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Correlation between single nucleotide polymorphism of rs3811047 in IL-1 F7 gene and rheumatoid arthritis susceptibility among Han population in central plains of China

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ABSTRACT

Objective: To discuss the association between single nucleotide polymorphism (SNP) of rs3811047 in IL-1 F7 gene and rheumatoid arthritis (RA) susceptibility among the Han population in central plains of China. **Methods:** A total of 276 RA patients admitted to our hospital from December 2009 to December 2011 together with 276 healthy physical examinees in the same period were chosen as the subjects. The typing for rs3811047 SNP in IL-1 F7 gene was carried out by using ligase detection reaction and polymerase chain reaction technique. And the frequency of each allele and genotypes distribution was calculated so as to evaluate the association between genotype distribution and RA susceptibility. **Results:** The frequency of A allele of rs3811047 in IL-1 F7 gene in RA group and control group was 16.27% and 17.68%, respectively, and that of G allele in two groups was 83.73% and 82.32%, respectively. The difference between two groups wasn't statistical significant (P >0.05). The frequency of genotype AA, AG and GG in RA group was 2.19%, 27.84% and 69.97%, respectively, while that in control group was 2.94%, 29.78% and 67.28%, respectively. The difference of distribution of three genotypes was not statistically significant (P >0.05). RA patients with A allele were better than those without A allele in joint swelling index, rest pain, HAQ scoring and blood sedimentation. There was significant difference between two groups in above indexes (P<0.05/P<0.01). **Conclusions:** No significant correlation between RA susceptibility among the Han population in central plains of China and rs3811047 SNP inIL-1 F7 gene is observed. However, A allele of rs3811047 has certain influence on the condition of RA patients.

1. Introduction

Rheumatoid arthritis (RA) is a form of autoimmune diseases mainly characterized by symmetry chronic inflammation of multi-joints, whose mobidity in China is around 1%-2%[1]. Though the onset of RA is not fully known so far, it is confirmed that RA seems to be inheritable, and hereditary features between different regions or different races are evident[2]. In order to explore the association between rs3811047 SNP in IL-1 F7 gene and RA susceptibility, this study, with the Han population in central plains of China as subjects, detects the SNP of rs3811047 in IL-1 F7 genes among RA patients by ligase detection reaction (LDR) and polymerase chain reaction (PCR) technique and compares the results with that of healthy participants so as to provide new clues for the genetics study of RA.

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2. Material and methods

2.1. General material

A total of 276 RA patients admitted to our hospital from December 2009 to December 2011 were enrolled, including 78 males and 198 females from 18 to 78 years old with the mean age of 46.7±14.6. All patients meet the RA diagnosis criteria amended by American Rheumatism Association in 1987. Meanwhile, another 276 healthy participants from the Medical Examination Center of our hospital were chosen as the control group, including 81 males and 195 females from 19 to 76 years old with the mean age of 47.4±13.9. There was no statistical difference between the two groups in gender or age (P>0.05). All subjects are Han population from central plains of China without blood relationship and informed consent was obtained.

2.2. Methods

2.2.1. Peripheral blood DNA extraction

2 mL of fasting peripheral venous blood was taken from all subjects and 0.2% of EDTA-Na_2 was used for anticoagulation. Modified salt fractionation[3] was used for DNA extraction (genome DNA rapid-extract kit, Beijing SBS genetech Co.,Ltd) and the extraction was reserved in refrigerator at -70 ℃.

2.2.2. Primer design

Primer and probe were synthesized by ABI Company with forward primer being 5′-AGCCCCCTGGAACCA-GGCCCAAGCCTCCCC-3′ and reverse primer being 5′-CCAT GAATTTTGTTCACACAAGTAAGGCCT-3′.

2.2.3. DNA amplification and purification

PCR reaction system was 20 μL, containing 100 ng of genomic DNA. The reaction conditions were as follows: pre-denaturing at 95 ℃ for 15 min, followed by 35 cycles of denaturing at 94 ℃ for 30 s, annealing at 59 ℃ for 1min and extension at 72 ℃ for 1 min, with a final extension at 72 ℃ for 7 min. TaKaRa (Dalian) Gel DNA Purification Kit was used for the purification of amplified production.

2.2.4. Genotyping

PCR products were mixed with formamide deionized solutions and heated for 2 min. After cooling down, they were put into mixed liquor of polyacry lamide and carbamide for electrophoresis for 3 h at 3 000 V. Data were collected by GENESCANTM672 scan analysis software and Gene Mapper fragment analysis software[4] was used for genome typing.

2.3. Statistical methods

All data were processed by SPSS software version 15.0 to calculate the frequency of each allele and genotype distribution. Goodness-of-fit χ^2 was used to test whether the genotype distribution conforms to Hardy-Weinberg balance. The clinical and experimental indexes of different genotypes were compared by analysis of variance (ANOVA), *t* test or χ^2 test. *P* value of <0.05 was taken as statistically significant.

3. Results

3.1. Allele frequency of IL-1 F7 (rs3811047 locus)

The frequency of A allele of rs3811047 in RA group and control group was 16.27% and 17.68%, respectively, and that of G allele in two groups was 83.73% and 82.32%, respectively. The difference between two groups was not statistically significant (*P* >0.05), indicating that there was no correlation between variant alleles and the incidence of RA.

3.2. Genotypes distribution of IL-1 F7(rs3811047 Locus)

The frequency of genotype AA, AG and GG in RA group was 2.19%, 27.84% and 69.97%, respectively while that in the control group was 2.94%, 29.78% and 67.28%, respectively. The difference of the distribution of three genotypes was not statistically significant (χ^2=0.61, *P* >0.05).

