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Research Article

Analysis of Adiponectin Gene Polymorphisms in Chinese Population with Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease. Adiponectin is an adipocyte-derived cytokine with anti-inflammatory, antidiabetic, and antiatherogenic properties. No study has reported on the association between adiponectin (ADIPOQ) gene and SLE. Our aim is to investigate the association between single-nucleotide polymorphisms in ADIPOQ gene and SLE. We examined 179 SLE patients and 237 age- and gender-matched controls from Sichuan province in China. Genotypes were determined using polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing. Results show that there was no significant difference in the allele frequencies of rs1501299 ($P = .311$, OR = 1.17, 95% CI: 0.86–1.59) and rs2241766 ($P = .929$, OR = 0.99, 95% CI: 0.74–1.33) in ADIPOQ gene between SLE patients and controls. The same results were seen in their genotypes ($P < .05$). The allele frequencies of rs1501299 and rs2241766 polymorphisms of ADIPOQ may not be associated with SLE risk.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem, complex autoimmune disease characterized by autoantibody production and tissue injury. The formation of immune complexes with these autoantibodies and their deposition in multiple organs contribute to eventual end-organ damage. The disease commonly affects young women and occurs with different frequencies in racial and ethnic groups [1, 2]. Although many studies have shown that environmental and genetic factors contribute to SLE, its etiopathogenisis remains unclear [3, 4].

Adiponectin, a product of the adiponectin (ADIPOQ) gene, is the most abundant human adipose-specific protein [5]. It belongs to a family of adipocytokines, including adiponectin, leptin, resistin, and visfatin [6]. Adipocytokines are soluble mediators derived mainly from adipocytes and are thought to play an important role in inflammation and immunity [7]. Different adiponectin levels were found to be related to risks of type 2 diabetes, atherosclerosis, and coronary artery disease, among others [8, 9]. Similarly, plasma, urine, and renal levels and expression of adiponectin were found to be associated with insulin resistance, body mass index, c-reactive protein, and nephritis in patients with SLE [10, 11]. Although adiponectin has been suggested as a biomarker for SLE and SLE nephritis, other potential factors may prompt its elevation in SLE [12]. Thus, adiponectin has not been validated as a true SLE biomarker.

A group of researchers recently found that plasma adiponectin levels are significantly lower in obesity, type 2 diabetes, hypertension, atherosclerosis, and other diseases [13], while in SLE, diabetic nephropathy, and chronic renal failure, the plasma and urine levels of adiponectin are significantly raised [11, 14]. These phenomena exist for a number of reasons. First, insulin resistance in obesity, type 2 diabetes, hypertension, atherosclerosis, and other diseases is a process of attaining a state of energy equilibrium. Low serum adiponectin levels can be viewed as a pathophysiological process in the initial event of insulin resistance. The negative energy balance of insulin resistance in SLE can stimulate the secretion of adiponectin to overcome the inhibiting factor, and serum adiponectin levels increase

significantly. Second, proteinuria in SLE patients causes simultaneous loss of large amounts of plasma albumin, certain immunoglobulin, complement components, and metal-binding proteins. This loss may cause degradation of the adiponectin enzymes and clear adiponectin disorder. Third, as a protective cytokine, adiponectin may reduce the metabolic disorder and endothelial vascular injury caused by other risk factors in SLE patients. It stimulates secretion of adiponectin to overcome the inhibiting factor, so that serum adiponectin level is significantly raised.

Adiponectin levels have a strong genetic component, with an additive genetic heritability of 46% [15]. The ADIPOQ gene consists of three exons and two introns spanning a 17-kb region and has been located on chromosome 3q27 [16]. The ADIPOQ gene was found to be the only major gene responsible for plasma adiponectin [17]. While majority of studies on ADIPOQ gene polymorphisms have focused on coronary artery disease, type 2 diabetes, and obesity [18–21], no study, to date, has examined the association between single-nucleotide polymorphisms (SNPs) of the ADIPOQ gene and SLE. Therefore, in this study, we investigated two SNPs (rs1501299 and rs2241766) in ADIPOQ gene and their possible association with SLE susceptibility in the Chinese Han population.

