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ORIGINAL ARTICLE

# Strong association of common variants in the miRNA-binding site of *NOD2* gene with clinicopathological characteristics and disease activity of systemic lupus erythematosus

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## Abstract

**Introduction/objectives** Systemic lupus erythematosus (SLE) is a multifactorial systemic autoimmune disease, in which genetic susceptibility plays a pivotal role. The nucleotide oligomerization domain 2 (*NOD2*) gene is one of the main regulators of chronic inflammatory conditions and could be involved in SLE pathogenesis. Single nucleotide polymorphisms (SNPs) in miRNA binding sites which are located in 3′UTR of the *NOD2* gene could be associated with SLE risk by dysregulation of *NOD2* expression. In the present study, we assessed the possible association between SNPs rs3135500 and rs3135499 in the *NOD2* gene with SLE risk in the Iranian population.

**Methods** A case–control study using 110 SLE patients and 120 control subjects was undertaken to estimate rs3135500 (G > A) and rs3135499 (A > C) genotypes via real-time PCR high-resolution melting method (HRM).

**Results** No significant association was observed between allele and genotype frequencies of rs3135500 and rs3135499 polymorphisms and SLE risk in this population ($P > 0.05$). However, there was an obvious association between rs3135500 (A allele) with laboratory factors that are associated with disease activity ($P < 0.05$) and some clinical manifestations that are associated with disease severity such as neurological symptoms, skin manifestations, renal involvements, and higher serum concentration of creatinine ($P < 0.05$). Besides, rs3135499 (C allele) was correlated with renal involvement and also the concentration of creatinine ($P < 0.05$). Moreover, in the patients group, the risk alleles in these polymorphisms were associated with lower age of onset ($P < 0.05$).

**Conclusions** Our results suggest a substantial association between NOD2 polymorphisms with clinicopathological characteristics and SLE disease activity.

**Key Points**

- *Single nucleotide polymorphisms (SNPs) in miRNA binding sites which are located in 3'UTR of the NOD2 gene could be associated with SLE risk by dysregulation of NOD2 expression.*
- *Our results suggested that two miRSNPs (rs3135500 and rs3135499) in the NOD2 gene were meaningfully correlated with clinicopathological characteristics and disease activity of SLE.*

**Keywords** miRNA · *NOD2* gene · Single nucleotide polymorphism · Systemic lupus erythematosus

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## Introduction

Systemic lupus erythematosus (SLE) is a multifactorial disorder and characterized by dysregulation in the innate and adaptive immune system and high-level production of autoantibodies [1, 2]. Numerous studies have revealed that genetic factors exert an important role in the occurrence of SLE. Based on twin studies, the heritability of SLE is approximately 66% [3]. Newly, genome-wide association studies (GWAS) discovered several single nucleotide polymorphisms (SNPs) and loci in various genes that have an immune and inflammatory function in correlation with SLE risk [4–6]. Emerging evidence emphasizes the role of genes that are involved in the inflammatory response in SLE pathogenesis [7]. Furthermore, some studies mentioned that infections are involved in the pathogenesis of SLE and known as a risk factor for this disease. On the other hand, uncovering the role of pattern recognition receptors (PRRs) in SLE triggering underscores the role of microbes in the pathogenesis of SLE [8–10].

The nucleotide oligomerization domain 2 (*NOD2*) gene is one of the main regulators of chronic inflammatory conditions [11, 12]. *NOD2* is an intracellular PRR and is involved in the recognition of certain bacteria. For meditation of innate immune response, *NOD2* detects intracellular muramyl dipeptide (MDP), a component of the bacterial wall, to trigger proinflammatory responses by activation of nuclear factor (NF)-κB, mitogen-activated protein kinases (MAPKs), and STAT1 [12–14]. Mutation or dysregulation in the expression of the *NOD2* gene has been found in patients with Blau syndrome (BS), early-onset sarcoidosis (EOS), Crohn's disease (CD), rheumatoid arthritis (RA), and especially in SLE disease [15–22].

MicroRNAs (miRNAs) with 19–25 nucleotides in length are the most studied classes of non-coding RNAs. These molecules regulate gene expression via binding to the 3′-untranslated regions (3′-UTRs) of mRNAs and are involved in various aspects of biological processes including immune cell functions and inflammatory responses [23]. Several investigations have indicated that miRNAs are involved in the pathogenesis and multiple aspects of various autoimmune diseases such as SLE [24, 25]. Numerous functional studies revealed that SNPs located in the 3′UTR of mRNAs may influence the function of miRNAs by affecting the secondary structure (target structure features) of 3′UTR and thermodynamic features of the binding site, which consequently dysregulate the expression of the target gene by changing the binding capacity of miRNAs [26, 27]. Disturbance in the gene expression regulatory activity of miRNAs predisposes the individuals to the diseases such as SLE [26, 28].

By investigation of literature and miRSNPs databases including MirSNP (http://bioinfo.bjmu.edu.cn/mirsnp/), and Polymirts (http://compbio.uthsc.edu/miRSNP), we selected 2 SNPs at miRNA binding sites (so-called miRSNP), which can modulate miRNA–*NOD2* mRNA interactions. rs3135500 (G > A) and rs3135499 (A > C) polymorphisms are two miRSNPs in the 3′UTR of *NOD2*. These variants are located in the miRNA binding site or vicinity of multiple miRNAs such as miR-192, miR-495, miR-671, miR-122, miR-158, miR-215, miR-3202, and miR-4747. Regarding our hypothesis, these variants could influence the miRNA-mRNA interaction and finally dysregulate *NOD2* expression [26, 29]. According to these data, for the first time, we intended to assess the possible association between rs3135500 and rs3135499 polymorphisms with risk of SLE occurrence and any other associations with clinicopathological characteristics and disease activity in the Iranian population.

