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The rs6427384 and rs6692977 Single Nucleotide Polymorphisms of the Fc Receptor-Like 5 (FCRL5) Gene and the Risk of Ankylosing Spondylitis: A Case Control Study in a Single Center in China

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

A 1 Hua-Wei Liu
AB 2 Dai-Xu Wei
AD 1 Da-Wei He
DE 1 Jiu-Zheng Deng
DE 1 Jian-Jin Zhu
AD 1 Kai Xu
DF 1 Dong Hu
BDF 3 Jing Li
ACF 3 Wen-Hao Hu
BDE 3 Yan Wang
EFG 1 Song-Hua Xiao
ACDE 1 Li-Gong Fu

1 Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, P.R. China
2 Department of Life Sciences and Medicine, Key Laboratory of Resource Biology and Modern Biotechnology in Western China, Ministry of Education, Northwest University, Xian, Shaanxi, P.R. China
3 Department of Orthopedics, Chinese People's Liberation Army (PLA) General Hospital, Beijing, P.R. China

Corresponding Authors: Song-Hua Xiao, e-mail: xiaosh301@163.com, Li-Gong Fu, e-mail: fuligong002@163.com
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Background: The study aimed to explore the genetic association of Fc receptor-like 5 (*FCRL5*) gene variants (rs6427384 and rs6692977) with ankylosing spondylitis risk in Chinese Han population.

Material/Methods: Genotyping for *FCRL5* gene variations rs6427384 and rs6692977 was implemented among 130 ankylosing spondylitis cases and 135 healthy persons, through polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Frequency dissimilarity for 2 polymorphisms was compared between 2 groups using chi-square test. The association strength of *FCRL5* gene polymorphism with ankylosing spondylitis risk was estimated by odds ratios with 95% confidence intervals.

Results: The frequencies of rs6427384 CC genotype and C allele were significantly lower in the case group than that in the control group ($P<0.05$), which suggested that C allele of rs6427384 polymorphism might offer protection against ankylosing spondylitis onset. Whereas only 2 genotypes of rs6692977 were detected in the control group, and no significant association was found with ankylosing spondylitis susceptibility.

Conclusions: *FCRL5* gene polymorphism rs6427384 was correlated to ankylosing spondylitis occurrence among Chinese Han population, while rs6692977 was not.

MeSH Keywords: Polymorphism, Genetic • Receptors, Fc • Spondylitis, Ankylosing

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Background

Ankylosing spondylitis stands for a popular chronic autoimmune disease, which usually occurs in 20- to 30-year-old young males [1]. Ankylosing spondylitis is marked by insidious onset and slow progression with minimal symptomology, mainly involving the joint tissue such as sacroiliac joint, joint axis, spine, and peripheral joints [2,3]. Ankylosing spondylitis shows a high degree of familiarity and heritability, with the average onset age of 25.6 years old [4]. The onset of ankylosing spondylitis shows a world popular trend, and certain differences exist among different races and regions. In China, the incidence of ankylosing spondylitis has reached about 0.26% [5]. In Turkey, it has reached 0.49%, and it also affects approximately 0.5% of white Europeans, but ankylosing spondylitis is rare in African populations [6,7]. Despite of the unclear pathogenesis, it is generally accepted that ankylosing spondylitis may result from the interaction between genetic and environmental elements [8]. At present, the diagnosis of ankylosing spondylitis is mainly based on the clinical criteria of inflammatory back pain with arthritis, imaging findings, and genetic tests, and several clinical guidelines are recommended such as the guidelines from the European Spondyloarthropathy Study Group, the Amor criteria, the New York criteria, and the Rome criteria. Several genetic factors play important roles in pathogenesis of ankylosing spondylitis. HLA-B27 and its subtypes are present in 80% to 95% of patients with ankylosing spondylitis in the USA, compared with 6% of the general population, and HLA-B27 has a recognized role in the pathogenesis of ankylosing spondylitis [2,9–11], and tumor necrosis factor (TNF) and interleukin (IL)-17A may be potential therapeutic targets [12,13]. Therefore, with further elucidation for human genome, detection of multiple genetic polymorphism that would contribute to the predisposition to ankylosing spondylitis is anticipated.

