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*Original Article*

# *PTPN22* and *TYK2* genes polymorphisms contribute to neuropsychiatric systemic lupus erythematosus (NPSLE) in Chinese Han

Jianming Li[1,2,3], Deliang Lei[2], Yan Wang[3,5], Fang Li[3,4], Meihua Bao[3,4], Ju xiang[3,4], Liang Tang[3,4], Beisha Tang[1]

*[1]Department of Neurology, Xiangya Hospital, Central South University, Changsha, PR China; [2]Department of Anatomy and Neurobiology, Central South University Xiangya Medical School, Changsha, PR China; [3]Neuroscience Research Center, [4]Department of Anatomy, [5]School of Basic Medical Science, Changsha Medical University, Changsha, PR China*

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**Abstract:** Background: Neuropsychiatric manifestations pose diagnostic and therapeutic challenges in systemic lupus erythematosus (SLE). The aim of this study was to investigate whether the *PTPN22*, *IRF5* and *TYK2* polymorphisms are involved in the development of NPSLE. Material and methods: The association was investigated between three immune-associated genes (*PTPN22, IRF5* and *TYK2*) polymorphisms and SLE patients with/without NP symptoms in Chinese Han population. And the relationship between clinical manifestations of neuropsychiatic SLE and SNPs were also assessed. Results: The allele distributions of *TYK2* rs280500 and *PTPN22* rs1217418 in the NPSLE group was significantly lower than that in the non-NPSLE group (rs280500: OR = 0.44, 95% CI = 0.27-0.74, $P = 0.001$, $P_{adj} = 0.005$; rs1217418: OR = 0.41, 95% CI = 0.23-0.74, $P = 0.002$, $P_{adj} = 0.014$). Significant association were detected between the allele distributions of *TYK2* rs280500 and *PTPN22* rs1217418 and SLE (NPSLE and non-NPSLE) compared with the controls (rs280500: OR = 0.62, 95% CI = 0.47-0.83, $P = 0.0009$, $P_{adj} = 0.0045$; rs1217418: OR = 0.63, 95% CI = 0.47-0.84, $P = 0.001$, $P_{adj} = 0.007$). $Hap_{GAGGT}$ in *TYK2* might confer protective effect on NPSLE compared to non-NPSLE group (OR = 0.43, 95% CI = 0.09-1.99, $P = 0.006$, $P_{adj} = 0.042$). Moreover, no correlation between any clinical manifestations (Polyneuropathy, Cognitive disorders, Seizures, Anxiety disorders, Psychosis, Autonomic disorder, headache) and the allele frequencies for *PTPN22*, *TYK2* and *IRF5* polymorphisms was detected. Conclusions: Our findings implied that the genetic polymorphisms (rs280500 in *TYK2* and rs1217418 in *PTPN22*) might be protective factors in the development of NPSLE in Chinese Han.

**Keywords:** Genetic association, neuropsychiatric lupus (NPSLE), systemic lupus erythematosus (SLE), Chinese Han

## Introduction

Systemic lupus erythematosus (SLE) is known as a complex chronic systemic autoimmune disorder. Clinical approaches have shown that neuropsychiatric (NP) symptoms such as depression, psychosis, seizures, headache, cognitive disorder and stroke occur in up to 75% of patients and the mortality is up to 20%. A condition representing a particularly severe form of the disease is known as neuropsychiatric lupus (NPSLE) [1]. Little is known about the pathophysiological bases of NPSLE. Recent researches have implicated that increased blood brain barrier (BBB) permeability, production of autoantibodies and proinflammatory cytokines were reported to participate in the pathogenesis of NPSLE [2].

Increased levels of proinflammatory cytokines including tumor necrosis factor α (TNF-α), Interleukin-1 (IL-1), Interferon γ (IFN-γ), IL-10 and IL6 have been reported in the cerebral spinal fluids (CSF) of patients with NPSLE. Up-regulated expression of proinflammatory cytokines genes for Interleukin 1 (IL-1), Interferon γ (IFN-γ)-, IL-10 and IL-6 in the hippocampus of MRL/lpr mice were investigated [3]. Moreover, microarray studies showed that 13 genes regulated by IFN were up-regulated in patients with lupus

