The Polymorphism of Osteopontin Gene and Receptors are associated with Allergic Rhinitis in a Chinese Han Population

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Abstract

Osteopontin (OPN) is a pro-inflammatory cytokine involved in chronic inflammatory diseases. In this study, we evaluated the potential association of OPN polymorphisms with Allergic Rhinitis (AR) in a Chinese Han population. Three single-nucleotide polymorphisms (SNPs) in OPN—rs9138, rs1126616, and rs1126772, and eight SNPs of its receptor—rs1448623, rs3770136, rs3738919, rs3911238, rs5918, rs2056131, rs187116, rs16927061 were genotyped in 1020 AR patients and 1173 healthy controls by using a PCR-restriction fragment length polymorphism assay. The allele, genotype, and haplotype frequencies in the patients and the controls were compared using a χ² test. Moreover, we performed haplotype analysis by using the online software platform SHEsis. The results revealed a significant association between five SNPs—rs9138, rs1126616, rs1126772, rs1449263, rs187116—and AR in the studied Chinese Han population. Furthermore, the ATG, CCG haplotype were associated with a significantly higher prevalence of AR than were the CTA haplotypes. However, no significant differences were detected in either the genotype or allele frequencies of the other SNPs between the AR and control groups. Overall, this study has identified a strong association between OPN polymorphisms and increased risk of AR in a Chinese Han population.

Keywords: OPN; Allergic rhinitis; SNP.

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa mediated by IgE, characterized by 4 major symptoms: nasal congestion, rhinorrhea, nasal itching, and sneezing. It affects 10-20% of the population worldwide [1]. For the prevalence of AR in China, it has increased markedly over the last 2 decades, especially in western regions [2]. For example, the overall incidence of AR in Chongqing in 2008 was estimated to be 32.3%, and is predicted to increase in the following years.

Currently, environment and gene are regarded as the main etiologic factors of AR. For environmental factors, pollens, house dust mite maybe the most popular allergens. While for genetic predisposition, single nucleotide polymorphisms (SNPs) in numerous genes may play an important role [3,4]. Genes such as IL-23R, MRPL4, and TNF-α have been reported relevant to the susceptibility to AR [5,6].

Osteopontin (OPN) is a Th1 cytokine with multiple functions in inflammation, immunity, and bone metabolism. Recent studies have reported that persistence of OPN expression may exacerbate autoimmune or inflammatory diseases such as multiple sclerosis (MS) [7], Crohn’s disease [8], systemic lupus erythematosus (SLE) [9] and rheumatoid arthritis (RA) [10]. In addition, genetic polymorphisms of OPN and its receptors were also reported to be associated with RA [11], MS [12,13], asthma [14,15,16], and SLE [17]. While whether they are involved in the development of AR is not yet known and were therefore the subject of the current study.
Materials and Methods

Ethics statement
This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. Written informed consent was obtained from all adult participants and from parents or legal guardians of minors.

Patients
From May 2011 to June 2014, 1020 AR patients aged 18 to 60 years were enrolled in this study. All patients were identified by and treated at the outpatient clinic of the Department of Otolaryngology at the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. The diagnosis of AR was based on the patients’ medical history, symptoms and the presence of a positive skin pricking test (SPT, Allergopharma, Hamburg, Germany) in response to a panel of common allergens defined by the ARIA 2008 guidelines [18]. The SPT results were diagnosed in accordance with the recommendations of the Subcommittee on Allergen Standardization and Skin Tests of the European Academy of Allergy and Clinical Immunology [19].

A positive SPT result was defined as the formation of a wheal larger than or equal to one half the diameter of the histamine control wheal, and at least 3 mm larger than the diameter of the negative control wheal. A total of 18 inhaled allergens were tested, including house dust, grass, tree, mold, food, and cat and dog dander et al. In contrast, patients with accompanying systemic disease were excluded from the study. A total of 1173 hereditarily-unrelated healthy individuals were enrolled as controls. They did not show clinical features or family history of allergies and had not experienced an upper respiratory tract infection within the 4 weeks prior to the study. All subjects of this study were of Chinese Han ethnic origin and from the Chongqing city of China.

