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Genetic association of aromatic hydrocarbon receptor and its repressor gene polymorphisms with risk of rheumatoid arthritis in Han Chinese populations

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Abstract

The goal of this study was to evaluate the potential relationship among polymorphisms of aromatic hydrocarbon receptor, aromatic hydrocarbon receptor repressor, and rheumatoid arthritis (RA) susceptibility as well as the association among the polymorphisms of aromatic hydrocarbon receptor, aromatic hydrocarbon receptor repressor, and their expression.

We performed a hospital-based, case–control study of 400 patients with RA and 726 healthy controls in Han Chinese populations. Two single-nucleotide polymorphisms were selected for genotyping including aromatic hydrocarbon receptor (rs2066853) and aromatic hydrocarbon receptor repressor (rs2292596).

To single-nucleotide polymorphism rs2292596, a statistically significantly increased risk of RA was found to be associated with the G allele of rs2292596; the odds ratio was 2.170 (95% confidence interval: 1.820–2.587). Unfortunately, no significant differences exhibited in the allelic and the genotype frequencies of rs2066853 between 2 groups. We failed to find any association between rs2066853, rs2292596 genotypes and their expression of patients, respectively. No statistical relationship was found between aromatic hydrocarbon receptor, aromatic hydrocarbon receptor repressor at messenger Ribonucleic acid levels and clinical data, either.

This study demonstrated that the polymorphisms of rs2292596 was significant with genetic susceptibility to RA patients; furthermore, it suggests the G allele of rs2292596 might be associated with a dangerous effect on RA in Han Chinese populations.

Abbreviations: AHR = aryl hydrocarbon receptor, AHRR = aryl hydrocarbon receptor repressor, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, PBMC = peripheral blood mononuclear cell, RA = rheumatoid arthritis, RT-PCR = real-time polymerase chain reaction, SNPs = single-nucleotide polymorphisms.

Keywords: arthritis rheumatoid, aryl hydrocarbon receptor, aryl hydrocarbon receptor repressor, single-nucleotide polymorphism

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that primarily manifests as polyarthritis, and it affects approximately 1% of the worldwide population.[1] The pathogenesis of RA is complicated and remains largely unknown, and what may predominantly attribute to the combination of genetic and environmental factors. To date, aromatic hydrocarbon receptor (AHR) is thought to be a key factor that regulates immune diseases including RA and then accelerates the development and progression of the diseases.[2–7]

AHR is a ligand-dependent transcription factor that belongs to a member of basichelix–loop–helix/period-AHR nuclear translocator (ARNT)-single minded family. AHR combines with the chaperonins in the cytoplasm and keeps inactive in the absence of its ligands. Once activated, AHR dissociates with its chaperonins, translocates into the nucleus, and binds ARNT. The AHR/ARNT heterodimers bind to specific deoxyribonucleic acid (DNA) sequence and control the transcriptional activities of responsive genes. Aromatic hydrocarbon receptor repressor (AHRR), a characteristic factor used to differentiate AHR activation, which is particularly responsive to AHR activation. Accumulating evidence from recent studies has shown that AHR plays crucial roles in several immune diseases including ulcerative colitis,[2,3] experimental autoimmune encephalomyelitis,[4,5] collagen-induced arthritis,[6] and Sjögren syndrome.[7] In our and other previous study, we obtain that the expressions of AHR and AHRR are higher in RA than that in matched group. Given the central effect of AHR and AHRR in the regulation of RA, we speculated that mutations of the *AHR* and *AHHR* gene might confer the incidence of RA. In order to prove this hypothesis, we genotyped for 1 AHR and 1 AHRR the most widely studied single-nucleotide polymorphisms (SNPs) in based case–control

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study of 400 RA patients without other rheumatic diseases and 726 age and sex frequency matched healthy controls in Han Chinese population.

2. Methods and materials

2.1. Study subjects

A total of 1126 subjects from Anhui Provincial Hospital, Anhui Medical University and the second hospital of Anhui Medical University between April 2013 and June 2015 were included, 400 of which were RA patients and the others were healthy controls. Patients diagnosed with RA according to the American Rheumatism Association 1987 revised criteria for the classification.[8] The included criteria for RA group: definite diagnosis and undergoing no other diseases. The RA-free control participants selected randomly from health center had no underlying diseases and were matched with RA patients by age, sex, and residential area (urban or rural areas). In the corresponding period, the expressions of AHR and AHRR were tested randomly from 71 RA cases. Venous blood samples of 5 mL were collected into heparinized tubes from donors. The clinical data and the disease activity score were calculated in detail as well. The Ethics Committee of the Hospital approved the study. Informed consent was obtained from the patients or from their relatives if the patients were incapable of giving consent.