**Table 1.** Clinical characteristics of NPSLE and non-NPSLE patients

| Clinical characteristics | | NPSLE (N = 126) (Mean ± SD) | % | Non-NPSLE (N = 264) (Mean ± SD) | % |
|---|---|---|---|---|---|
| | Male/Female | 18/108 | | 24/242 | |
| | Age (years) | 47.3±12.5 | | 44.3±10.5 | |
| | $Sm^+$, % | 78 | 61.9% | 38 | 14.3% |
| | $dsDNA^+$, % | 90 | 71.4% | 34 | 12.7% |
| | $Pro^+$, % | 66 | 52.3% | 138 | 52.4% |
| | $ANA^+$, % | 74 | 58.1% | 71 | 26.9% |
| | $RNP^+$, % | 54 | 42.9% | 113 | 42.9% |
| | $ACA^+$, % | 42 | 33.3% | 25 | 9.5% |
| | $APL^+$, % | 40 | 31.7% | 67 | 25.4% |
| CNS involvement | Headache | 26 | 21.4% | - | - |
| | Cognitive disorders | 28 | 22.9% | - | - |
| | Seizures | 16 | 12.4% | - | - |
| | Anxiety disorders | 12 | 9.4% | - | - |
| | Psychosis | 12 | 9.4% | - | - |
| | Acute confusional state | 6 | 4.7% | - | - |
| PNS involvement | Polyneuropathy | 6 | 4.7% | - | - |
| | Cranial neuropathy | 4 | 3.1% | - | - |
| | Mononeuropathy | 6 | 4.7% | - | - |
| | Autonomic neuropathy | 18 | 14.1% | - | - |
| Others | Cerebrovascular disorder | 44 | 35% | - | - |

Abbreviation: SD: Standard Deviation; Sm: Anti-Sm antibody; ANA: Antinuclear antibodies; dsDNA: Anti-double-stranded DNA; Pro: Urine protein; RNP: Ribonucleoprotein; ACA: Anticardiolipin; APL: Anti-phospholipid antibodies.

[4]. Genetic research in SLE supplied a tool for finding clues about the pathogenesis of the disease and of its manifestations. Previously, we have found significant genetic association of *PTPN22*, *IRF5* and *TYK2* with SLE in Han Chinese [5]. Although genetic risks have been identified in SLE, the involvement of various variants in NPSLE remains uncertain. A latest meta-analysis study by RC Ho *et al.* [6] has shown that *FcγRIIIa, FcγRIIIb*, and *ITGAM* genotypes are potential susceptibility genes for NPSLE. Novel approaches by Zou *et al.* identified *IRF5* cis-SNP rs4728142 was a risk factor for SLE [7]. Whether *IRF5* variants are involved in the pathogenesis of cognitive dysfunction or other neuropsychiatric features in SLE patients remains to be investigated.

In previous study, we aimed to investigate the three immune-associated genes (*PTPN22, IRF5* and *TYK2*) polymorphisms in our NPSLE population compared to SLE patients without NP symptoms and a healthy cohort. And the relationship between clinical manifestations of NPSLE and single nucleotide polymorphisms (SNPs) were also accessed.

## Materials and methods

### *Samples*

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. Informed consent was obtained from all individual participants included in the study. The study covered 390 unrelated Chinese Han SLE patients fulfilling the American College of Rheumatology 1982 criteria for SLE [8], at the age of 46.2± 11.8 years, treated at the First Affiliated Hospital to Changsha Medical University. The neuropsychiatric manifestations were classified according to the American College of Rheumatology (ACR) case definitions for NPSLE syndromes and the developed algorithm by Bortoluzzi A. [9]. And a total of 126 NPSLE patients (47.3±12.5 years) were identified. The control group consisted of 310 gender-, age- and ethnicity-matched controls who were with no history of autoimmune and psychiatric disorders. All the patients and controls were Chinese Han. The clinical manifestations of SLE and NPSLE groups were described in **Table 1**.

*SNP and genotyping*

SNPs (minor allele frequency [MAF] > 5%) in *PTPN22* and *TYK2* genes were searched from the Han Chinese database (International HapMap Consortium, http://www.hapmap.org/index.html.ja). Then the Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview) was used to select tag SNPs with an $r^2 \geq 0.85$. Five tag SNPs (rs1217414, rs1217418, rs1746853, rs1970559 and rs3765598) in *PTPN22* gene were identified. In addition, the common functional SNP R620W (rs2476601) and rs3811021 in the 3'-UTR of *PTPN22* gene were also selected. For *TYK2* gene, five tag SNPs (rs280500, rs280519, rs2304256, rs8108236, rs12720253) were selected. For *IRF5* gene, 4 common SNPs including exon6 (in/de), rs2004640, rs2070197 and rs10954213 were also selected for genotyping.

The standard phenol-chloroform method was used to extract Genomic DNA from peripheral leukocytes. The multiplex PCR were carried out on the ABI Veriti Thermal Cycler (Applied Biosystems, USA). Individual genomic DNAs were genotyped by direct sequencing by the ABI 3730XL DNA Sequencer (Applied Biosystems). The sequencing primers were listed in Table S1.

*Statistical analysis*

Hardy-Weinberg equilibrium (HWE) was assessed using a classic chi-square test with 1 degree of freedom. Individual analyses of associations between PTPN22, IRF5 and TYK2 polymorphisms and NPSLE, as well as clinical manifestations were performed by comparing genotypes and allele in cases and controls using Fisher's exact test. The corresponding ORs and 95% confidence intervals (CI) were assessed using a standard logistic regression analysis. If a significant relationship was found, stringent Bonferroni's correction was carried out to correct the *p* value in multiple comparisons. Linkage disequilibrium (LD) analysis was performed in NPSLE patients using Haploview program. Analysis of haplotype diversity was performed using the expectation-maximization algorithm (EM). Specific *P* values and ORs and 95% confidence intervals (CI) were obtained by comparing each haplotype with the more common haplotype in the population using Fisher's exact test. Statistical significance was set at $P < 0.05$. PLINK 1.09 was used to perform the statistical analysis (http://pngu.mgh.harvard.edu/~purcell/plink/) [10]. Power analysis was performed by using the Quanto 1.2 (http://biostats.usc.edu/Quanto.html).