## Materials and methods

In this case–control study, a total of 110 unrelated patients were selected among individuals referred to the rheumatology division of Alzahra Hospitals, Isfahan, Iran, according to European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) for SLE (2019). One hundred twenty unrelated SLE-free individuals from the same hospital with no signs and symptoms of SLE based on negative clinical and laboratory examination constituted our normal control group. Furthermore, healthy subjects had no personal and family history of SLE or other immunological and autoimmune disorders. This case–control study was confirmed by the AJA University of Medical Science Research Ethics Committee (UREC) and all contributors provided written informed consent. The participants were interviewed to fill up a structured questionnaire to find the data including gender, age of onset and age at sampling time, height and weight to calculate body mass index (BMI, calculated as weight [kg] divided by height [m] squared), blood pressure (SBP; systolic blood pressure and DBP; diastolic blood pressure), and family history of SLE and other autoimmune disorders. Moreover, we recorded clinical manifestations of SLE such as the presence of neurological symptoms, skin manifestations, hematological disorders, arthritis, and renal involvement. Likewise, we documented laboratory characteristics such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), anti-dsDNA antibodies, complement component 3 and 4 (C3 and C4), white blood cell (WBC), hemoglobin, blood urea nitrogen (BUN), platelet count test (PLT), creatinine, fasting blood sugar (FBS), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Eventually, about 3 ml of peripheral blood

was collected from cases and controls and drawn into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes and stored at − 20 °C for DNA extraction.

### Single nucleotide polymorphism selection

For selection of miRSNPs, in the first step, we investigated the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) to find SNPs in the 3′UTR of *NOD2* and then 1000 Genomes Project was used to screen SNPs according to the criteria of a minor allele frequency (MAF) more than or equal to 0.05 (MAF ≥ 0.05). Then, we found potential miRNA binding sites of the selected SNPs by some databases such as miRdSNP (http://mirdsnp.ccr.buffalo.edu/), miRSNP (http://cmbi.bjmu.edu.cn/mirsnp), and Polymirts (http://compbio.uthsc.edu/miRSNP). Consequently, based on these criteria, we reached to 2 polymorphisms (rs3135500 (G > A) and rs3135499 (A > C)) which existed in or in vicinity of miRNA response elements (MREs).

### Single nucleotide polymorphism genotyping

Genomic DNA was extracted from 200 µl of blood samples using the GeNet Bio DNA extraction Kit (Korea) according to the manufacturer's protocol. The DNA purity, concentration, and suitability of extracted DNA for polymerase chain reaction high-resolution melting (HRM) method were assessed by electrophoresis and spectroscopy. The forward and reverse primer sequences for amplification of the fragments around polymorphisms in the *NOD2* gene are located in Table 1. The HRM method was used to determine polymorphism genotypes in this study under the following conditions: 5 min at 95 °C for initial denaturation of the template DNA for the first cycle, 36 cycles of denaturation at 95 °C for the 20 s, annealing at 59 °C (for both polymorphisms) for 30 s and extension at 72 °C for 20 s. This method was carried out using HOT FIREPol EvaGreen HRM Mix (no ROX) HRM PCR kit and analysis accomplished with Rotor-Gene 6000™ (Corbett Research, Mortlake, New South Wales, Australia). Then, HRM analysis was performed by fluorescence acquisition during a temperature ramp from 60 to 95 °C using 0.1 °C intervals. To find sample genotypes in HRM analysis as a standard, specific samples (with different melting curves) were subjected to direct Sanger sequencing and their genotypes were distinguished.

**Table 1** Primer sequences for the amplification of fragments around of the two polymorphisms of the *NOD2* gene

| SNP ID | Primer sequence | PCR product length (bp) | Annealing temperature |
|---|---|---|---|
| rs3135500 | F: AATTGTCAG ATGCTGTGC AAATG<br>R: GCATAAAGT TCACGGCCA TGTT | 134 | 59 °C |
| rs3135499 | F: ACTGAGTGC CTTTTGGTGGA<br>R: GCCTGGATG GATGAGTCGAG | 132 | 59 °C |

### Statistical analyses

The SPSS 25 (Armonk, NY: IBM Corp) was used for statistical analyses. Genotype frequencies in patients and controls were tested for Hardy–Weinberg equilibrium using the $\chi^2$ test. Logistic regression analysis was performed to examine the correlation between genotypes and SLE and compute specific odds ratios (ORs), 95% confidential intervals (CIs), and *P* values. For demographic, clinical, and laboratory characteristics, *P* values were calculated using the independent Pearson $\chi^2$ test for categorical variables and t-test for continuous variables test with the significance level of < 0.05.

