Association study of AFF1 gene polymorphism (rs340630) with Iranian systemic lupus erythematosus patients

Arabi E1, Garshasbi M2, Jamshidi AR1, Khalesi R1, Ahmadzadeh N1, Akbarian M1, Mahmoudi M1

ABSTRACT

Aim: To investigate whether AFF1 polymorphism was associated with susceptibility and clinical features of SLE in Iranian patients.

Methods: A total of 320 patients with SLE and 330 age, sex and ethnically matched healthy control subjects were enrolled in the present study. Both cases and healthy controls were genotyped for rs340630 polymorphism located inside the AFF1 using Amplification Refractory Mutation System-PCR (ARMS-PCR). In order to investigate the association of this SNP with clinical features of SLE, clinical manifestations of the patients were recorded. The distribution of rs340630 genotypes were tested for deviation from Hardy-Weinberg in healthy controls. Genotypic and allelic distribution between patients and controls were assessed by chi-square test.

Major results: Both A and G alleles of rs340630 were seen among our cohort of Iranian SLE and healthy control samples. All three genotypes of rs340630 were found, i.e., homozygous A/A (OR=1/01, 95%CI=72 – 1/42, P= %99), homozygous G/G (OR=92, 95%CI= 63 – 1/35, P= %77) and heterozygous A/G (OR=1/03, 95%CI= 76 – 1/41, P=87). No significant differences were observed between allele frequency of this polymorphism in SLE patients and healthy controls. However, in the SLE group, the number of patients with renal disorder was significantly increased for the AG genotype compared to the other genotypes of AFF1 polymorphism (P= 0.05).

Conclusion: There was no association between AFF1 polymorphism (rs340630) and SLE. However, our findings indicated that the AG genotype of AFF1 polymorphism (rs340630) was significantly (P=0.0045) correlated with renal disorder in Iranian population.

Keywords: Systemic lupus erythematosus; Autoimmune rheumatic disease; Chronic immune diseases

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease. It causes multiple organ damage with different clinical manifestations ranging from mild cases with limited skin and joint manifestations to life-threatening conditions with renal impairment, severe cytopenia, central nervous system disease, or thromboembolic events. This disease is characterized by autoantibodies to nuclear antigens, immune complex deposition, and subsequent tissue destruction.

There are various reports from different parts of the world on the prevalence and characteristics of SLE. These reports have revealed that there are gender, age, and racial variations affecting the prevalence of SLE. The SLE prevalence in the African-American, Spanish, and Asian population is higher than the Caucasian population. Familial aggregation of the disease and studies on monozygotic twins have provided strong evidence to support the role of genetic factors along with environmental and hormonal factors in the pathogenesis of SLE. Since the epidemiology of SLE has demonstrated that the prevalence of the disease substantially differs among populations, genetic backgrounds of SLE should be also heterogeneous across populations. In addition to genetic differences across ethnicities, variations in the environment, culture, socioeconomic status, and methodological factors could also underlie the differences in the disease prevalence and manifestations among ethnic groups.
Since 2008, more than 25 potential loci for SLE have been identified by genome wide association studies (GWASs) performed in Caucasian populations. The investigations have demonstrated that several genes associated with SLE in Caucasians are also risk factors in Asians: HLA-II, STAT4, BANK1, BLK, IRF5, TNFSF4, ITGAM, etc. These risk alleles account for about 8–15% of the total genetic susceptibility to the disease. Therefore, it is still important to examine the sub-loci of GWAS in order to reveal the entire picture of the genetic etiology.

In a recent GWAS, a novel association has been detected between a variant in the AF4/FMR2 family member 1 (AFF1) gene at 4q21, and SLE susceptibility (rs340630; P = 8.36×10^{-29}, OR = 1.21) in a Japanese population suggesting that AFF1 is involved in the etiology of SLE through the regulation of development and activity of lymphocytes. Their results showed that accumulation of quantitative changes in the gene expression would accelerate the onset of SLE.

Therefore, in this study, the association of AFF1 polymorphism (rs340630) with susceptibility and clinical features of SLE in Iranian patients was investigated.