2. Materials and Methods

2.1. Subjects. A total of 179 unrelated Han SLE patients from West China Hospital, Sichuan University, were enlisted from July 2006 to March 2009. Patients (18 males and 161 females) with an average age of 33.5 ± 13.1 years had typical clinical SLE symptoms and were diagnosed according to the American Rheumatism Association Criteria for SLE classification [22]. A total of 237 controls (24 men and 213 women) with an average age of 34.5 ± 11.8 years had healthy blood with no family history of SLE. The controls were taken from the same geographic areas as the patients, whose gender ratio and mean ages were matched with the SLE group. A written informed consent was obtained from all the subjects, and the study was performed with the approval of the ethics committee of the Chinese Human Genome.

2.2. Genotyping of ADIPOQ. Genomic DNA was extracted from peripheral blood cells using an extraction kit (Bioteke Corporation, Perking, China) according to the manufacturer's instructions. Polymorphisms of adiponectin gene were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primer sequences and reaction conditions are shown in Table 1. To further confirm the genotyping results, PCR-amplified DNA samples were examined by DNA sequencing.

2.3. Statistical Analysis. Genotype and allele frequencies of rs1501299 and rs2241766 in ADIPOQ gene were compared in two groups using a χ^2 test and Fisher's exact test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated to assess the relative risk conferred by a particular allele and genotype. Demographic and clinical data between groups were analyzed by χ^2 test and the Student's t-test. Hardy-Weinberg equilibrium was tested with a goodness of fit χ^2 test with one degree of freedom to compare the observed genotype frequencies among subjects with the expected genotype frequencies. Statistical significance was assumed at the $P < .05$ level. The Statistical Package for Social Sciences (SPSS 11.5 Chicago, IL, U.S.A.) was used for all of the statistical analyses.

3. Results

The genotype and allele frequencies of rs1501299 and rs2241766 polymorphisms are shown in Table 2. The genotyping results by PCR-RFLP and DNA sequencing were 100% concordant. The frequencies of the AA, AC, and CC genotypes of rs1501299 were 5.6%, 47.5%, and 46.9% in patients and 6.8%, 38.8%, and 54.4% in the controls, respectively. The frequencies of the A and C alleles of rs1501299 were 29.3% and 70.7% in patients and 26.2% and 73.8% in the controls, respectively. The frequencies of the GG, GT, and TT genotypes of rs2241766 were 8.4%, 46.4%, and 45.3% in patients and 8.0%, 47.7%, and 44.3% in the controls, respectively. The frequencies of G and T alleles of rs2241766 were 31.6% and 68.4% in patients and 31.9% and 68.1% in the controls, respectively. No significant difference was observed in the allele frequencies of the rs1501299 and rs2241766 polymorphisms between the patients and controls (for rs1501299: $P = .311$, OR = 1.17, 95% CI: 0.86–1.59; for rs2241766: $P = .929$, OR = 0.99, 95% CI: 0.74–1.33). The same results were seen in the genotypes (Table 2).

4. Discussion

To the best of our knowledge, this is the first study that investigated the association between the rs2241766 and rs1501299 polymorphisms of ADIPOQ gene and SLE. In this study, no significant difference was found in the distribution of ADIPOQ gene polymorphisms between SLE patients and controls, suggesting that ADIPOQ gene polymorphisms may not be a significant contributor to SLE susceptibility.

Adiponectin is an adipocyte-derived peptide expressed exclusively in adipocytes. Adiponectines are also called gelatin-binding protein-28 (GBP28), AdipoQ, ACRP30, or apM1. Low plasma adiponectin concentration is associated with metabolic disorders and an increased risk of cardiovascular events [23]. However, circulating adiponectin levels have been reported to be significantly increased in patients with SLE, while patients with high plasma adiponectin had poor prognosis in lupus nephritis [10, 24]. Serum levels of adiponectin were significantly and inversely correlated with insulin resistance in SLE patients. Elevated levels of adiponectin in SLE suggest the possible involvement of adiponectin in insulin resistance and alteration of insulin sensitivity [25].

Some studies showed that the plasma level, expression, and biological effects of adiponectin are associated with polymorphism in the ADIPOQ gene [26–28]. Polymorphisms in the ADIPOQ gene have also been shown to correlate

Table 1: Primer sequences and reaction conditions for genotyping adiponectin polymorphisms.