## Results

### Demographic and clinical characteristics

To estimate the correlation between risk factors (environmental and genetic) with SLE development, we evaluated a total of 230 participants in patient and control groups; 110 patients (25 males and 85 females with a mean age of 43.62 ± 13.41) in the case and 120 (32 males and 88 females with a mean age of 45.01 ± 12.83) in the control group. There was no significant relationship among patients and control subjects in terms of age ($P = 0.423$) and sex ($P = 0.542$), indicating that for these characteristics matching was adequate. The mean age of onset in the case group was 26.23 ± 10.85. Concerning BMI and blood pressure (SBP and DBP), there was a remarkable difference between the two groups of participants ($P < 0.05$). From all patients, twenty-seven patients (24.5%) with neurological symptoms,70 patients (63.65%) with skin manifestations, 56 patients (50.9%) with hematological manifestations, 84 patients (76.4%) with oral ulcers, 98 patients (89.1%) with arthritis, and 48 patients (43.6%) with renal involvement were documented. The characteristics of subjects with SLE and healthy individuals are presented in Table 2. The results of laboratory tests discovered that the mean concentration of ESR, CRP, creatinine, BUN, and anti-dsDNA was expressively higher in the patient group compared with healthy controls ($P < 0.05$). However, the concentration of hemoglobin, PLT, C3, and C4 levels was expressively higher in

**Table 2** Baseline characteristics of SLE patients and control subjects participated in the study

| Characteristics | Patients | Controls | *P* |
|---|---|---|---|
| Total number | 110 | 120 | |
| Age at sampling time (mean ± SD) | 43.62 ± 13.41 | 45.01 ± 12.83 | 0.423 |
| Gender n (%) | | | |
| Male | 25 (22.7%) | 32 (26.7%) | 0.542 |
| Female | 85 (77.3%) | 88 (73.3%) | |
| Age of onset (mean ± SD) | 26.23 ± 10.85 | – | – |
| BMI (mean ± SD) | 25.75 ± 2.34 | 24.33 ± 3.20 | < 0.001* |
| SBP (mean ± SD) | 125.46 ± 16.00 | 120.71 ± 9.64 | 0.008* |
| DBP (mean ± SD) | 82.64 ± 5.90 | 79.00 ± 8.34 | < 0.001* |
| Positive family history n (%) | 20 (18.18%) | 0 | – |
| Neurological symptoms n (%) | 27 (24.5%) | 0 | – |
| Skin manifestations n (%) | 70 (63.65%) | 0 | – |
| Hematological manifestations n (%) | 56 (50.9%) | 0 | – |
| Oral ulcers n (%) | 84 (76.4%) | 0 | – |
| Arthritis n (%) | 98 (89.1%) | 0 | – |
| Renal involvement n (%) | 48 (43.6%) | 0 | – |

*$P$ value < 0.05. *SLE*, systemic lupus erythematosus; *BMI*, body mass index; *SD*, standard deviation; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure

controls than in patients' subjects ($P < 0.05$). The details of the laboratory characteristics of patients with SLE and control groups are listed in Table 3.

## Genotype and allele distribution of rs3135500

The genotype frequencies in case and control groups were in agreement with those predicted via Hardy–Weinberg equilibrium. The frequencies of GG, AG, and AA genotypes in the RA patients were 33.6%, 43.6%, and 22.7%, respectively, and the genotype frequencies in the control group were 38.4%, 45.8%, and 15.8%, respectively. In addition, the frequencies of G and A alleles were 61.3% and 38.8% in controls, and 55.5% and 44.5% in cases, respectively. Statistical analysis demonstrated that no significant association exists between SNP rs3135500 and risk of SLE ($P$ for genotype and allele distribution > 0.05). Further analysis demonstrated that the comparison of combined genotypes, AG + AA genotypes, compared to the GG genotype was not

**Table 3** Laboratory characteristics of patients with SLE and controls group

| | Patients (110) | Controls (120) | *P* |
|---|---|---|---|
| ESR (mm/h) | 41.33 ± 22.81 | 15.35 ± 6.91 | < 0.001* |
| CRP (mg/l) | 16.30 ± 9.70 | 4.39 ± 2.60 | < 0.001* |
| White blood cell ($10^9$/1) | 6820.91 ± 1780.71 | 6485.33 ± 1325.94 | 0.105 |
| Hemoglobin | 11.87 ± 1.40 | 14.20 ± 1.48 | < 0.001* |
| PLT ($10^9$/1) | 225.91 ± 63.28 | 247.02 ± 67.1 | 0.015* |
| Creatinine (mg/dl) | 1.02 ± 0.24 | 0.84 ± 0.17 | < 0.001* |
| BUN | 19.59 ± 11.90 | 16.12 ± 4.13 | 0.003* |
| FBS | 89.70 ± 12.71 | 93.14 ± 22.10 | 0.140 |
| HDL | 51.05 ± 8.91 | 50.24 ± 11.26 | 0.546 |
| LDL | 102.74 ± 26.14 | 107.20 ± 31.35 | 0.245 |
| TG | 157.23 ± 46.50 | 156.83 ± 59.72 | 0.955 |
| Anti-dsDNA (IU/ml) | 198.91 ± 181.70 | 10.91 ± 4.35 | < 0.001* |
| C3 level (mg/dl) | 50.28 ± 32.03 | 141.53 ± 35.12 | < 0.001* |
| C4 level (mg/dl) | 10.52 ± 7.20 | 19.87 ± 5.84 | < 0.001* |