PATIENTS AND METHODS

SAMPLE COLLECTION
A total of 650 Iranians including 320 SLE patients (40 males and 280 females, mean ± SD age 36.96 ± 12.025 years) and 330 age, sex and ethnically matched healthy controls (42 males and 288 females, mean ± SD age 37.62 ± 12.320 years) without any signs or symptoms of autoimmune diseases were studied. SLE patients were recruited from the outpatient rheumatology clinic of Shariati Hospital, Tehran, Iran, from February 2012 to April 2014, and healthy controls participated in this study voluntarily at the same time. All the SLE patients met the 1997 American College of Rheumatology (ACR) revised classification criteria for SLE. The study was approved by the local ethics committee of Tehran University of Medical Sciences. Informed consent was obtained from all patients and healthy controls.

DNA ANALYSIS
10-ml peripheral blood sample was collected from each participant and genomic DNA was extracted from the white blood cells (WBCs) using the phenol-chloroform method. The quantity of the extracted DNA was measured at 280 nm wavelength by the Thermo 2000 Nano-drop spectrophotometer.

ARMS-PCR
SNP Genotyping was performed using Amplification Refractory Mutation System-PCR (ARMS-PCR) method. The PCR reaction was carried out in 20 μl of the solution containing Taq DNA polymerase, standard 10× PCR buffer, 200 ng DNA, 0.2 μM of each primer, 1.5mM of MgCl2 and 200 μM of dNTP mix and 0.5 U Taq polymerase. Cycling conditions was initial denaturation (94ºC, 3 min) followed by 40 cycles at 94ºC for 30 sec, 64ºC for 30 sec, 72ºC for 30 sec, and final extension at 72ºC for 10 min. Both alleles were amplified in separated PCR reactions with allele specific primers for A and G alleles (Table I). The amplified DNA fragments were separated on 2% (w/v) agarose gel and viewed after staining with ethidium bromide. A 100 bp DNA ladder was used as a marker to estimate the size of the PCR products. The samples were genotyped and classified into one of the three possible genotypes AA, AG, GG. In order to confirm ARMS-PCR results, the DNA of 3 samples (one sample from each of the AA, AG and GG genotypes) was sequenced by the Sanger sequencing method and analyzed with the Codoncode Aligner software (Version. 6.0.2).

STATISTICAL ANALYSIS
Statistical analyses were performed using SPSS V: 19 Software. Statistical calculations in this study included allelic and genotypic frequencies (n), Chi-square and odds ratio (OR). P values were calculated for each allele and genotype in the patient and control groups. The genotype distributions of AFF1 (rs340630) were tested for deviation from the Hardy-Weinberg equilibrium in control group using 2 test in Package ‘genetics’ of R-Software, Version. R-3.2.0 (http://cran. um.ac.ir/web/packages/genetics/ genetics.pdf). To adjust for multiple testing the Benjamini-Hochberg method to control the False Discovery Rate (FDR) was used. P value of less than 0.05 was considered to be statistically significant.

RESULTS
Two PCR products were observed on the agarose gel. The expected size of the normal and mutant alleles was 198 and 355 bps, respectively. Both A and G alle-
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In addition, we investigated the association between this polymorphism and clinical manifestations of the SLE group using the Chi-square test (Table III). In the SLE group, the number of patients with renal disorder after adjusting for the Bonferroni multiple comparison correction was significantly higher for the AG genotype compared to the other genotypes of AFF1 polymorphism (P = 0.05).

Discussion

TABLE I. PRIMERS AND AMPLICON SIZES USED IN THIS STUDY

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Amplicon Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. AFF1</td>
<td>5’-CCCAAAATCAGTTTCAGAGC-3’</td>
<td>434</td>
</tr>
<tr>
<td>R. AFF1</td>
<td>5’-AGAACTGCTTACCACCCAGGA-3’</td>
<td></td>
</tr>
<tr>
<td>Rwt</td>
<td>5’-TTTGGGAGCATTGTAGTTAC-3’</td>
<td>198</td>
</tr>
<tr>
<td>Rmt</td>
<td>5’-TTTGGGAGCATTGTACT-3’</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 1.** ARMS-PCR gel electrophoresis for AFF1 A/G polymorphism in 7 samples. Samples 1 and 2 are heterozygote (AG), sample 3 is homozygote for A allele (AA) and samples 4-7 are homozygote for G allele (GG). Sample 8 is negative control. The expected size of internal control and allele specific products are 434 bp and 198 bp, respectively. B is an empty lane.