## Results

The SLE population consisted of 126 NPSLE patients (18 Men, 108 women, aged 47.3±12.5 years (mean ± SD), and ranged 18-75 years) and 264 non-NPSLE patients (24 Men, 242 women, aged 44.3±10.5 years (mean ± SD), and ranged 21-78 years). The most frequent manifestations in our NPSLE group were cognitive disorders and headache, which were similar to other NPSLE populations [11, 12]. 54 patients (42.9%) suffered from one NPSLE syndromes only. 30 (23.8%) had two and 24 (19%) had three NPSLE syndromes. 18 patients (14.2%) developed four syndromes.

The *PTPN22, IRF5* and *TYK2* allele and genotype frequencies are given in **Table 2**. All variants in case and control were in HWE ($P > 0.05$). For the comparison of NPSLE and non-NPSLE, SLE (NPSLE and non-NPSLE) and healthy control, our sample size is large enough to detect an association at an odds ratio of 1.5, since it had > 71.8% and > 92.1% power at the 5% significant level (2-tailed) respectively. No significant difference in either *IRF5* allele or genotype distributions between NPSLE, non-NPSLE and control group ($P > 0.05$). The genotype distributions of rs1217418 in *PTPN22* in SLE (NPSLE and non-NPSLE) were significantly different from that in the control group ($P = 0.006$). However, the genotype distributions of *TYK2* rs280500 *in* NPSLE, not non-NPSLE, were significantly different from that in the control group ($P = 0.001$). The allele frequencies of rs280500 in *TYK2* gene and rs1217418 in *PTPN22* gene in the NPSLE group was significantly different from that in the non-NPSLE group (rs280500: OR = 0.44, 95% CI = 0.27-0.74, $P = 0.001$, $P_{adj} = 0.005$; rs1217418: OR = 0.41, 95% CI = 0.23-0.74, $P = 0.002$, $P_{adj} = 0.014$). Significant association were detected between the allelic distributions of *PTPN22* rs1217418 and *TYK2* rs280500 and SLE (NPSLE and non-NPSLE) compared with the healthy controls (rs280500: OR = 0.62, 95% CI = 0.47-0.83, $P = 0.0009$, $P_{adj} = 0.0045$; rs1217418: OR = 0.63, 95% CI = 0.47-0.84, $P = 0.001$, $P_{adj} = 0.007$).

**Table 2.** The association between the allele and genotype distribution of *PTPN22, IRF5* and *TYK2* polymorphisms and NPSLE