*$P$ value < 0.05. *SLE*, systemic lupus erythematosus; *SD*, standard deviation; *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein; *BUN*, blood urea nitrogen; *PLT*, platelet; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *TG*, triglyceride; *FBS*, fasting blood sugar; *C3*, complement component 3; *C4*, complement component 4; *dsDNA*, double-stranded DNA

significantly different between case and control groups ($P = 0.494$) (Table 4). Moreover, stratification based on some laboratory and clinical characteristics illustrated that patients with risk allele (A) have lower age of onset ($P < 0.001$). Furthermore, patients with different genotypes have a significantly different mean concentration of CRP, ESR, C3, C4, anti-dsDNA, and creatinine ($P < 0.05$). In detail, patients with risk allele (A) had a higher concentration of CRP, ESR, anti-dsDNA, and creatinine. But, the mean concentration of C3 and C4 was lower in SLE subjects with the A allele. Besides, our analysis demonstrated that patients with different genotypes have significantly different clinical manifestations. In detail, from all patients with AA genotype, 48.0% have neurological symptoms while just 20.8% and 13.5% of patients with AG and GG genotypes, respectively, have this symptom ($P = 0.006$). Additionally, most of the patients with AA genotypes (96.0%), 75.0%, and 27.0% of patients with AG and GG genotypes, respectively, had skin manifestations ($P < 0.001$). Also, 76.0%, 47.9%, and 16.2% of patients with AA, AG, and GG genotypes, respectively, had renal involvement ($P < 0.001$). However, there was no significant association between the stratification of the hematological manifestations, oral ulcers, and arthritis with different genotypes of this polymorphism ($P > 0.05$) (Table 5).

## Genotype and allele distribution of rs3135499

In our study, the frequencies of the AA, AC, and CC genotypes in the control group were 45.0%, 34.2%, and 20.8%, respectively. In the same vein, in the case group, these frequencies were 41.8% for AA, 30.9% for AC, and 27.3% for CC. Additionally, the frequencies of A and C alleles were 57.3% and 42.7% in cases, and 62.1% and 37.9% in the control group, respectively. There was not a significant difference between case and control groups regarding genotypes and allele frequency ($P > 0.05$). Also, the comparison of combined genotypes uncovered that the CC + AC genotypes compared to the AA genotype were not significantly different between case and control groups ($P > 0.05$) (Table 4). Our stratification analysis in the case group revealed that the C allele was significantly correlated with lower age of onset ($P = 0.004$). In this study, patients with CC, AC, and AA genotypes have $20.70 \pm 6.70$, $27.88 \pm 12.04$, and $28.61 \pm 11.03$ mean age of onset, respectively. Likewise, patients with risk allele (C) had a slightly higher mean concentration of anti-dsDNA ($P = 0.04$). Moreover, between patients, the C allele was meaningfully associated with creatinine concentration ($P = 0.003$) and renal involvement ($P < 0.001$). In detail, 73.3%, 35.3%, and 30.4% of patients with CC, AC, and AA genotypes had renal involvement, respectively. Consistently, the concentrations of creatinine in patients with CC, AC, and AA genotypes were $1.10 \pm 0.24$,

**Table 4** Association between genotypes and allele frequency of NOD2 polymorphisms with SLE risk

| Genotype group | Patients (n = 110) n (%) | Controls (n = 120) n (%) | OR (95%CI) | *P* value |
|---|---|---|---|---|
| rs3135500 | | | | |
| GG | 37 (33.6%) | 46 (38.4%) | Reference | –- |
| AG | 48 (43.6%) | 55 (45.8%) | 1.10 (0.61,1.94) | 0.882 |
| AA | 25 (22.7%) | 19 (15.8%) | 1.64 (0.78,3.42) | 0.198 |
| Combined genotype | | | | |
| GG | 37 (33.6%) | 46 (38.3%) | Reference | –- |
| AG + AA | 73 (66.4%) | 74 (61.7%) | 1.23 (0.72,2.11) | 0.494 |
| Allele | | | | |
| G | 122 (55.5%) | 147 (61.3%) | Reference | –- |
| A | 98 (44.5%) | 93 (38.8%) | 1.27 (0.88,1.84) | 0.219 |
| rs3135499 | | | | |
| AA | 46 (41.8%) | 54 (45.0%) | Reference | –- |
| AC | 34 (30.9%) | 41 (34.2%) | 1.03 (0.56,1.87) | 0.999 |
| CC | 30 (27.3%) | 25 (20.8%) | 1.41 (0.73,2.73) | 0.320 |
| Combined genotype | | | | |
| AA | 46 (41.8%) | 54 (45.0%) | Reference | –- |
| AC + CC | 64 (58.2%) | 66 (55.0%) | 1.14 (0.68,1.92) | 0.690 |
| Allele | | | | |
| A | 126 (57.3%) | 149 (62.1%) | Reference | –- |
| C | 94 (42.7%) | 91 (37.9%) | 1.22 (0.84,1.80) | 0.297 |