**TABLE II. COMPARISON OF ALLELE AND GENOTYPE FREQUENCIES OF AFF1 POLYMORPHISMS (RS340630) IN THE SLE PATIENTS AND HEALTHY CONTROLS**

<table>
<thead>
<tr>
<th>Alleles/Genotypes</th>
<th>SLE (n=320) N (%)</th>
<th>Control (n=330) N (%)</th>
<th>P</th>
<th>χ²</th>
<th>OR† (CI‡)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>370 (56.6)</td>
<td>357 (54.9)</td>
<td>0.39</td>
<td>0.72</td>
<td>1.11 (0.89%-1.38)</td>
</tr>
<tr>
<td>G</td>
<td>270 (43.4)</td>
<td>303 (45.1)</td>
<td>0.54</td>
<td>0.36</td>
<td>0.99 (0.74%-1.35)</td>
</tr>
<tr>
<td>GG</td>
<td>64 (65.25)</td>
<td>70 (69.55)</td>
<td>0.77</td>
<td>0.81</td>
<td>0.99 (0.63%-1.35)</td>
</tr>
<tr>
<td>AG</td>
<td>161 (158.50)</td>
<td>163 (163.90)</td>
<td>0.87</td>
<td>0.24</td>
<td>1.03 (0.76%-1.41)</td>
</tr>
<tr>
<td>AA</td>
<td>95 (96.25)</td>
<td>97 (96.55)</td>
<td>0.99</td>
<td>0</td>
<td>1.01 (0.72%-1.42)</td>
</tr>
<tr>
<td>HWE§</td>
<td></td>
<td></td>
<td>0.78</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

†OR, odds ratio; ‡CI, confidence interval; §HWE, Hardey-Weinberg Equilibrium

Les of rs340630 were seen among the Iranian samples. All three genotypes of rs340630 were found, i.e., homozygous A/A, homozygous G/G and heterozygous A/G, as shown in Figure 1. The genotypic and allelic frequency of AFF1 A/G polymorphism is shown in Table II. Genotypic distribution of this polymorphism did not deviate from that expected Hardey-Weinberg Equilibrium (HWE), and the heterozygous A/G genotype showed the highest frequency among both SLE (50.3%) and healthy control (49.3%) groups (Table II). Allele frequency of this polymorphism was not significantly different between SLE patients and healthy controls.

In addition, we investigated the association between this polymorphism and clinical manifestations of the SLE group using the Chi-square test (Table III). In the SLE group, the number of patients with renal disorder after adjusting for the Bonferroni multiple comparison correction was significantly higher for the AG genotype compared to the other genotypes of AFF1 polymorphism (P = 0.05).
Autoimmune diseases, such as SLE, are a varied group of complex disorders with a strong genetic component, caused by breakdown of self-tolerance. Identification of genes involved in multifactorial diseases has been classically approached by association studies such as those conclusively demonstrating the presence of SLE susceptibility factors in the HLA region. SLE is a relatively common disorder with a prevalence of 40 per 100000 in Iran. Relatively high prevalence, high mortality rate (approximately 20.2%), clinical heterogeneity of the disease, and involvement of vital organs such as the brain, blood, and kidneys in most patients that are mostly women of child bearing age, necessitate efforts to develop diagnostic tools and effective therapies. On the other hand, early detection by finding potential susceptibility genes and their molecular interactions may help to prevent its occurrence or progression and could provide targets for more focused therapies in SLE.

Familial aggregation demonstrates that both genetic and environmental factors play an important role in the pathogenesis of SLE. Genetic studies have been performed using candidate gene approaches and recently, genome-wide association studies (GWAS). However, most of these studies have been conducted in European populations, and few studies have been performed in Asian populations and especially the status of SLE in Iran has remained indistinct. Since the epidemiology of SLE has demonstrated that the prevalence of the disease substantially differs among populations, genetic backgrounds of SLE should also be heterogeneous across populations.