3.3. Association between genotypes distribution and disease condition

Among 276 patients, the frequency of genotype AA, AG and GG of rs3811047 was 2.19%, 27.84% and 69.97%, respectively. ANOVA analysis showed that differences among all clinical and experimental indicators were not statistically significant (*P*>0.05). However, since genotype AA accounts for a small proportion (2.19%), it was put into the same group with genotype AG, namely, comparing those containing A allele with those without A allele. Results showed that RA patients with A allele was better than those without A allele in joint swelling index, rest pain, HAQ scoring and blood sedimentation. There was significant difference between two groups in above indexes (*P*<0.05/*P*<0.01) (Table 1).

4. Discussion

Genetic factor is the primary reason that causes RA susceptibility (about 60%). Epidemiological investigations indicated that RA has obvious familial aggregation tendency, and the prevalence rate in monozygotic twins is 21%-32%[5,6]. At present, the research about RA susceptibility gene was mainly focused on human leucocytic antigen (HLA) gene, tumor necrosis factor (TNF) gene and peptidylarginine deiminase 4 (PADI4) genes. It has been found that RA susceptibility is closely related to SNP of specific site in HLA-DR4, HLA-DRB1, HLA-DM and TNF gene[7]. The role that those genes play in RA mobility of familial aggregation only takes up 20%-33%[8]. More RA susceptibility genes have not been found yet, therefore, in recent years, people will turn to other RA susceptibility genes.

Large amount of $CD4^+$T cells exist in synovium of bone joint of RA patients. After activation, $CD4^+$T cells can produce IL-1, IL-6, IL-8, TNF, *etc*, which can cause chronic inflammation of synovium of bone joint. IL-1 can cause hypodynamia, low-grade fever, blood sedimentation *etc*. Some scholars[9] had verified that it is feasible to treat RA and relieve pathogenetic condition by IL-1receptor blocker. Zwerina *et al*[10] proved the important role that IL-1 played

Table 1

Association between genotypes distribution in RA group and disease condition.

Indicators	AA+AG(*n*=82)	GG(*n*=191)	*t*	*P* value
Joint swelling index	4.76±3.35	6.23±4.89	2.48	<0.05
Joint tenderness index	10.73±8.45	12.90±8.51	1.94	>0.05
Rest pain	3.42±2.33	4.49±2.90	2.96	<0.01
HAQ scoring	0.78±0.65	1.04±0.76	2.70	<0.01
CRP(mg/L)	33.25±40.93	41.02±45.34	1.34	>0.05
RF(RU/mL)	193.67±101.71	205.26±109.29	0.82	>0.05
Blood sedimentation (mm/h)	35.63±23.35	45.84±32.91	2.55	<0.05

in RA osteoclasia. They blocked the role that IL-1 plays in TNF inducing arthritis by knockouting the rats' IL-1 gene, compared with rats without knockout IL-1gene, and there was no significant difference in the aspect of inflammation. But in the aspects of erosion of bone and osteoclasia, the former was obviously lighter than the latter, which indicated that IL-1 has an important regulating effect on TNF inducing osteoclasia.

This study proved certain correlation exists between rs3811047 SNP of IL-1 F7 gene and RA susceptibility, that is, in the aspects of joint swelling index, rest pain, HAQ score and blood sedimentation, RA patients having A allele outweighs those don't have A allele ($P<0.05$ or $P<0.01$). It indicated that IL-1 F7 gene was closely related to clinical arthritis manifestation and daily living ability. It also indicated that different genetype of IL-1 F7 gene may lead to the end product difference in the aspects of biological function, structure, quality and quantity of expression, even receptor binding, which would finally lead to different role that IL-1 F7 plays in the inflammatory process. However, the exact mechanism needs further in-depth study.

SNP is DNA sequence polymorphism caused by single nucleotide (A, G, C, T) variation, approximately taking up over 90% of human DNA polymorphism[11-13]. SNP, a common type of human genetic variation, one of the bases of genetic material, is called a new generation genetic marker. Through the detection of genetype distribution of SNP site related to disease susceptibility, we can judge the correlation between a certain genetype and the susceptibility of this disease, which provided the theoretical basis for the precaution and individualized diagnosis and treatment for this disease. This study detected the rs3811047 site SNP of IL-1 F7 gene of Han population in the central plains of China by LDR and PCR technology. We found that the A allele frequency of rs3811047 site in IL-1 F7 gene of RA group and control group was 16.27% and 17.68%, respectively, and G allele frequency was 83.73% and 82.32%, respectively. The difference between the two groups was not statistically significant ($P>0.05$). The frequency of genotype AA, AG and GG in RA group was 2.19%, 27.84% and 69.97%, respectively, while that in the control group was 2.94%, 29.78% and 67.28%, respectively. The difference of three genotypes frequency distribution between the two groups was not statistically significant ($P>0.05$). This indicated that there was no significant correlation between of rs3811047 site SNP in IL-1 F7 gene and RA susceptibility of Han population in the central plains of China.

In summary, we proved that rs3811047 site SNP in IL-1 F7 gene was independent of RA susceptibility of Han population in the central plains of China. However, in the aspects of joint swelling index, rest pain, HAQ score and blood sedimentation, RA patients having A allele in this site outweighs those don't have A allele, which indicated that A allele of rs3811047 site in IL-1 F7 gene has certain influence on pathogenetic condition. This provides certain theoretical basis for the research on the pathogenesis of RA in Han population in the central plains of China and RA diagonosis with IL-1 gene as the target. We will carry out more IL-1 gene site research in order to provide theoretical basis for the prediction, early diagnosis and gene therapy of RA.

Conflict of interest statement

We declare that we have no conflict of interest.

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