*$P$ value < 0.05; *SLE*, systemic lupus erythematosus

**Table 5** Association of *NOD2* rs3135500 polymorphism with various parameters of SLE (110 patients)

| Genotype group | GG (n = 37) | AG (n = 48) | AA (n = 25) | *P* value |
|---|---|---|---|---|
| Age of onset | 32.49 ± 12.35 | 23.92 ± 7.70 | 23.28 ± 8.33 | < 0.001* |
| ESR (mm/h) | 32.96 ± 16.94 | 45.38 ± 26.15 | 46.48 ± 20.38 | 0.015* |
| CRP (mg/l) | 11.40 ± 6.04 | 16.67 ± 9.29 | 22.92 ± 10.95 | < 0.001* |
| C3 level (mg/dl) | 82.38 ± 33.56 | 41.01 ± 26.16 | 20.58 ± 13.73 | < 0.001* |
| C4 level (mg/dl) | 16.20 ± 4.80 | 9.10 ± 7.33 | 4.88 ± 2.39 | < 0.001* |
| Anti-dsDNA (IU/ml) | 46.53 ± 43.12 | 185.41 ± 121.93 | 450.36 ± 126.48 | < 0.001* |
| Creatinine (mg/dl) | 0.90 ± 0.18 | 1.03 ± 0.18 | 1.16 ± 0.31 | < 0.001* |
| Hemoglobin (HB) | 11.80 ± 1.32 | 11.75 ± 1.38 | 12.20 ± 1.52 | 0.386 |
| Neurological symptoms n (%) | 5 (13.5%) | 10 (20.8%) | 12 (48.0%) | 0.006* |
| Skin manifestations n (%) | 10 (27.0%) | 36 (75.0%) | 24 (96.0%) | < 0.001* |
| Hematological manifestations n (%) | 23 (62.2%) | 22 (48.8%) | 11 (44.0%) | 0.241 |
| Oral ulcers n (%) | 32 (86.5%) | 33 (68.8%) | 19 (76.4%) | 0.162 |
| Arthritis n (%) | 34 (91.9%) | 42 (87.5%) | 22 (88.0%) | 0.608 |
| Renal involvement n (%) | 6 (16.2%) | 23 (47.9%) | 19 (76.0%) | < 0.001* |

Data are mean ± SD, or n (%). *$P$ value < 0.05; *SLE*, systemic lupus erythematosus; *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein; *BMI*, body mass index; *SD*, standard deviation

1.01 ± 0.23, and 1.00 ± 0.20, respectively. Nevertheless, there was no significant association between the stratification of some laboratory characteristics such as ESR, CRP, C3, C4, hemoglobin concentration, and other clinical manifestations with this polymorphism ($P > 0.05$) (Table 6).

## Discussion

Several studies have emphasized the notion that a large number of SNP loci affect the susceptibility to autoimmune disorders [30, 31]. GWAS have reported several genetic susceptibility loci associated with increased risk of SLE [5, 32]. Between these polymorphisms, functional polymorphisms such as SNPs located in the 3′UTR of immune and inflammatory-related genes could contribute to the disruption of miRNA recognition elements (MREs) or create new sites leading to up- or downregulation of these genes and consequently may be remarkably associated with SLE risk [26, 33].

NOD2, a cytosolic protein that is expressed in monocytes and macrophages, acts as an activator of nuclear factor (NF)-κB, mitogen-activated protein kinases (MAPKs), and STAT1 by sensing bacterial lipopolysaccharide (LPS), ultimately, resulting in the production of pro-inflammatory mediators [34, 35]. Several studies reported

**Table 6** Association of *NOD2* rs3135499 polymorphism with various parameters of SLE (110 patients)

| Genotype group | AA (n = 46) | AC (n = 34) | CC (n = 30) | *P* value |
|---|---|---|---|---|
| Age of onset | 28.61 ± 11.03 | 27.88 ± 12.04 | 20.70 ± 6.70 | 0.004* |
| ESR (mm/h) | 38.28 ± 23.66 | 39.88 ± 23.38 | 47.63 ± 20.15 | 0.198 |
| CRP (mg/l) | 15.35 ± 8.74 | 15.27 ± 9.10 | 18.67 ± 11.49 | 0.220 |
| C3 level (mg/dl) | 54.46 ± 34.30 | 54.52 ± 42.52 | 38.58 ± 28.34 | 0.113 |
| C4 level (mg/dl) | 11.84 ± 8.04 | 10.10 ± 7.00 | 9.01 ± 5.57 | 0.220 |
| Anti-dsDNA (IU/ml) | 162.13 ± 159.60 | 187.66 ± 178.70 | 268.10 ± 203.43 | 0.04* |
| Creatinine (mg/dl) | 1.00 ± 0.20 | 1.01 ± 0.23 | 1.10 ± 0.24 | 0.003* |
| Hemoglobin (HB) | 12.03 ± 1.40 | 11.86 ± 1.43 | 11.63 ± 1.37 | 0.479 |
| Neurological symptoms n (%) | 10 (17.4%) | 9 (26.5%) | 8 (33.3%) | 0.274 |
| Skin manifestations n (%) | 25 (54.3%) | 24 (70.6%) | 21 (70.0%) | 0.229 |
| Hematological manifestations n (%) | 23 (50.00%) | 17 (50.00%) | 16 (53.3%) | 0.312 |
| Oral ulcers n (%) | 36 (78.3%) | 23 (67.6%) | 25 (83.8%) | 0.216 |
| Arthritis n (%) | 44 (95.7%) | 28 (82.4%) | 26 (86.7%) | 0.149 |
| Renal involvement n (%) | 14 (30.4%) | 12 (35.3%) | 22 (73.3%) | < 0.001* |

Data are mean ± SD, or n (%). *$P$ value < 0.05. *SLE*, systemic lupus erythematosus; *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein; *BMI*, body mass index; *SD*, standard deviation

that dysregulation in this gene is correlated with different immune and autoimmune diseases [19, 21, 36]. Also, researches revealed that mutations in this gene are associated with different diseases such as Blau syndrome, Yao syndrome, and Crohn's disease [37–39]. Based on previous reports about dysregulation of NOD2 in SLE patients and some overlap between the clinical manifestation of SLE with diseases associated with *NOD2* mutations such as symmetric arthritis and dermatitis and chronic inflammation, we hypothesized that impairment of NOD2 function partakes in the SLE pathogenesis [21, 40].