2.2. SNP selection and genotyping

Two tagging SNPs in *AHR* and *AHRR* genes were selected with $r^2 > 0.8$ and low minor allele frequency >5% in Chinese population from HapMap project,[9] including rs2066853 and rs2292596. As a result, the SNPs of rs2066853 and rs2292596 were selected in our study. Genomic DNA was extracted from 1 mL of 400 RA patients and 726 healthy controls of the peripheral blood sample and DNA isolated by AxyPrep DNA Purification kit from AXYGEN Company (New York). For each sample, reading of the genotype was confirmed by 2 individuals at least. The polymerase chain reaction (PCR) restriction technology was used to determine each SNP. Moreover, Takara QuickCut Enzyme (Beijing, China) was applied in our present study. The specific primers designed were as follows: for rs2066853, forward-5′-CATTGATTTTGAAGACCTCA-3′, rs2066853-reverse 5′-CTGAAGGTATGAAGGGAG-3′"; rs2292596 forward-5'-AGGTTTGGTTGGCAGGACT-3′ and reverse 5′-GCTCAGATGGTTGGCTGTTC-3′. The conditions of PCR are as follows: initial denaturation at 95°C for 15 minutes, 94°C for 30 seconds, 59°C for 60 seconds, 72°C for 1 minute, then 40 cycles and final extension at 72°C for 7 minutes. All genotyping was performed at the same site on ABI 7500 real-time PCR (RT-PCR) system (Applied Biosystems, Foster City, CA).

2.3. Isolation and reverse transcription of total RNA

Total Ribonucleic acid (RNA) from RA peripheral blood mononuclear cells (PBMCs) was isolated using Trizol reagent (Invitrogen). The RNA extraction of 71 subjects was from the rest of 4-mL blood sample. The synthesis of complementary deoxyribonucleic acid about the procedure was performed under the following conditions: amplification was performed in a 20-μL reaction volume comprising 4-μL template RNA, 4-μL buffer, 1-μL RT Master Mix, 1-μL oligo dT primer, 1-μL random 6 mers, and 9-μL RNase-free water, and the conditions were as follows: 37°C for 15 minutes and 85°C for 5 seconds. RT-PCR was severely performed according to the gene protocol of Applied Biosystems Company. Products were sequenced.

Table 1

Age and gender of patients with RA and controls.

	Cases (n = 400)	Controls (n = 726)	*P*
Age, y			
Mean ± SD	44.9 ± 12.8	42.2 ± 10.8	0.622
Gender (n, %)			
Male	196 (49)	381 (52.5)	0.264
Female	204 (51)	345 (47.5)	

P value based on 2-sided χ^2 test.

2.4. Statistical analysis

A statistical analysis was carried out by the Statistical Package for Social Sciences 10.0 software (SPSS Inc.; 2000, Chicago). A chi-squared test or a Fisher exact test was used to compare genotype frequencies and each allele in patients and controls. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated using logistic regression analyses with adjustments for age and sex. Kolmogorov–Smirnov test was applied in cases that have a normal distribution. Abnormal distribution of continuous variables was analyzed by the Mann–Whitney *U* test. Pearson or Spearman correlation analysis was applied in our study. $P < 0.05$ was thought to be statistical significance. Hardy–-Weinberg equilibrium (HWE) test was conducted in both groups by using an exact chi-squared test. If the *P* value <0.05, the subjects were deviated from HWE.

3. Results

3.1. Characteristics of the study population

As shown in Table 1, age and gender are well matched between RA patients and controls. The frequency of 2 groups was appropriate on age and sex ($P = 0.622$ and 0.264, respectively).

3.2. Genotype distribution and gene frequency of rs2066853 between RA patients and controls

The genotype distributions of rs2066853 were in HWE in controls ($\chi^2 = 0.297$, $P = 0.586$), as well as in RA patients ($\chi^2 = 3.219$, $P = 0.073$). As shown in Table 2, AA, AG, and GG were expressed between patients and controls, and there were no significant differences exhibited in the allelic and the genotype frequencies of rs2066853 between them. The detailed results were shown in Table 2.