| Gene | SNPs, (A > B) | Number (MAF, minor allele frequency) | | | OR, 95% CI | $P^a$ | $P_{adj}^a$ | OR, 95% CI | $P^b$ | $P_{adj}^b$ | Genotype | | | $P^c$ | $P^d$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NPSLE (N = 126) | Non-NPSLE (N = 264) | Control (N = 310) | | | | | | | NPSLE (N = 126) | Non-NPSLE (N = 264) | Control (N = 310) | | |
| | | | | | | | | | | | AA/AB/BB | AA/AB/BB | AA/AB/BB | | |
| *PTPN22* | rs2470601 (C > T) | 0 | 0 | 0 | - | - | - | - | - | - | 126/0/0 | 264/0/0 | 310/0/0 | - | - |
| | rs1217414 ( C > T) | 6 (0.024) | 13 (0.025) | 15 (0.024) | 1.04 [0.39-2.76] | 1.00 | - | 0.99 [0.50-1.97] | 1.00 | - | 120/6/0 | 251/13/0 | 295/15/0 | 0.54 | 0.18 |
| | rs1217418 (A > G) | 15 (0.059) | 90 (0.170) | 122 (0.181) | 0.41 [0.23-0.74] | 0.002 | 0.014 | 0.63 [0.47-0.84] | 0.001 | 0.007 | 112/13/1 | 180/78/6 | 200/96/13 | 0.43 | 0.006* |
| | rs1746853 (T > G) | 63 (0.250) | 128 (0.242) | 167 (0.269) | 0.96 [0.68-1.36] | 0.82 | - | 1.14 [0.89-1.45] | 0.30 | - | 72/45/9 | 155/90/19 | 169/115/26 | 0.06 | 0.16 |
| | rs1970559 (T > C) | 5 (0.019) | 14 (0.027) | 25 (0.40) | 1.35 [0.48-3.78] | 0.57 | - | 1.68 [0.92-3.09] | 0.09 | - | 121/5/0 | 250/14/0 | 285/25/0 | 1.00 | 1.00 |
| | rs3765598 (C > T) | 49 (0.194) | 105 (0.198) | 146 (0.235) | 1.03 [0.71-1.50] | 0.46 | - | 1.25 [0.97-1.62] | 0.08 | - | 84/35/7 | 173/77/14 | 183/108/19 | 0.06 | 0.04 |
| | rs3811021 (T > C) | 45 (0.179) | 110 (0.28) | 135 (0.217) | 1.21 [0.82-1.78] | 0.33 | - | 1.12 [0.87-1.45] | 0.38 | - | 87/33/6 | 168/82/14 | 195/95/20 | 0.70 | 0.91 |
| *IRF5* | Exon6 (de/in) (D > I) | 77 (0.306) | 145 (0.275) | 156 (0.252) | 0.86 [0.62-1.19] | 0.17 | - | 0.85 [0.67-1.07] | 0.17 | - | 60/55/11 | 133/117/14 | 171/122/17 | 0.31 | 0.11 |
| | rs2070197 (C > T) | 0 | 0 | 0 | - | - | - | - | - | - | 126/0/0 | 264/0/0 | 310/0/0 | - | - |
| | rs10954213 (G > A) | 38 (0.151) | 78 (0.148) | 95 (0.153) | 0.98 [0.64-.49] | 0.92 | - | 1.04 [0.77-1.39] | 0.82 | - | 90/34/2 | 191/68/5 | 222/81/7 | 0.20 | 0.35 |
| | rs2004640 (C > A) | 76 (0.302) | 149 (0.282) | 180 (0.290) | 0.91 [0.66-1.27] | 0.58 | - | 1.01 [0.79-1.27] | 0.92 | - | 60/56/10 | 138/103/23 | 158/124/28 | 0.09 | 0.79 |
| *TYK2* | rs280500 (A > G) | 21 (0.083) | 89 (0.169) | 129 (0.208) | 0.44 [0.27-0.74] | 0.001 | 0.005 | 0.62 [0.47-0.83] | 0.0009 | 0.0045 | 105/19/1 | 188/63/13 | 194/103/13 | 0.001 | 0.21 |
| | rs280519 (A > G) | 127 (0.508) | 260 (0.492) | 302 (0.487) | 0.96 [0.71-1.29] | 0.76 | - | 0.96 [0.78-1.19] | 0.74 | - | 34/56/36 | 65/138/61 | 79/160/71 | 0.60 | 0.22 |
| | rs2304256 (G > T) | 65 (0.258) | 142 (0.269) | 173 (0.279) | 1.06 [0.75-1.49] | 0.74 | - | 1.07 [0.85-1.36] | 0.57 | - | 67/53/6 | 136/114/14 | 158/131/21 | 0.09 | 0.08 |
| | rs8108236 (G > A) | 69 (0.274) | 117 (0.222) | 158 (0.255) | 0.76 [0.53-1.07] | 0.11 | - | 1.09 [0.86-1.39] | 0.48 | - | 63/57/6 | 155/101/8 | 169/124/17 | 0.06 | 0.07 |
| | rs12720253 (T > G) | 18 (0.071) | 45 (0.085) | 57 (0.092) | 1.21 [0.69-2.14] | 0.51 | - | 1.15 [0.79-1.68] | 0.46 | - | 108/18/0 | 219/45/0 | 253/57/0 | 0.36 | 0.06 |

Abbreviations: NPSLE, Neuropsychiatric systemic lupus erythematosus; SNP, Single nucleotide polymorphism; OR, Odds ratio; 95% CI, 95% confidence intervals; B: Risk allele, A: Non-risk allele. -: Not calculated. [a,c]*P* values were calculated by Fisher's exact test using PLINK 1.09 (Comparison: NPSLE *vs.* non-NPSLE). [b,d]*P* values were calculated by Fisher's exact test using PLINK 1.09 (Comparison: SLE (NPSLE and non NPSLE) *vs.* controls).