Chuang et al. with work on miRNAs that target the *NOD2* gene revealed that overexpression of miR-192, miR-495, miR-512, and miR-671 leads to downregulation of the *NOD2* gene in HCT116 cells, and more functional analysis demonstrated that rs3135500 (A allele) could meaningfully reduce the suppression effect of miR-192 on NOD2 gene expression [29]. Furthermore, Karimzadeh et al. with bioinformatics analysis and based on the high amount of change in energy (ΔΔG), suggested that rs3135500 and especially rs3135499 have a significant effect on the function of miRNAs and consequently could affect *NOD2* expression [26]. To the best of our knowledge, the present study is the first report in the Iranian population that investigates the correlation between *NOD2* polymorphisms (rs3135500 and rs3135499) with SLE risk.

No significant association is established between allele and genotype frequencies of rs3135500 polymorphism and SLE risk in our population ($P > 0.05$) (Table 4). However, there was a significant correlation between laboratory factors that are associated with disease activity and different genotypes. In the present study, the A allele in rs3135500 polymorphism was correlated with a higher amount of CRP, ESR, anti-dsDNA, and low level of C3 and C4, leading us to conclude this variant is obviously correlated with disease activity ($P < 0.05$) [41]. Furthermore, our results demonstrated that this variant was considerably associated with disease severity on the account of some clinical symptoms such as neurological, skin manifestations, and renal involvements were significantly common in patients with A allele ($P < 0.05$) (Table 5).

Moreover, although there was not any connection between rs3135499 and the risk of SLE in the Iranian population ($P > 0.05$), this variant was correlated with the frequency of renal involvement in patients. Patients with the C allele were more susceptible to represent renal involvement ($P < 0.05$). Also, these patients had a higher concentration of creatinine. In addition, the risk allele in both variants showed lower age of onset in our analysis ($P < 0.05$) (Tables 5 and 6).

Although several works have evaluated the association between polymorphisms with different diseases, a small number of studies worked on these two SNPs, i.e., rs3135500 and rs3135499. For instance, the A allele at rs3135500 was significantly associated with an increased risk of asthma in the adult population of Germany [42]. Cao and coworkers assessed the peripheral blood mononuclear cells (PBMCs) from multiple system atrophy (MSA) patients and uncovered that patients with the "A" allele of rs3135500 had higher levels of mRNA *NOD2* which might increase the risk of MSA [43]. In a similar way, a study in the Turkish population indicated that this polymorphism could be related to chronic obstructive pulmonary disease (COPD) progression [44]. In the Iranian population, Ahangari et al. reported that AA genotype and A allele were correlated with increased risk of CRC [45], while Chaleshi et al., similar to our results, observed a lack of association between this polymorphism and risk of CRC in the Iranian population [46]. Enevold and colleagues reported that rs3135499 polymorphism was associated with the increase in the time to relapse of multiple sclerosis [47]. In the other study, Cai et al. demonstrated that the C allele of rs3135499 was correlated with an increased risk of asthma in the Chinese population [48].

Our results suggested that although two miRSNPs (rs3135500 and rs3135499) in the *NOD2* gene were not correlated with the risk of SLE in the Iranian population, these variants were meaningfully correlated with clinicopathological characteristics and disease activity of SLE. However, because of limited sample size and different genetic background, replicative studies in the Iranian population and different populations are a necessity to validate these results.

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## Declarations

**Disclosures** None.

## References

1. Lu R, Munroe ME, Guthridge JM, Bean KM, Fife DA, Chen H, Slight-Webb SR, Keith MP, Harley JB, James JA (2016) Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. J Autoimmun 74:182–193
2. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, van Vollenhoven R, Ruiz-Irastorza G, Hughes G (2016) Systemic lupus erythematosus. Nat Rev Dis Primers 2(1):16039. https://doi.org/10.1038/nrdp.2016.39
3. Guerra SG, Vyse TJ, Cunninghame Graham DS (2012) The genetics of lupus: a functional perspective. Arthritis Res Ther 14(3):211. https://doi.org/10.1186/ar3844
4. Martínez-Bueno M, Alarcón-Riquelme ME (2019) Exploring impact of rare variation in systemic lupus erythematosus by a genome wide imputation approach. Front Immunol 10:258
5. Julià A, López-Longo FJ, Pérez Venegas JJ, Bonàs-Guarch S, Olivé À, Andreu JL, Aguirre-Zamorano MÁ, Vela P, Nolla JM, de la Fuente JLM, Zea A, Pego-Reigosa JM, Freire M, Díez E,