Fc receptor-like molecules (FCRLs) are a class of proteins, resembling Fc receptors imposing vital influences on maintaining the homeostatic balance in immune system [14]. *FCRL* genes belong to the immunoglobulin gene superfamily, which was discovered by several groups using different strategies, with scholars finally designating them as a uniform nomenclature [15]. FCRLs contains 8 function gene, including *FCRL1-6*, FCRX, and one pseudogene *FCRY* [16]. Recent years the domestic and foreign researches unveil *FCRLs* gene poses crucial impacts on immunodeficiencies, lymphoid malignancies and autoimmune illnesses [17].

In humans, the 6 *FCRL* genes (*FCRL1-6*) are located on chromosome 1q21-23, which is a region has been identified as a candidate locus for multiple autoimmune disorders in both human and murine models [18]. Fc receptor-like 5 (FCRL5) is an orphan immunoregulatory protein highly expressed by innate B lymphocytes, which regulates B cell Ag receptor signaling and binds aggregated IgG [19,20]. Genetic variants in *FCRL5* gene may modify its protein function, thus influencing immune reactions. Rs6427384 is located on the coding exon of *FCRL5* gene, and participates in the biosynthesis of amino acids, in which the allele mutation C to T results in the amino acids variations of valine (Val) to isoleucine (Ile). For rs6692977 polymorphism, Gu et al. reported that CT genotype and T allele might contribute to the risk of asthma with comorbid allergic rhinitis in a Chinese Han population [21]. Ankylosing spondylitis, as one of the human autoimmune disease, its association with FCRL5 is excepted further study.

In this study, we investigated the genetic association of single nucleotide polymorphisms (SNPs) of *FCRL5* gene polymorphisms rs6427384 and rs6692977 with ankylosing spondylitis risk in Chinese Han population.

Material and Methods

The participants

The study participants included 130 unrelated ankylosing spondylitis patients and 135 healthy controls. The sample collection was performed according to the ethics criteria of national human genome research. All the participants signed written informed consents. Our research was approved by the Ethics Committee of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University.

The patients with ankylosing spondylitis were diagnosed by Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University from December 2012 to January 2015. The case group included 81 males and 49 females, with a mean age of 26.41 years. All cases were diagnosed according to New York criteria for ankylosing spondylitis which was revised by the American Rheumatism Association in 1984 [22]. Patients who had histories of inflammation or who had other systemic diseases were excluded. The 135 healthy individuals were recruited as the control group who had a medical examination in Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University during the same period, including 85 males and 50 females. The control group was matched with case group in age and gender. Additionally, the healthy controls who had a history of blood pressure, diabetes, hyperlipidemia, smoking, and obesity would be excluded from the study.

Sample collection

We collected 5 mL blood samples from 130 ankylosing spondylitis patients and 135 healthy controls who fasted for more

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Table 1. Primer sequences of *FCRL5* gene rs6427384 and rs6692977 polymorphisms.

SNP		Primer sequences	Annealing temperature
rs6427384	Forward	5'-GAGCATTCAGGGAACTACTA -3'	53°C
	Reverse	5'-TGGAGGAGGATATTAGGTTG-3'	
rs6692977	Forward	5'-CGGTCTCACTGGGCTAAA-3'	53°C
	Reverse	5'-TGACTTTGCTGGCTTTGG-3'	

FCRL5 – Fc receptor-like 5; SNP – single nucleotide polymorphism.

than 12 hours. Samples were stored in ethylenediamine tetra acetic acid (EDTA) containing tubes (Thermo Fisher, Shanghai, China). Then the genomic DNA was extracted by Genome DNA Extraction Kit (Takara, Dalian, China) according to the manual, and then stored at –20°C for future use.