**Table 3.** The association between the haplotypes conducted by *PTPN22, IRF5* and *TYK2* genes polymorphisms and NPSLE

| Gene | Haplotypes[a] | Number (Frequency) | | | OR [95% CI] | $P^b$ | $P_{adj}{}^b$ | OR [95% CI] | $P^c$ | $P_{adj}{}^c$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NPSLE | Non-NPSLE | Control | | | | | | |
| *PTPN22* | CAGTTC | 10 (0.085) | 47 (0.176) | 37 (0.118) | 0.43 [0.11-1.67] | 0.21 | - | 0.67 [0.18-2.40] | 0.53 | - |
| | CAGTTT | 19 (0.154) | 26 (0.100) | 0 (0.000) | 1.67 [0.45-6.24] | 0.44 | - | - | - | - |
| | CATTCT | 65 (0.513) | 160 (0.605) | 209 (0.675) | 0.67 [0.26-1.69] | 0.39 | - | 0.44 [0.19-0.97] | 0.04 | 0.24 |
| | CGTTCT | 9 (0.071) | 9 (0.034) | 0 (0.000) | 2.25 [0.28-17.58] | 0.43 | - | - | - | - |
| | CATTCC | 10 (0.082) | 0 (0.000) | 11 (0.036) | - | - | - | 2.36 [0.47-11.66] | 0.28 | - |
| | TATTCT | 3 (0.024) | 0 (0.000) | 11 (0.038) | - | - | - | 0.59 [0.06-5.85] | 0.66 | - |
| *IRF5* | DTAA | 36 (0.286) | 29 (0.109) | 33 (0.106) | 3.28 [1.01-10.66] | 0.04 | 0.2 | 3.36 [1.28-8.82] | 0.01 | 0.05 |
| | DTAC | 44 (0.349) | 83 (0.313) | 109 (0.350) | 1.18 [0.47-2.93] | 0.72 | - | 0.99 [0.45-2.17] | 0.99 | - |
| | DTGC | 10 (0.079) | 10 (0.036) | 21 (0.067) | 2.33 [0.32-16.91] | 0.39 | - | 1.18 [0.29-4.85] | 0.81 | - |
| | ITAC | 28 (0.222) | 36 (0.135) | 84 (0.271) | 1.83 [0.58-5.75] | 0.29 | - | 0.76 [0.32-1.83] | 0.54 | - |
| | ITGC | 8 (0.064) | 23 (0.088) | 23 (0.073) | 0.70 [0.13-3.61] | 0.67 | - | 0.86 [0.19-3.82] | 0.84 | - |
| *TYK2* | AAGGT | 17 (0.136) | 35 (0.131) | 32 (0.102) | 0.98 [0.27-3.48] | 0.98 | - | 1.35 [0.43-4.18] | 0.60 | - |
| | AGGAT | 4 (0.030) | 31 (0.119) | 0 (0.000) | 0.22 [0.03-1.61] | 0.10 | - | - | 0.11 | - |
| | GAGAT | 11 (0.088) | 13 (0.048) | 16 (0.050) | 1.84 [0.31-10.89] | 0.49 | - | 1.77 [0.42-7.56] | 0.43 | - |
| | GAGGT | 5 (0.045) | 15 (0.131) | 109 (0.351) | 0.43 [0.09-1.99] | 0.006 | 0.042 | 0.12 [0.03-0.45] | 0.0003 | 0.0021 |
| | GGTGG | 6 (0.047) | 13 (0.048) | 0 (0.000) | 0.94 [0.12-7.05] | 0.95 | - | - | 0.04 | 0.28 |
| | GGTGT | 6 (0.051) | 13 (0.048) | 0 (0.000) | 1.03 [0.14-7.42] | 0.97 | - | - | 0.03 | 0.21 |
| | GGGGT | 32 (0.252) | 16 (0.060) | 71 (0.230) | 2.08 [1.18-21.84] | 0.01 | 0.07 | 1.09 [0.45-2.59] | 0.84 | - |

[a]The program, Plink, was used to estimate common (frequency > 0.01). Haplotype structure of *PTPN22* was rs1217414, rs1217418, rs1746853, rs1970559, rs3765598, rs3811021, Haplotype structure of *IRF5* was exon6 (in/de), rs10954213, rs2004640; Haplotype structure of *TYK2* was rs280500, rs280519, rs2304256, rs8108236, rs12720270. [b]*P* values were calculated by Fisher's exact test using PLINK 1.09 (Comparison: NPSLE VS. non-NPSLE). [c]*P* values were calculated by Fisher's exact test using PLINK 1.09 (Comparison: SLE (NPSLE and non-NPSLE) VS. controls). Abbreviations: OR, Odds ratio; 95% CI, 95% confidence intervals.

Pairwise LD for *the* 6 *PTPN22* SNPs (except for rs2476601), 5 *TYK2* SNPs (rs280500, rs280519, rs2304256, rs8108236, rs12720253) and 4 *IRF5* SNPs (exon6 (in/de), rs2004640, rs2070197, rs10954213) was defined using the genotype data from NPSLE cases, and the summary statistics $r^2$ was calculated using the HaploView program separately (Tables S2, S3, S4). Haplotype analysis shown that the frequency of $Hap_{GAGGT}$ in *TYK2* was found to be significantly higher in non-NPSLE than that in the NPSLE group (OR = 0.43, 95% CI = 0.09-1.99, $P$ = 0.006, $P_{adj}$ = 0.042), even after the Bonferroni correction. Significant association was also detected between $Hap_{GAGGT}$ in *TYK2* and SLE (non-NPSLE and NPSLE) compared to healthy controls (OR = 0.12, 95% CI = 0.03-0.45, $P$ = 0.0003, $P_{adj}$ = 0.0021). None of other haplotypes conducted on *PTPN22, IRF5* and *TYK2* genes alleles was associated with the development of NPSLE (**Table 3**).

No correlation between any clinical manifestations (Polyneuropathy, Cognitive disorders, Seizures, Anxiety disorders, Psychosis, Autonomic disorder, headache) and the allelic distributions for *PTPN22*, *TYK2* and *IRF5* polymorphisms was observed ($P$ > 0.05, **Table 4**).

## Discussion

In this study, the *TYK2* rs280500 and *PTPN22* rs1217418 were firstly found to be associated with NPSLE in Chinese Han. While, our analysis failed to find association between the polymorphisms and the neuropsychiatric syndromes.