Rodríguez-Almaraz E, Carreira P, Blanco R, Taboada VM, López-Lasanta M, Corbeto ML, Mercader JM, Torrents D, Absher D, Marsal S, Fernández-Nebro A (2018) Genome-wide association study meta-analysis identifies five new loci for systemic lupus erythematosus. Arthritis Res Ther 20(1):100. https://doi.org/10.1186/s13075-018-1604-1
6. Cui Y, Sheng Y, Zhang X (2013) Genetic susceptibility to SLE: recent progress from GWAS. J Autoimmun 41:25–33. https://doi.org/10.1016/j.jaut.2013.01.008
7. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC (2017) Pathogenesis of human systemic lupus erythematosus: a cellular perspective. Trends Mol Med 23(7):615–635
8. Jung J-Y, Suh C-H (2017) Infection in systemic lupus erythematosus, similarities, and differences with lupus flare. Korean J Intern Med 32(3):429
9. Cooper GS, Dooley MA, Treadwell EL, St. Clair EW, Parks CG, Gilkeson GS (1998) Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. Arthritis Rheum 41(10):1714–1724
10. de Koning HD, Simon A, Zeeuwen PL, Schalkwijk J (2012) Pattern recognition receptors in immune disorders affecting the skin. J Innate Immun 4(3):225–240. https://doi.org/10.1159/000335900
11. Joosten LA, Heinhuis B, Abdollahi-Roodsaz S, Ferwerda G, LeBourhis L, Philpott DJ, Nahori M-A, Popa C, Morre SA, van der Meer JW (2008) Differential function of the NACHT-LRR (NLR) members Nod1 and Nod2 in arthritis. Proc Natl Acad Sci 105(26):9017–9022
12. Negroni A, Pierdomenico M, Cucchiara S, Stronati L (2018) NOD2 and inflammation: current insights. J Inflamm Res 11:49
13. Kawai T, Akira S (2009) The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol 21(4):317–337
14. Feerick CL, McKernan DP (2017) Understanding the regulation of pattern recognition receptors in inflammatory diseases–a 'Nod'in the right direction. Immunology 150(3):237–247
15. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411(6837):603–606
16. Janssen CE, Rose CD, De Hertogh G, Martin TM, Meunier BB, Cimaz R, Harjacek M, Quartier P, Ten Cate R, Thomee C (2012) Morphologic and immunohistochemical characterization of granulomas in the nucleotide oligomerization domain 2–related disorders Blau syndrome and Crohn disease. J Allergy Clin Immunol 129(4):1076–1084
17. Khubchandani RP, Hasija R, Touitou I, Khemani C, Wouters CH, Rose CD (2012) Blau arteritis resembling Takayasu disease with a novel NOD2 mutation. J Rheumatol 39(9):1888–1892
18. La Torre F, Lapadula G, Cantarini L, Lucherini OM, Iannone F (2015) Early-onset sarcoidosis caused by a rare CARD15/NOD2 de novo mutation and responsive to infliximab: a case report with long-term follow-up and review of the literature. Clin Rheumatol 34(2):391–395
19. Franca R, Vieira S, Talbot J, Peres R, Pinto L, Zamboni D, Louzada-Junior P, Cunha F, Cunha T (2016) Expression and activity of NOD1 and NOD2/RIPK2 signalling in mononuclear cells from patients with rheumatoid arthritis. Scand J Rheumatol 45(1):8–12
20. Ospelt C, Brentano F, Jüngel A, Rengel Y, Kolling C, Michel BA, Gay RE, Gay S (2009) Expression, regulation, and signaling of the pattern-recognition receptor nucleotide-binding oligomerization domain 2 in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 60(2):355–363
21. Yu S-L, Wong C-K, Wong PT-Y, Chen D-P, Szeto C-C, Li EK, Tam L-S (2011) Down-regulated NOD2 by immunosuppressants in peripheral blood cells in patients with SLE reduces the muramyl dipeptide-induced IL-10 production. PLoS One 6(8):e23855
22. Ehtesham N, Alani B, Mortazavi D, Azhdari S, Kenarangi T, Esmaeilzadeh E, Pakzad B (2021) Association of rs3135500 and rs3135499 polymorphisms in the microRNA-binding site of nucleotide-binding oligomerization domain 2 (NOD2) gene with susceptibility to rheumatoid arthritis. Iran J Allergy Asthma Immunol 20(2):178–187
23. Raisch J, Darfeuille-Michaud A, Nguyen HTT (2013) Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol: WJG 19(20):2985
24. Qu B, Shen N (2015) miRNAs in the pathogenesis of systemic lupus erythematosus. Int J Mol Sci 16:9557–9572. https://doi.org/10.3390/ijms16059557
25. Long H, Wang X, Chen Y, Wang L, Zhao M, Lu Q (2018) Dysregulation of microRNAs in autoimmune diseases: pathogenesis, biomarkers and potential therapeutic targets. Cancer Lett 428:90–103. https://doi.org/10.1016/j.canlet.2018.04.016
26. Karimzadeh MR, Zarin M, Ehtesham N, Khosravi S, Soosanabadi M, Mosallaei M, Pourdavoud P (2020) MicroRNA binding site polymorphism in inflammatory genes associated with colorectal cancer: literature review and bioinformatics analysis. Cancer Gene Ther 27(10):739–753. https://doi.org/10.1038/s41417-020-0172-0
27. Mosallaei M, Simonian M, Esmaeilzadeh E, Bagheri H, Miraghajani M, Salehi AR, Mehrzad V, Salehi R (2019) Single nucleotide polymorphism rs10889677 in miRNAs Let-7e and Let-7f binding site of IL23R gene is a strong colorectal cancer determinant: report and meta-analysis. Cancer Genet 239:46–53
28. Saunders MA, Liang H, Li W-H (2007) Human polymorphism at microRNAs and microRNA target sites. Proc Natl Acad Sci 104(9):3300–3305
29. Chuang AY, Chuang JC, Zhai Z, Wu F, Kwon JH (2014) NOD2 expression is regulated by microRNAs in colonic epithelial HCT116 cells. Inflamm Bowel Dis 20(1):126–135
30. Gregersen PK, Olsson LM (2009) Recent advances in the genetics of autoimmune disease. Annu Rev Immunol 27:363–391
31. Ye J, Gillespie KM, Rodriguez S (2018) Unravelling the roles of susceptibility loci for autoimmune diseases in the post-GWAS era. Genes 9(8):377
32. Su C, Johnson ME, Torres A, Thomas RM, Manduchi E, Sharma P, Mehra P, Le Coz C, Leonard ME, Lu S, Hodge KM, Chesi A, Pippin J, Romberg N, Grant SFA, Wells AD (2020) Mapping effector genes at lupus GWAS loci using promoter Capture-C in follicular helper T cells. Nat Commun 11(1):3294. https://doi.org/10.1038/s41467-020-17089-5
33. de Almeida RC, Chagas VS, Castro MA, Petzl-Erler ML (2018) Integrative analysis identifies genetic variants associated with autoimmune diseases affecting putative microRNA binding sites. Front Genet 9:139
34. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278(11):8869–8872
35. Caruso R, Warner N, Inohara N, Núñez G (2014) NOD1 and NOD2: signaling, host defense, and inflammatory disease. Immunity 41(6):898–908
36. Vanaki N, Golmohammadi T, Jamshidi A, Akhtari M, Vojdanian M, Mostafaei S, Poursani S, Ahmadzadeh N, Mahmoudi M (2018) Increased inflammatory responsiveness of peripheral blood mononuclear cells (PBMCs) to in vitro NOD2 ligand stimulation in patients with ankylosing spondylitis. Immunopharmacol Immunotoxicol 40(5):393–400
37. Snyers B, Dahan K (2006) Blau syndrome associated with a CARD15/NOD2 mutation. Am J Ophthalmol 142(6):1089–1092
38. Yang X, Wu D, Li J, Shen M, Zhang W (2018) A Chinese case series of Yao syndrome and literature review. Clin Rheumatol 37(12):3449–3454. https://doi.org/10.1007/s10067-018-4274-0