Genotyping

The 2 single nucleotide polymorphisms (SNPs) was genotyped through polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Primer sequences for *FCRL5* gene rs6427384 and rs6692977 were designed with Primer Premier 5.0, and synthesized in Shanghai Sangon Biotech Co., Ltd. (Table 1). The PCR reaction system was in a total volume of 25 μL, including 5.0 μL DNA template, 2.5 μL 10×buffer, 2.0 μL $MgC1_2$, 0.5 μL dNTPs, each 0.5 μL of forward and reverse primers, 0.2 μL Taq enzyme (Thermo Fisher, Shanghai, China), 13.8 μL ddH_2O. The amplification reaction was carried out in Applied Biosystems (ABI, America), and the PCR procedure was set as follows, pre-denaturation at 94°C for 2 minutes; followed by 36 cycles of 94°C degeneration for 20 seconds, 53°C annealing for 30 seconds, 72°C extension for 30 seconds; and finally 72°C extension for 10 minutes. Then the purity for PCR products was examined adopting 2% agarose gel electrophoresis (AGE) (Thermo Fisher, Shanghai, China).

Then the qualified PCR products of rs6427384 and rs6692977 were digested by specific restriction enzyme (Sangon Biotech, Shanghai, China) (*MaeIII* for rs6427384 and *CviRI* for rs6692977) respectively. The system of restriction digestion was 20 μL, including 10 μL PCR products, 2 μL 10×buffer and 10 U restriction enzyme. Finally, the digestion reaction was performed at 37°C for 4 hours. Digested products were handled with 2.5% agarose gel electrophoresis, and genotypes were observed under ultraviolet lamp. Random samples of the PCR products were selected for direct sequencing to be verified the accuracy and reliability of the genotyping results.

Statistical analysis

All data were analyzed by inputting PASW statistics 18.0 statistical software. All genotype and allele frequencies of 2 SNPs were performed Hardy-Weinberg equilibrium (HWE) test to detect the representativeness of control group. The differences of the genotype and allele distributions of *FCRL5* gene rs6427384 and rs6692977 polymorphisms were compared by chi-square test between 2 groups. The relative risk of AS was evaluated via odds ratios (ORs) accompanied by 95% confidence intervals (CIs). Significance level lay at $P<0.05$.

Results

HWE test

As shown in Table 2, the distributions of the 2 polymorphisms rs6427384 and rs6692977 were all confirmed to HWE, indicating that our study population were from the same Mendelian population.

Distributions of *FCRL5* gene polymorphisms

For rs6427384, CT and CC genotype frequencies of rs6427384 were decreased in case group compared with control group (28.15% versus 31.54%; 2.96% versus 8.46%), while TT genotype frequency was increased (68.89% versus 60.00%). Via chi-square test, the difference of CC genotype distribution between 2 groups reached statistically significant level ($P<0.05$). We summarized that *FCRL5* gene rs6427384 polymorphism might hold strong relation to AS onset. Besides, we also noted that C allele frequency was decreased in case group compared with control group (17.04% versus 24.23%), showing remarkable dissimilarity ($P<0.05$). Individuals carrying C allele had low risk of ankylosing spondylitis (OR=0.642, 95%CI=0.420–0.983). All results suggested that *FCRL5* gene rs6427384 was correlated with ankylosing spondylitis risk, and the ancestral C allele might reduce the risk of ankylosing spondylitis onset in the Chinese Han population.

For rs6692977, CC, CT, and TT genotype frequencies were 78.46%, 19.23%, and 2.31% in each case group, respectively. Whereas only 2 genotypes of CC and CT were detected in the control group, with the frequencies of 80.74% and 19.26%. We noted that the mutant T allele carriers were more in case groups

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Table 2. Comparison of the genotype and allele distributions of *FCRL5* gene rs6427384 and rs6692977 polymorphisms between case and control groups.