Neuropsychiatric manifestations pose diagnostic and therapeutic challenges in systemic lupus erythematosus (SLE). Pathways that containing candidate genes for SLE susceptibility are related to IFN, TCR and TLR pathways. As many as 100 genes were predicted to be involved in those pathways. Large-scale association studies have identified several variants within the Human Leukocyte Antigen (HLA) and non-HLA loci that confer susceptibility to SLE [13]. Although most studies are under-powered to detect distinct relationships between genotype and phenotype, there is some evidence for increased genetic burden in NPSLE [14]. Recently, Koga *et al.* [14] investigated alleles in seven genes *(IRF5*, *BLK*, *FCγ RIIb*, *STAT4*, *TNFAIP3*, *HLA-DRB1* and *TNFSF13)* and found that SLE patients who carries more than 10 risk alleles showed a greater risk of neuropsychiatric manifestations compared with those carrying fewer than 10 risk alleles. Guerra *et al.* [15]

**Table 4.** The association between *PTPN22, IRF5* and *TYK2* polymorphisms and clinical characteristics in NPSLE

| Genes | SNPs (minor allele) | Polyneuropathy ($p^a$, *OR, 95% CI*) | Cognitive disorders ($p^a$, *OR, 95% CI*) | Seizures ($p^a$, *OR, 95% CI*) | Anxiety disorders ($p^a$, *OR, 95% CI*) | Psychosis ($p^a$, *OR, 95% CI*) | Autonomic disorder ($p^a$, *OR, 95% CI*) | Headache ($p^a$, *OR, 95% CI*) |
|---|---|---|---|---|---|---|---|---|
| *PTPN22* | rs1217418 (G) | 0.89, 0.88 [0.13-5.87] | 0.89, 0.88 [0.13-5.87] | - | - | 0.55, 1.76 [0.26-11.82] | 0.50, 0.46 [0.05-4.57] | 0.18, 0.23 [0.02-2.32] |
| | rs1746853 (G) | 0.77, 0.82 [0.22-3.07] | 0.70, 1.30 [0.34-4.94] | 0.63, 0.72 [0.18-2.82] | 0.83, 1.16 [0.28-4.88] | 0.06, 0.27 [0.07-1.09] | 0.64, 0.72 [0.18-2.82] | 0.43, 1.71 [0.45-6.51] |
| | rs3765598 (T) | 0.21, 2.50 [0.58-10.69] | 0.83, 0.86 [0.20-3.64] | 0.61, 1.47 [0.34-6.37] | 0.91, 0.91 [0.19-4.33] | 0.10, 3.41 [0.74-15.67] | 0.61, 1.47 [0.34-6.38] | 0.86, 1.13 [0.27-4.69] |
| | rs3811021 (C) | 0.91, 0.92 [0.21-4.21] | 0.91, 0.92 [0.21-4.21] | 0.42, 0.54 [0.12-2.46] | 0.72, 1.33 [0.27-6.49] | 0.83, 1.17 [0.26-5.17] | 0.42, 0.54 [0.12-2.46] | 0.59, 0.66 [0.15-2.93] |
| *IRF5* | Exon6 (de/in) (I) | 0.92, 0.93 [0.24-3.62] | 0.55, 1.50 [0.39-5.77] | 0.15, 2.75 [0.68-11.1] | 0.05, 0.25 [0.06-1.06] | 0.38, 1.83 [0.47-7.10] | 0.15, 2.75 [0.68-11.1] | 0.06, 0.25 [0.06-1.13] |
| | rs10954213 (A) | 0.70, 1.40 [0.25-7.91] | 0.61, 0.63 [0.10-3.86] | 0.35, 0.35 [0.04-3.36] | 0.49, 2.20 [0.23-21.1] | 0.89, 1.12 [1.19-6.29] | 1.00, 1.00 [0.16-6.26] | 0.45, 0.50 [0.08-3.08] |
| | rs2004640 (A) | 0.55, 0.67 [0.17-2.56] | 0.43, 1.75 [0.43-7.08] | 0.47, 0.60 [0.15-2.40] | 0.67, 1.38 [0.32-5.85] | 0.63, 1.40 [0.36-5.41] | 0.47, 1.74 [0.39-7.81] | 0.38, 0.55 [0.14-2.12] |
| *TYK2* | rs280500 (G) | 0.09, 0.24 [0.04-1.39] | 0.40, 0.50 [0.09-2.58] | 0.24, 3.55 [0.38-32.81] | 0.17, 0.30 [0.05-1.76] | 0.17, 0.30 [0.05-1.76] | 0.14, 0.30 [0.06-1.59] | 0.58, 0.63 [0.12-3.24] |
| | rs280519 (G) | 0.13, 0.38 [0.11-1.34] | 0.37, 0.57 [0.1-1.96] | 0.08, 3.33 [0.84-13.52] | 0.75, 1.22 [0.36-4.11] | 0.75, 1.22 [0.36-4.11] | 0.27, 2.07 [0.55-7.78] | 0.36, 0.57 [0.17-1.93] |
| | rs2304256 (T) | 0.36, 0.52 [0.13-2.11] | 0.22, 2.50 [0.56-11.23] | 0.62, 1.47 [0.32-6.68] | 0.38, 1.87 [0.45-7.69] | 0.38, 1.87 [0.45-7.69] | 0.62, 1.47 [0.32-6.68] | 0.22, 0.41 [0.10-1.71] |
| | rs8108236 (A) | 0.55, 0.67 [0.17-2.56] | 0.55, 0.67 [0.17-2.56] | 0.47, 1.74 [0.39-7.81] | 0.85, 0.88 [0.23-3.34] | 0.85, 0.88 [0.23-3.34] | 0.47, 0.60 [0.15-2.40] | 0.63, 1.40 [0.36-5.41] |
| | rs12720253 (G) | 0.73, 1.55 [0.13-18.10] | 0.39, 0.35 [0.03-4.17] | - | 0.61, 1.90 [0.16-22.71] | 0.61, 1.90 [0.16-22.71] | 1.00, 1.00 [0.08-12.07] | 0.61, 1.90 [0.16-22.71] |

