Association between IKZF1 related gene polymorphism, DNA methylation and rheumatoid arthritis in Han Chinese: A case-control study.

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Abstract

Background: Rheumatoid arthritis (RA) is a systematic autoimmune disease with evidence of genetic predisposition. The IKZF1 (IKAROS family zinc finger 1 (Ikaros)) gene is located at chromosome 7, encodes a transcription factor related tochromatin remodeling, regulates lymphocyte differentiation, and is associated with some autoimmune diseases. However, there were few studies reported the association between IKZF1 and the risk of RA in China. For this, we determined to investigate the possibility of association between the IKZF1 locus and RA. Methods: we selected one single nucleotide polymorphisms (SNP) in the IKZF1 locus, rs1456893, based on a detailed analysis of genome-wide association study (GWAS) data and performed genotyping in 410 RA patients and 421 healthy controls in Han Chinese. We studied the systematic genome-wide interrogation of DNA methylation between RA group and control group and we also studied the association between CpGs and RA. Results: The results showed that the rs1456893 locus was correlated with RA in different models(P¡0.05). Through comparison with methylation levels determined in their equivalent healthy counterparts we have identified and validated a restricted set of CpGs that show distinct methylation differences between patients with RA and control group. The Multiple logistic regression results showed CpG_3 (OR=3.17,95%CI=1.05-9.60,P=0.041) and CpG_13.14(OR=4.96,95%CI=1.77-13.86,P=0.002)were associated with RA. Conclusion: IKZF1 can be used as a marker for the diagnosis of RA. Higher CpG_3 and CpG_13.14 methylation levels would increase the risk of RA.

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Key words: IKZF1, DNA methylation, Rheumatoid arthritis

Summary

Rheumatoid arthritis (RA) is a systematic autoimmune disease with evidence of genetic predisposition. The IKZF1 (IKAROS family zinc finger 1 (Ikaros)) gene is located at chromosome 7, encodes a transcription factor related tochromatin remodeling, regulates lymphocyte differentiation, and is associated with some autoimmune diseases. However, there were few studies reported the association between IKZF1 and the risk of RA in China. For this, we determined to investigate the possibility of association between the IKZF1 locus and RA. we selected one single nucleotide polymorphisms (SNP) in the IKZF1 locus, rs1456893, based on a detailed analysis of genome-wide association study (GWAS) data and performed genotyping in 410 RA patients and 421 healthy controls in Han Chinese. We studied the systematic genome-wide interrogation of DNA methylation between RA group and control group and we also studied the association between CpGs and RA. The results showed that the rs1456893 locus was correlated with RA in different models(P ¡0.05). Through comparison with methylation levels determined in their equivalent healthy counterparts we have identified and validated a restricted set of CpGs that show distinct methylation differences between patients with RA and control group. The Multiple logistic regression results showed CpG_3 (OR=3.17,95%CI=1.05-9.60,P=0.041) and CpG_13.14(OR=4.96,95%CI=1.77-13.86,P =0.002)were associated with RA. IKZF1 can be used as a marker for the diagnosis of RA. Higher CpG_3 and CpG_13.14 methylation levels would increase the risk of RA.

Introduction

Rheumatoid arthritis (RA) is a unexplained autoimmune disease with characteristic chronic articular synovial inflammation[1].The global prevalence of RA was 0.24% ,with no discernible change from 1990 to 2010[2]. Between 70-80% of RA patients have autoantibodies such as rheumatoid factor and anti-citrullinated protein antibodies, suggesting that RA is an autoimmune disease[3]. Several meta-analyses of genome-wide association studies (GWAS) have identified close to 40 genetic variants that confer risk for the citrullinated protein antibody-associated (ACPA) subtype of RA[4-7]. However, the fact that these discoveries can only explain less than 20% of disease variance suggests that other factors are likely involved in the disease[6].