3.3. Prevalence of AHRR rs2292596 alleles and genotypes between RA patients and controls

The genotype distributions of rs2292596 on RA patients ($\chi^2 = 3.656$, $P = 0.056$) and healthy subjects ($\chi^2 = 2.400$, $P = 0.121$) were in HWE. As shown in Table 3, GG, GC, and CC were emerged in RA and RA-free cases. To SNP rs2292596, genotype frequencies for GG, CC, and GC were 33.3%, 14.0%, and 52.8% in the RA group and 15.0%, 34.0%, and 51.0% in the control group, respectively. By using 3×2 contingency table χ^2 test, we found an increased frequency of GG genotype in RA patients as compared with healthy controls ($P < 0.001$). Allelic frequencies for G and C were 59.6%, 40.4% and 40.5%, 59.5% in the RA and the control groups. Statistical differences were

Table 2

Allele and genotype frequencies of rs2066853 in RA patients and controls.

Polymorphism (rs2066853)	RA (n = 400) n, %	Control (n = 726) n, %	χ^2	*P*	OR (95% CI)
AA	35 (8.8)	73 (10)	1.433	0.488	NA
GG	173 (43.3)	330 (45.5)			
AG	192 (48)	323 (44.5)			
Allele					
A	262 (32.8)	469 (32.3)	0.048	0.827	1.021 (0.849–1.227)
G	538 (67.3)	983 (67.7)			

CI = confidence interval, NA = not available, OR = odds ratio, RA = rheumatoid arthritis.

Table 3

Allele and genotype frequencies of rs2292596 in RA patients and controls.

Polymorphism (rs2292596)	RA (n = 400) n, %	Control (n = 726) n, %	χ^2	*P*	OR (95% CI)
GG	133 (33.3)	109 (15)	78.488	<0.001*	NA
CC	56 (14.0)	247 (34)			
GC	211 (52.8)	370 (51)			
Allele					
G	477 (59.6)	588 (40.5)	75.721	<0.001*	2.170 (1.820–2.587)
C	323 (40.4)	864 (59.5)			

CI = confidence interval, NA = not available, OR = odds ratio, RA = rheumatoid arthritis.
* Statistically significant ($P < 0.05$).

Table 4

The correlation between the expression of AHR/AHRR mRNA and gene polymorphism.

Genotype	N	$2^{\Delta\Delta CT}$ M (P25, P75)	*P*
rs2066853			
AA	4	0.669 (0.224, 1.15)	
AG	38	0.766 (0.356, 1.396)	0.875
GG	29	0.558 (0.305, 1.133)	
rs2292596			
GG	27	2.434 (0.952, 5.664)	
GC	37	1.335 (0.613, 3.178)	0.222
CC	7	0.967 (0.416, 2.540)	

AHR = aryl hydrocarbon receptor, AHRR = aryl hydrocarbon receptor repressor, mRNA = messenger Ribonucleic acid, N = numbers of cases.

shown in the allelic distributions between them; a statistically significantly increased risk of RA was found to be associated with the G allele of rs2292596, and the OR was 2.170 (95% CI: 1.820–2.587; $P < 0.05$).

3.4. Association between the expression of AHR/AHRR gene polymorphism and AHR/AHRR mRNA expression among RA patients

There is no significant difference between genotypes of rs2066853/rs2292596 and messenger Ribonucleic acid (mRNA) expressions ($P > 0.05$). The detail was shown in Table 4.

3.5. Correlations between the expression of AHR and AHRR and clinical data

As shown in Table 5, no obvious correlations between the expression of AHR, AHRR at mRNA levels and clinical data ($P > 0.05$ for all) are detected.

4. Discussion

In the present RA case–control study, the major aim was to determine whether the SNPS of rs2066853 and rs2292596 were involved in the individual susceptibility to be RA patients. Thus, the associations of rs2066853 and rs2292596 in the *AHR* and *AHRR* genes with the risk of RA in Han Chinese populations

Table 5

Correlations between the expression of AHR, AHRR at mRNA levels and clinical data.