[a]The alleles were compared between the clinical features-positive group and clinical features-negative group in NPSLE group. *P* value were calculated using Fisher's exact test. Abbreviations: SNP, Single nucleotide polymorphism; OR, Odds ratio; 95% CI, 95% confidence intervals; -, Not calculated.

reported that the *ITGAM* and *FcγR* genes may lead to programmed cell death in neurons and result in NPSLE. Yang *et al.* [16] found that *ITGAM* was associated with severe manifestations of SLE. May *et al.* [17] indicated that *TNF-α* causes local damage in the brain and results in demyelination. In particular, mutations in *TREX1*, which encodes repair exonuclease 1 (also known as DNase III), have been identified to be associated with the manifestations of CNS involvement in patients with NPSLE [18, 19]. Together, immunological genes may be susceptible to NPSLE.

Cytokines play a central role in cognitive function, as well as in learning and memory in the hippocampus [20]. Proinflammatory cytokines including IFN-γ and IFN-α were reported to be marked increased in the cerebrospinal fluid (CSF) of NPSLE compared to the SLE patients without psychiatric disorder [21]. Therefore, the importance of the type I interferon pathway in the initiation and pathogenesis of SLE and its manifestations are worth mention. IRF5 is a transcription factor that can induce transcription of the IFN transcript itself as well as many IFN-α induced genes [22]. And it was confirmed to be a SLE susceptible gene. Sigurdsson [23] firstly reported a significant association between *IRF5* rs2004640T and SLE in Swedish and Finnish. Similar results were detected in Korean [24], Caucasian [25], Mexicans [26] and Kuwaiti [27] populations. Notably, more susceptible SNPs including exon6 (insertion/deletion), rs10954213, rs4728142 have also been found to be associated with SLE in multiple ethnics. Furthermore, *IRF5* was also identified to be correlated with SLE and autopsied patients with Alzheimer's disease [7]. However, we failed to detected association between *IRF5* alleles, genotypes and haplotypes and NPSLE in this study. Although no significant association was found, we could not draw out the effect of *IRF5* polymorphisms and NP manifestations. Thus, a larger number of SNPs of *IRF5* with the correlation to NPSLE should be involved in further studies.

Interestingly, significant association was observed between the *PTPN22* rs1217418 and *TYK2* rs280500 and SLE (including NPSLE). *TYK2* is a type I interferon (IFN) signaling pathway gene. And the *PTPN22* gene is involved in signaling that helps control the activity of T cells. Both the genes were susceptible to SLE in Caucasian and Asian populations. Number of polymorphisms in the *TYK2* and *PTPN22* genes has been identified, and these polymorphisms were reported to be associated with the pathogenesis of SLE. One of the remarkable SNPs in *TYK2* and *PTPN22* should be the rs2304256 and rs2470601 separately. The *TYK2* rs2304256 causes a Val/Phe substitution at position 362 in the JH4 region and was shown to be a SLE risk factor in Finnish, Swedish and the UK populations [23, 28-30], but not in Chinese Hong Kong [31] and Japanese populations [32]. And the *PTPN22* rs2470601 leads to a tryptophan (W) for arginine (R) transition at codon 620 [33], which cause reducing in the interaction between the protein and C-terminal Src tyrosine kinase (Csk) and resulting in loss of negative regulation of T-cell activation, and thus autoimmune disorders. While, significant association was found between *TYK2* rs280500 and *PTPN22* rs1217418, but not *TYK2* rs2304256 and *PTPN22* rs2470601, and NPSLE in this study. To our knowledge, this was the first time that the association between intron variant (*TYK2* rs280500) and NPSLE in Chinese cohorts was reported. Recent evidence suggested that many disease-related intronic SNPs have been reported to be responsible for aberrant splice processes [34, 35]. Several researches have also reported significant association between intron variants and diseases including ankylosing spondylitis (AS) [36], rheumatoid arthritis (RA) [37] and type I psoriasis [38]. These findings suggest that the intronic SNPs (*TYK2* rs280500 and *PTPN22* rs1217418) might affect the splice processes of TYK2 or LYP, which lead to aberrant expression of *TYK2* or *LYP* and thereby influence the susceptibility to NPSLE. Moreover, although the *TYK2* rs280500 and *PTPN22* rs1217418 may be non-functional itself, it may be linked to some other functional SNPs in *TYK2* and *PTPN22* genes.