In SLE, for example, the 26 risk loci identified by the previous GWASs explain only around 8% of the total genetic susceptibility to the disease. Therefore, it is still important to examine the sub-loci of GWAS in order to reveal the entire picture of genetic etiology.

In a GWAS study performed in the Japanese population (2012), a novel association is found between AFF1 gene polymorphism at 4q21 and SLE susceptibility (rs340630; P = 8.3610^{-29}, OR = 1.21). Because expression quantitative trait loci (eQTLs) have been implicated in genetic risks for autoimmune diseases, an eQTL study was integrated into the results of the GWAS. By this, it was confirmed that A allele of rs340630 had a cis-eQTL effect on the AFF1 transcript with enhanced expression levels (P = 0.05). AFF1 transcripts are prominently expressed in CD4+ and CD19+ peripheral blood lymphocytes, up-regulation of AFF1 may cause abnormalities in these lymphocytes, and therefore leading to the disease onset.

Surprisingly, our findings were contradictory to this GWAS study. In line with our findings, no association between rs340630 and SLE susceptibility was found in a Chinese population (for A versus G, P = 0.79, OR 0.98, 0.82).

### Table III. Frequencies of rs340630 Genotypes with Various Clinical Features

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Number</th>
<th>rs340630 Genotypes distribution</th>
<th>Frequency N (%)</th>
<th>P-Value</th>
<th>Adj. P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo sensitivity</td>
<td>205</td>
<td>AA (56 (27.3), AG (107 (52.2), GG (42 (20.5)</td>
<td>0.753</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Malar rash</td>
<td>111</td>
<td>AA (30 (27), AG (54 (48.6), GG (27 (24.3)</td>
<td>0.610</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Discoid rash</td>
<td>15</td>
<td>AA (4 (26.7), AG (7 (46.7), GG (4 (26.7)</td>
<td>0.870</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>79</td>
<td>AA (20 (25.3), AG (40 (50.6), GG (19 (24.1)</td>
<td>0.686</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>201</td>
<td>AA (51 (23.4), AG (100 (49.8), GG (50 (24.9)</td>
<td>0.046</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Pleuritis</td>
<td>52</td>
<td>AA (17 (32.7), AG (26 (50.0), GG (9 (17.3)</td>
<td>0.594</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Pericarditis</td>
<td>15</td>
<td>AA (7 (46.7), AG (6 (40.0), GG (2 (13.3)</td>
<td>0.238</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Serositis</td>
<td>19</td>
<td>AA (4 (21.1), AG (13 (68.4), GG (2 (10.5)</td>
<td>0.262</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Anti-ds DNA</td>
<td>261</td>
<td>AA (69 (26.4), AG (136 (52.1), GG (56 (21.5)</td>
<td>0.022</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>ANA†</td>
<td>274</td>
<td>AA (76 (27.7), AG (139 (50.7), GG (59 (21.5)</td>
<td>0.661</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Renal disorder</td>
<td>120</td>
<td>AA (19 (13.8), AG (73 (60.8), GG (28 (23.3)</td>
<td>0.0045</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Hematologic disorder</td>
<td>176</td>
<td>AA (55 (31.3), AG (86 (48.9), GG (35 (19.9)</td>
<td>0.233</td>
<td>0.52</td>
<td></td>
</tr>
</tbody>
</table>

*FDR-adjusted P-value for multiple testing using the Benjamini-Hochberg method, †Anti-nuclear Antibody
95% CI 0.86-1.12)\textsuperscript{88}. The allelic distribution of AFF1 A/G polymorphism in the Iranian population was similar to what was found in the Chinese population but different from the Japanese population.

**CONCLUSION**

In our present study, it was observed that the AFF1 polymorphism (rs340630) was correlated with renal disorder, a clinical feature observed in patients with SLE. Furthermore, our results showed that AFF1 polymorphism was not significantly associated with SLE susceptibility. Further studies on different genetic risk factors in various ethnicities of Iran are warranted to shed more light on the genetic background of this disease in Iranian patients.

**REFERENCES**