		CRP	ESR	RF	Anti-CCP	DAS28 score	Course
AHR mRNA	*r*	−0.126	−0.135	−0.151	0.048	−0.165	−0.329
	P	0.297	0.263	0.209	0.696	0.169	0.125
AHRR mRNA	*r*	−0.182	−0.143	−0.041	−0.218	−0.103	0.265
	P	0.129	0.236	0.735	0.068	0.391	0.222

AHR = aryl hydrocarbon receptor, AHRR = aryl hydrocarbon receptor repressor, Anti-CCP = anticyclic citrulline polypeptide, CRP = C-reactive protein, DAS28 = disease activity score in 28 joints, ESR = erythrocyte sedimentation rate, mRNA = messenger Ribonucleic acid.

were investigated by our group. We found that the variant genotypes of rs2292596 in AHRR were significantly associated with RA risk. To best of our knowledge, this is the first time that we performed this association between rs2292596 polymorphism and RA. Furthermore, the allele G of rs2292596 was found to be associated with RA that possibly increased individual susceptibilities. In addition, the relationship about the expression of AHR, AHRR and the polymorphism of rs2066853, rs2292596 were never focused on though no statistical significances were found between them in our experiment.

As far as we know, AHR ubiquitously existed in cells and tissues of humans and has progressed for a long time[10–12] such as the lung, the kidney, the liver, the placenta, the kidney, the skin, the spleen, cells, and so on and is thought to be a significant factor that regulates the development of many diseases. Previous literature[13] and our study have proved a fact that the higher levels of AHR and AHRR expression in RA patients than that in controls. Currently, a large number of studies have discussed the association of the genetic polymorphism of AHR and related diseases such as male infertility,[14,15] lung cancer,[16,17] non-Hodgkin lymphoma,[18] urinary 1-hydroxypyrene in polycyclic aromatic hydrocarbon-exposed workers,[19] and vitiligo.[20] In addition, we selected the rs2066853 in intron10 for it widely being studied in a list of diseases.[16,17,20] Going even further, the A allele of rs2066853 was found to be associated with lower AHR mRNA expression, which could account for certain individual susceptibilities.[21] However, no statistical relations between rs2266853 and the risk of RA were tested in our results, and what were consistent with the single-locus in some related diseases.[17,20,22]

Evidence of AHR activation was indicated by AHRR, and what based on the selected genetic polymorphisms of AHRR (rs2292596) in the aryl hydrocarbon receptor (AHR) signaling pathway which were significantly associated with insomnia.[23] GG, GC, and CC were expressed in RA cases and healthy controls of rs2292596. AHRR (rs2292596) CC genotype was found associated with micropenis and male infertility in humans.[24–26] Given these outcomes, we identify the links between rs22925596 and RA, that is, GG genotype was associated with the higher risk in RA and people with G allele of rs2292596 may increase the risk of RA when compared with C allele.

However, we failed to detect any correlation between mutation of rs2266853 and rs2292596 and the mRNA levels of AHR and AHRR expression in PBMCs of RA patients. At the same time, no significant association was observed between the expression of AHR, AHRR and clinical data. The reasons for this outcome are probably multifactorial. One of which, other susceptible polymorphisms in AHR and AHRR genotype may influence the expression of them under our consideration. The hypothesis needs further cohort studies of follow-up.

Some limitations are existed in our study and must be focused. First, sample size of this case–control study was relatively small, which may lead to the false-positive or false-negative results. Second, there was only 1 locus selected in the genotype of AHR and AHRR, respectively. Therefore, larger population and more SNPs about AHR and AHRR are needed to certify the findings.

In conclusion, we found strong evidence that the rs2292596 polymorphism is related to susceptibility of RA in Han Chinese populations, which may broaden our horizons in the etiology of RA and provide new insights into characterization of RA. However, larger studies and more genetic locus may be needed to elucidate these results in the future study. Moreover, the environmental risk factors of RA are little analyzed for the incomplete information. Furthermore, the mechanisms of these effects may need functional studies to clarify this issue.

5. Conclusion

Our observations suggested that genetic variation in AHRR rs2292596 might be related to RA patients, and the G allele of rs2292596 was associated with a dangerous effect on RA in Han Chinese populations. However, no significant association was observed between rs2066853/rs2292596 genotypes and the expression of AHR and AHRR in RA patients.

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