Limitation exists in this study. The number included in the research was relatively small, which may affect the statistic power. Therefore, we can neither completely ascertain the effect of these polymorphisms in NPSLE nor exclude that the association between the described *TYK2* and *PTPN22* variants and NPSLE. To identify the correction, larger number of NPSLE

and SLE individuals is needed in the further study.

Three well known SLE susceptibility genes were detected for the first time in NPSLE in Han Chinese population in this study. Our findings implied the involvement of the genetic polymorphisms (rs280500 in *TYK2* and rs12174-18 in *PTPN22*) in the development of NPSLE. For the complication of the NPSLE pathogenesis, additional studies to examine the function of *TYK2* and *PTPN22* genes polymorphisms in NPSLE are required.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Beisha Tang, Department of Neurology, Xiangya Hospital, Central South University, No. 87, Xiangya Road, Kaifu Area, Changsha 410008, PR China. Tel: +86 0731-432-7398; E-mail: bstang7398@163.com; Liang Tang, Neuroscience Research Center, Changsha Medical University; Department of Anatomy, Changsha Medical University, No. 1501, Leifeng Road, Wangcheng Area, Changsha 410219, PR China. Tel: +86 0731-88602602; E-mail: tlcool318@163.com

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**Table S1.** The sequencing primers of *PTPN22, IRF5* and *TYK2* genes SNPs

| Gene | SNPs | Sequencing primers (5'-3') |
|---|---|---|
| *PTPN22* | rs2470601 | CATCTGCTATCCGGTACTCGA |
| | rs1217414 | GAAATTACACGGGGTGACTGC |
| | rs1217418 | GCTATTTCAAGTCCACAAGTCA |
| | rs1746853 | AATTGCATTGCACTTGTAGATC |
| | rs1970559 | ATTTTTGACATTTTGGATAGCA |
| | rs3765598 | ATAAGGATTTGGGAACATTTGG |
| | rs3811021 | TCAGGTGGATTCTTTGTAAAATC |
| *IRF5* | Exon6 (de/in) | TGGGAGGCAGTTCGTGGAG |
| | rs2070197 | TCTGGGTTTCCTGGAAGTAGAT |
| | rs10954213 | CTCACTTCCTCATCTCCCTGTC |
| | rs2004640 | ATGAAGACTGGAGTAGGGCGG |
| *TYK2* | rs280500 | CAACTCAAGCAAGCCACTTA |
| | rs280519 | CCCAACCAGGAGGGTCGA |
| | rs2304256 | GCTCCCTGGAAGGTGGTC |
| | rs8108236 | TGGGATTACAGGTGTGAGGAA |
| | rs12720253 | ATGGGGGCTCCTCAACGG |

Abbreviations: SNP, Single nucleotide polymorphism.

**Table S2.** The patterns of linkage disequilibrium (LD) in *PTPN22* gene

| $r^2$ | NPSLE cases (n = 126) | | | | | |
|---|---|---|---|---|---|---|
| | rs1214414 | rs1214418 | rs1746853 | rs1970559 | rs3765598 | rs3811021 |
| rs2470601 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| rs1214414 | - | 0.064 | 0.012 | 0.144 | 0.041 | 0.010 |
| rs1214418 | - | - | 0.080 | 0.035 | 0.039 | 0.032 |
| rs1746853 | - | - | - | 0.051 | 0.796 | 0.213 |
| rs1970559 | - | - | - | - | 0.001 | 0.001 |
| rs3765598 | - | - | - | - | - | 0.219 |

Abbreviations: NPSLE, Neuropsychiatric systemic lupus erythematosus.

**Table S3.** The patterns of linkage disequilibrium (LD) in *IRF5* gene

| $r^2$ | NPSLE cases (n = 126) | | |
|---|---|---|---|
| | rs2070197 | rs10954213 | rs2004640 |
| Exon6 (de/in) | 0.075 | 0.065 | 0.063 |
| rs2070197 | | 0.021 | 0.036 |
| rs10954213 | | | 0.035 |

Abbreviations: NPSLE, Neuropsychiatric systemic lupus erythematosus.

**Table S4.** The patterns of linkage disequilibrium (LD) in *TYK2* gene

| $r^2$ | NPSLE cases (n = 126) | | | |
|---|---|---|---|---|
| | rs280519 | rs280521 | rs8108236 | rs12720253 |
| rs280500 | 0.065 | 0.054 | 0.027 | 0.047 |
| rs280519 | | 0.037 | 0.028 | 0.046 |
| rs280521 | | | 0.058 | 0.022 |
| rs8108236 | | | | 0.029 |

Abbreviations: NPSLE, Neuropsychiatric systemic lupus erythematosus.