The zinc finger protein IKAROS (also termed IKZF1) is encoded by the IKZF1 gene that regulates lymphocyte differentiation and proliferation as well as self-tolerance through the regulation of B-cell receptor signaling, located in chromosome subband 7p12.2. Previous studies also found that IKZF1is significantly associated with several autoimmune diseases, such as Childhood Acute Lymphoblastic Leukemia ,systemic lupus erythematosus (SLE), Primary Sjogren's syndrome[8-10].However, there have been no reports about the association between IKZF1 and RA. The genetic factors contributing to the pathogenesis of RA are still unclear.

Recent genome-wide association (GWAS) and epigenome-wide association (EWAS) studies of RA have tagged more than one hundred genetic risk loci and ten putative differentially methylated positions (DMPs)[4,11-13]. In RA, genetic and epigenetic modifications can influence disease development and disease risk variation jointly[14].Epigenetic modifications play a central role in the cellular programming of gene expression. Epigenetics is defined as the study of heritable and reversible changes in gene function that do not involve alterations in the DNA sequence[15]. Epigenetic mechanisms are sensitive to external stimuli; therefore, they mediate in gene-environment interactions. Three main epigenetic mechanisms have been described, including non-coding RNA species, histone modification, and DNA methylation. Epigenetic factors have recently emerged as potential elements in explaining and redefining diseases. Among a variety of epigenetic regulatory mechanisms, DNA methylation is the most frequently studied factor because of relatively more mature detection technology [16]. Growing evidences have suggested that DNA methylation plays an important role in the pathogenesis of RA[17,18].

To our knowledge, there are no report about the associations of IKZF1 DNA methylation and the risk of RA. The aim of our present study was to explore the association between common variants (single nucleotide polymorphisms, SNPs) near the IKZF1 locus and RA risk in an independent Han Chinese sample.

Materials and methods

Study samples

The RA patients were recruited from rheumatology inpatients and outpatients of three hospitals in Shandong province, and the controls were selected from contemporaneous health checkup population above three hospitals. All the cases were diagnosed with RA by at least two rheumatologists according to the ACR/EULAR2010 RA criteria for RA[19]. They were not diagnosed with any other autoimmune diseases at the same time. People with family history of major diseases, prolonged use of hormones, Immunosuppressive drugs were excluded.

Subjects fasted in the morning and was collected 5 mL of elbow peripheral venous blood samples with a vacuum anticoagulation tube. Blood samples were stored at -20 degrees after centrifugal separation for DNA extraction, genotyping and methylation detection. For the genotyping procedure, we first extracted genomic DNA from peripheral blood samples from each individual using a Whole-Blood DNA Extraction Kit. Then, genotyping was performed using the iPLEX Mass ARRAY platform (Sequenom). For quality control, we checked the SNP call rate (¿95%), Hardy-Weinberg equilibrium (HWE) for controls (P -values¿1E-3), minor allele frequency (¿0.05) and call rate difference between cases and controls (P -values¿1E-5). The basic data was collected through the questionnaire, such as age, sex, and education level of the research subjects. This study was approved by the Internal Review Committee of Shandong First Medical University,and appropriate written informed consent was obtained from all participants at recruitment.

Statistical analyses

All data analysis were performed with SPSS 26.0. Normality was tested using the Shapiro-Wilk test. Normal distribution data was analyzed by t test. Chi square tests were used to compare categorical variables. Wilcoxon rank sum tests were used to compare continuous variables with skewed distributions. The HWE test for genotypic distributions of controls was assessed with χ^2 test for goodness of fit. Comparisons of genotype and allele frequencies between the two groups were performed for each polymorphism with PLINK v1.07[20]. Logistic regression models were used to analysis the relation of CpGs and the risk of RA, containing unadjusted and adjusted models, the adjusted factors were sex, age and BMI. And the results were presented as odds ratios (ORs) and 95% confidence intervals (CIs).P -values in all the association tests were two-sided, and$P$ ¡0.05 was considered having statistically significant.

Results

The results of characteristics of the study participants.