39. Cuthbert AP, Fisher SA, Mirza MM, King K, Hampe J, Croucher PJ, Mascheretti S, Sanderson J, Forbes A, Mansfield J (2002) The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. Gastroenterology 122(4):867–874
40. Li J, Wu S, Wang M-R, Wang T-T, Li B-k, Zhu J-M (2014) Potential roles of nucleotide-binding oligomerization domain 2 in the pathogenesis of systemic lupus erythematosus. Rheumatol Int 34(10):1339–1344
41. Giles BM, Boackle SA (2013) Linking complement and anti-dsDNA antibodies in the pathogenesis of systemic lupus erythematosus. Immunol Res 55(1–3):10–21. https://doi.org/10.1007/s12026-012-8345-z
42. Weidinger S, Klopp N, Rümmler L, Wagenpfeil S, Baurecht H, Gauger A, Darsow U, Jakob T, Novak N, Schäfer T (2005) Association of CARD15 polymorphisms with atopy-related traits in a population-based cohort of Caucasian adults. Clin Exp Allergy 35(7):866–872
43. Cao B, Chen Y, Zhou Q, Zhang L, Ou R, Wei Q, Wu Y, Shang H-F (2018) Functional variant rs3135500 in NOD2 increases the risk of multiple system atrophy in a Chinese population. Front Aging Neurosci 10:150
44. Icduygu FM, Erdogan MO, Ulasli SS, Yildiz HG, Celik ZS, Unlu M, Solak M (2017) Is there an association between NOD2 gene polymorphisms and chronic obstructive pulmonary disease progression? Int J Hum Genet 17(2):86–96
45. Ahangari F, Salehi R, Salehi M, Khanahmad H (2014) A miRNA-binding site single nucleotide polymorphism in the 3′-UTR region of the NOD2 gene is associated with colorectal cancer. Med Oncol 31(9):173
46. Chaleshi V, Tajali R, Savabkar S, Zali N, Mojarad N, E, Haghazali M, Pasha S, A Aghdaei H, R Zali M, Vahedi M, (2016) Lack of association between NOD2 rs3135500 and IL12B rs1368439 microRNA binding site SNPs and colorectal cancer susceptibility in an Iranian population. Microrna 5(2):152–156
47. Enevold C, Oturai AB, Sørensen PS, Ryder LP, Koch-Henriksen N, Bendtzen K (2010) Polymorphisms of innate pattern recognition receptors, response to interferon-beta and development of neutralizing antibodies in multiple sclerosis patients. Mult Scler J 16(8):942–949
48. Cai X, Xu Q, Zhou C, Zhou L, Dai W, Ji G (2019) The association of nucleotide-binding oligomerization domain 2 gene polymorphisms with the risk of asthma in the Chinese Han population. Mol Genet Genomic Med 7(6):e00675