Gene	Location	Primer sequence	Product (bp)	Annealing temperature (°C)	Restriction enzyme	Allele (bp)
rs1501299	Intron-2	5′-GTCTAGGCCTTAGTTAATAATGAAGG-3′ 5′-GTGAGAAAGGAGATCCAGGTAA-3′	106	56	STU I	A (106) C (80,26)
rs2241766	Exon-2	5′-TGGACGGAGTCCTTTGTAGG-3′ 5′-TTGAGTCGTGGTTTCCTGGT-3′	161	56	Sma I	G (134,27) T(161)

Table 2: Genotype and allele frequencies of rs2241766 and rs1501299 in ADIPOQ between patients with asthma and controls.

Polymorphisms	Patients $n = 179$ (%)	Controls $n = 237$ (%)	OR (95% CI)	P
rs1501299				
genotypes				
AA	10(5.6)	16(6.8)	1.00 (Ref)	
AC	85(47.5)	92(38.8)	0.68(0.29–1.57)	.362
CC	84(46.9)	129(54.4)	0.96(0.42–2.22)	.923
alleles				
A	105(29.3)	124(26.2)	1.00 (Ref)	
C	253(70.7)	350(73.8)	1.17(0.86–1.59)	.311
rs2241766				
genotypes				
GG	15(8.4)	19(8.0)	1.00 (Ref)	
GT	83(46.4)	113(47.7)	1.08(0.52–2.24)	.847
TT	81(45.3)	105(44.3)	1.023(0.49–2.14)	.951
alleles				
G	113(31.6)	151(31.9)	1.00 (Ref)	
T	245(68.4)	323(68.1)	0.99(0.74–1.33)	.929

For rs1501299, the AA genotype and A allele were used as reference [1.00 (ref)]; for rs2241766, the GG genotype and G were used as reference [1.00 (ref)].

with adiponectin serum levels, in which the expression of the G allele at SNP rs2241766 was consistently higher than the T allele among all study subjects [28]. Moreover, Hara et al. reported that the C allele at SNP rs1501299 is inversely associated with lower plasma adiponectin concentration in Japanese population. Yang and Chuang reported that the A allele at SNP rs1501299 is associated with lower serum adiponectin concentration in Italians [23, 27]. There are other factors that have been shown to regulate adiponectin levels. A Mediterranean diet or a diet rich in whole grain and fat was shown to produce increased adiponectin levels [29, 30]. Physical activity was also shown to influence adiponectin, and high levels of physical activity could elevate adiponectin levels [31].

Based on these studies, we started this study with the hypothesis that the ADIPOQ gene polymorphisms may be one of the genetic factors that affect SLE susceptibility. However, our results show that the SNP rs2241766 and rs1501299 in the ADIPOQ gene had no association with SLE in the Chinese Han population. No significant difference was found in allele or genotype frequencies between SLE patients and the controls in terms of the selected SNPs. This finding is contrary with our hypothesis and suggests that this polymorphism should be tested in groups of different ethnic origins.

Although deciphering the reasons for failure and finding a positive association is difficult, several possibilities should necessarily be considered. First, SLE is a multifactorial disease; different individuals could be exposed to various environmental factors and genetic susceptibility might lead to different results. Second, the disparity between our results and assumptions may be due to the relatively small number of SLE patients tested. A variation in ADIPOQ gene may contribute to susceptibility to SLE, but the effect should be minimal. Large, varied populations of SLE patients should be tested to avoid a statistical false negative. Third, although the investigated SNPs (rs2241766 and rs1501299) do not affect susceptibility to SLE in our study, further research on more SNPs in the ADIPOQ gene is needed to exclude the role of the adiponectin gene polymorphisms as a possible susceptibility factor for SLE. Finally, the inadequate study design, such as nonrandom sampling, should also be considered. The possibility of selection bias from the hospital-based case-control study is a relevant issue. Nevertheless, the results of this study provide additional information and motivation for further research

into the association of ADIPOQ gene polymorphisms and SLE.

In conclusion, we found that ADIPOQ gene polymorphisms were not associated with the risk of SLE in the Chinese Hans population. Further studies are needed to explore the complicated interaction between environmental factors and ADIPOQ gene polymorphisms in terms of susceptibility to SLE, especially in ethnically diverse populations.

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