The present study included 410 RA cases (mean age 53.73±11.67years) consisting of 348 females and 62 males. The control group included 421 healthy subjects (mean age 49.27±12.02 years), consisting of 264 female and 157 male. The demographic and clinical characteristics of the two groups (RA group and control group) were showing in Table 1. There were statistically significant differences in terms of their demographic and clinical information(P ¡0.05), such as age , sex , place of residence , BMI , marital status , education level, income , smoking status. The control group was tested by Hardy Weinberg, in line with Hardy-Weinberg Law(P ¿0.05,table 2). Only the genotype and allele frequencies of rs1456893 had statistically difference during case and control group (P ¡0.05). There were no statistically differences of rs1110701, rs4917014, and rs7089424 during two groups(P ¿0.05)(Table 3).

The association between rs1456893 and RA.

Correlation analysis using different genetic models showed that rs1456893 locus were associated with the risk of RA in the dominant(OR=1.457, 95%CI=1.103-1.924,P =0.008), codominant(OR=1.289, 95%CI=1.030-1.614,P =0.027) and over dominant model(OR=1.438, 95%CI=1.089-1.898,P =0.010). However, there was no statistically correlation in the recessive model(P ¡0.05)(Figure1). After adjusting for age, gender and BMI, the results showed that rs1456893 locus were also associated with the risk of RA in the dominant(OR=1.237, 95%CI=0.683-2.239,P =0.483),codominant (OR=1.365, 95%CI=1.064-1.752,P =0.014) and over dominant model(OR=1.433,95%CI=1.055-1.948,P =0.021). However, there was also no statistically correlation in the recessive model(P ¡0.05).

The association between methylation levels of CpGs and RA.

The methylation levels of CpG_3, CpG_13.14, CpG_17, CpG_6, CpG_20, and CpG_22 were different (P ¡0.05). The methylation level of RA group was greater than that of control group. There was no significant difference in the methylation level of the remaining sites. (Table 4). Correlation analysis of mQTL between IKZF1 gene CpGs site and rs1456893 site under different models. Using PLINK v1.07 software, the methylation level of CpG site was used as the dependent variable, and the SNP site (rs1456893) with significant difference between IKZF1 gene and RA was used as the independent variable. Linear regression was performed to analyze the IKZF1 gene CpGs site and rs1456893 site under different models. (Additive model, implicit model, explicit model). The results showed that CpG_3 and CpG_6 were statistically correlated with IKZF1 under the additive model, explicit model, and recessive model. CpG_8 and CpG_22 were only statistically correlated in the additive model, and CpG_9 and CpG_13.14 and CpG_29.30 are additive. There is statistical correlation between the model and the implicit model. CpG_15 and CpG_17 and CpG_25, CpG_27 and CpG_28 were statistically correlated under the additive model and the dominant model. The other sites CpG_4, CpG_5, CpG_11.12, CpG_16, CpG_19, CpG_20, CpG_23, CpG_24 and CpG_26 were not statistically significant under the three models. (Table 5). The Multiple logistic regression results showed that CpG_3 was associated with RA

(OR=3.79,95%CI=1.28-10.90, P =0.016) and CpG_13.14 was significantly associated with RA(OR=5.18,95%CI=1.91-14.07,P =0.001). After adjusting for age, gender and BMI,the results also showed that CpG_3 (OR=3.17,95%CI=1.05-9.60, P=0.041) and CpG_13.14(OR=4.96,95%CI=1.77-13.86, P =0.002) were associated with RA(Figure 2).

Discussion

Ikaros family zinc finger 1, the protein product encoded by IKZF1, is a member of the DNA-binding protein family that functions as a transcription factor in the thymus, spleen, peripheral blood lymphocytes and lymph nodes. Previous studies have reported that Ikaros plays an important role in the development of several lymphocytes, such as T and B cells[21].It was found that Ikaros is required in the early stages of T and B cell specification during fetal development, and different phases of the development of lymphoid lineages in adults are dependent on the regulation of Ikaros[22]. Moreover, in homozygous mutant mice in which the DNA-binding domain of the IKZF1gene was changed, erythroid and myeloid lineages were complete, but T, B and natural killer (NK) cells and their precursors were lacking. Thus, Ikaros is essential for normal lymphocyte occurrence, development and differentiation. Abnormal Ikaros may lead to paralysis of the immune system and further influences the occurrence of autoimmune diseases, such as RA.