SNPs	Genotype/allele	Case (%) (n=130)	Control (%) (n=135)	χ^2	*P*	OR (95% CI)
rs6427384	TT	93 (68.89)	78 (60.00)	–	–	1
	CT	38 (28.15)	41 (31.54)	0.856	0.355	0.777 (0.456–1.326)
	CC	4 (2.96)	11 (8.46)	4.246	0.039	0.305 (0.093–0.996)
	T	224 (82.96)	197 (75.77)	–	–	1
	C	46 (17.04)	63 (24.23)	4.196	0.041	0.642 (0.420–0.983)
rs6692977	CC	102 (78.46)	109 (80.74)	–	–	1
	CT	25 (19.23)	26 (19.26)	0.008	0.931	1.028 (0.557–1.895)
	TT	3 (2.31)	0 (0.00)	3.159	0.076	0.971 (0.940–1.004)
	C	229 (88.08)	244 (90.37)	–	–	1
	T	31 (11.92)	26 (9.63)	0.726	0.394	1.270 (0.732–2.205)

FCRL5 – Fc receptor-like 5; SNP – single nucleotide polymorphism; OR – odds ratio; CI – confidence interval.

than that in the control group. Via chi-square test, the differences of *FCRL5* gene rs6692977 genotype and allele distributions between groups did not reach significant level ($P>0.05$). These results demonstrated that *FCRL5* gene rs6692977 might had no obvious association with ankylosing spondylitis risk.

Discussion

Ankylosing spondylitis is a chronic inflammatory arthritis that affects young adults, manifesting predominantly with new bone formation, ankylosis, and inflammation of hip, sacroiliac joints, and spine [23]. It has been identified to be genetically heterozygous [24]. Recently, a number of twin studies have confirmed the role of genetic factors in inflammatory joint disease, especially ankylosing spondylitis [25]. Furthermore, various genetic mutations have been verified to influence the ankylosing spondylitis risk, such as TNF-like ligand 1A gene (*TNFST15*), endoplasmic reticulum amino peptidase 1 (*ERAP1*) and so on [5,26]. Until now, there is no effective method for early diagnosis of ankylosing spondylitis, and the treatment options available for ankylosing spondylitis patients have met with limited success.

FCRL5 is a novel IgG binding protein with the capacity to regulate Ag receptor signaling, which is expressed on B cells [27]. Recent researches have also proposed FCRL5 differentially affects innate-like B cell receptor function [28]. The human *FCRL5* gene is one of *FCRL* genes, which is located on chromosome 1q21-23, and has been identified as a candidate locus for multiple autoimmune disorders.

In this paper, we examined potential connection for *FCRL5* variants to ankylosing spondylitis susceptibility. Rs6427384 polymorphism was genotyped in 130 ankylosing spondylitis patients and 135 healthy controls. Via chi-square test, CC genotype and C allele frequencies showed significantly declining trend. We deduced that *FCRL5* gene polymorphism rs6427384 might affect ankylosing spondylitis initiation and its ancestral C allele might offer protection against the disease among Chinese Han people. In the previous study, Tang et al. had performed a similar investigation in HLA-B27-positive populations [29], and the results was in accordance with our conclusion. Meanwhile, rs6692977 polymorphism was also detected in our present study, but no significant association was found. However, Simmonds et al. have explored the role of rs6692977 for another autoimmune disease Graves' disease, and significant association has been found [30]. The divergence might be attributed to the differences in pathogenesis across the different types of inflammation disease. Therefore, further studies should be performed to confirm our results.

Conclusions

In conclusion, our present study suggested a tight relation for *FCRL5* gene rs6427384 to ankylosing spondylitis occurrence among Chinese Han people, while rs6692977 was not. However, several limitations still presented in our study. First, our study cohorts were selected from one hospital, which may cause selection bias in the data analysis. Second, our study sample was relatively small, which would reduce the statistical power. Thus, further genetic researches should be warranted to inspect this topic in other populations. Besides, other multivariate risk assessments should also be involved.

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