 20

Vector WT R109C C268F E373A E510V Q517H

Myc-RIG-I GAPDH

ISRE

0 10 20 30 40 50

Vector WT R109C C268F E373A E510V Q517H

NF-κB

0 20 40 60 80 100

Vector WT R109C C268F E373A E510V Q517H