Furthermore, previous studies have shown that interferon regulatory factors (IRFs) (e.g.IRF-5 and IRF-8) regulate the expression of IKZF1and Ikaros[23,24], thereby regulating the induction of inflammatory cytokines and type 1 interferons. Additionally, Ikaros is involved in the regulation of STAT4 in human T cells[25]. Interestingly, associations of IRF-5 and STAT4 with RA have been confirmed in previous GWAS of RA[26,27]. If further experimental evidence confirms our finding that IKZF1is associated with RA, then IKZF1, IRFs and STAT4 may be further demonstrated to play a role in disease pathogenesis by regulating immune-related cell activities of some signaling pathways involved in RA, such as the STAT4 and interferon (IFN) pathways.

Through replication and meta-analyses in Han Chinese subjects, our study identified one SNP, rs1456893, near the IKZF1 locus that were significantly associated with RA. Among these SNPs, rs1456893 reached the level of genome-wide significance. We found that the IKZF1 rs1456893 AA allele may increase the risk of RA, the result is consistent with Harney's research. A research showed the PADI4 rs2240340 AA allele may increase the risk of RA, particularly in patients who are ACPA-positive, indicating a gene-environment interaction. And the PADI4 rs2240340 AA allele may increase the risk of RA, especially in older patients[28].

There are some reports showing association between DNA methylation and diseases, including RA[11], cancer[29,30], and type 1 diabetes[31].A previous study with this dataset found a robust association between methylome modifications and risk of RA[11].In order to further determine the relationship between IKZF1

and RA, we now report a systematic genome-wide interrogation of DNA methylation in populations from patients with RA. Through comparison with methylation levels determined in their equivalent healthy counterparts we have identified and validated a restricted set of CpGs that show distinct methylation differences between patients with RA and control group, indicating that IKZF1 can be used as a marker for the diagnosis of RA.

Compared with DNA methylation change of cancer which usually contains thousand differential methylated loci, systemic autoimmune rheumatic diseases seems to have only few differential methylation regions. PCA analysis to our methylation dataset also revealed there is no significant separation between RA and control, indicating there would be not many different methylation regions in CD4 + T-cells between RA and normal individual[32]. Jeffries et al. identified 341 differential methylation loci in CD4 + between SLE and health control[33]. Altorok et al. identified 753 differential methylation loci in CD4 + between SjS and health control[34]. Although multiple test correction has been conducted in these papers, there are still large number of differential methylated loci would be false positive. Therefore, the candidate differential methylated loci underlying the pathogenesis of RA would be limited. There were 9 shared differential methylated CpG loci which might be very important in the pathogenesis of RA in my study, due to the small sample size. More genome-wide DNA methylation profiles from different ethnic populations may provide ethnic specific information of methylation changes of RA patients.

Several limitations of our study need to be addressed. First, this was a hospital-based case-control study, so selection bias was unavoidable and subjects were not fully representative of the general population. Second, the polymorphisms we investigated, based on their functional considerations, may not offer a comprehensive view of the genetic variability of IKZF1. Third, we selected only one SNP at the IKZF1locus for Methylation. Other causal variant(s) associated with RA may exist. Therefore, we need to further explore the relationship between other polymorphisms at the IKZF1gene locus and RA in the Han Chinese population. Finally, we did not obtain detailed information about the outcomes of treatment, which restricted our analyses.

Conclusion:

IKZF1 can be used as a marker for the diagnosis of RA. Higher CpG_3 and CpG_13.14 methylation levels would increase the risk of RA.

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