SNP selection and geno typing
We studied 3 SNPs (rs9138, rs1126616, rs1126772, ) in human OPN gene, which have been documented to be associated with several immune diseases. Mean while, we also chose its receptors SNP sintegrina4 (ITGA4) - rs11149263 and rs3770136, integrin αv(ITGAV)- rs3738919 and rs3911238, integrin β3(ITGB3) -rs5918 and rs2056131, and CD44 -rs1871176 and rs16927061 for gene polymorphism analysis.

The 11 SNPs were genotyped by restriction fragment length polymorphism (RFLP) analysis. Briefly, peripheral blood samples were collected from each subject and stored at -80°C for further use. Genomic DNA was isolated from blood leukocytes using the Qiagen DNA Blood Mini kit (Qiagen, Valencia, CA, USA). Amplification of target DNA was performed by PCR using the primers listed in Table 2. PCR conditions were as follows: initial denaturation at 95°C for 5 min, 37 cycles of denaturation at 95°C for 30 s, annealing at 58-62°C for 30 s, and extension at 72°C for 30 s, and final extension at 72°C for 5 min. The PCR products were digested with specific restriction enzymes (Table 2) at 37°C for at least 4 h. Digestion products were visualized on a 4% agarose gel and stained with Goodview (SBS Gene tech, Beijing, China). Genotypes were confirmed by direct sequencing by the Invitrogen Biotechnology Company (Guangzhou, China) using 20% of randomly selected PCR products.

Statistical analysis
Call rates for the SNPs studied were compared between AR patients and controls by the χ2 test. The χ2 test was also applied to compare demographic characteristics and the allele and genotype frequencies between the patients and controls. Estimation of genotype frequencies was performed by direct counting. The online software platform SHE was used to analyze the haplotype and probabilities. We used non-risk alleles as the reference, and tested all the other haplotypes. Logistic regression analysis was used to analyze the genotype allele, controlling for age, gender, and occupation as the co variables. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the association of SNPs with the risk of AR. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (SPSS Inc., Chicago, Ill. USA). P values were corrected (Pc) for the number of alleles tested using the Bonferroni correction method. A Pc value of <0.05 was considered statistically significant.

Results

Subject characteristics
Demographic and clinical characteristics of the participants are presented in Table 1. The patient group consisted of 493 males and 527 females, with a mean age of 31.58 years (SD = 7). The control subjects included 593 males and 580 females and had a mean age of 33.23 years (SD = 8). There was no significant difference between the 2 groups with respect to mean age and gender distribution. About 51.8% of the patients were allergic to house dust mite, 18.1% to pollens, and 30.1% to mixed allergens.

Genetic polymorphisms of OPN and its receptors in AR
The genotype distributions of the 11examined SNPs in the OPN gene and its receptors were in Hardy-Weinberg equilibrium in both the AR and control groups. The call rate for each SNP was 100%. There were no statistically significant differences in the proportions of missing genotype data between cases and controls (P>0.05). The results of genotypic and allelic frequency analysis are shown in Table 3 and 4.

For OPN, A significantly increased frequency of the AC, AA genotype and A allele of rs9138 in AR patients (p=6.761×10^-3. OR=1.281, 95%CI 1.072-1.531; p=6.693×10^-7.OR=2.117, 95%CI 1.570-2.853; p=4.39×10^-2. OR=1.257, 95%CI 1.150-1.374) (Table 3) were identified.

The results also showed obvious differences between the AR patients and control patients concerning the frequencies of rs1126616. A higher frequency of the CC, CT genotype (p=1.391×10^-6. OR=2.111, 95%CI 1.566-2.846; p=1.705×10^-3) was observed.
OR = 1.337, 95% CI 1.118-1.598) and the C allele (p = 2.834 × 10^{-7}, OR = 1.260, 95% CI 1.376-1.53) were found to be AR patients compared with the controls.

Meanwhile, a marked difference of AG, GG genotype and G allele of rs1126772 were identified and a higher frequency can be observed in AR patients (p = 5.111 × 10^{-4}, OR = 1.442, 95% CI 1.263-2.121; p = 1.525 × 10^{-3}, OR = 1.260, 95% CI 1.163-1.36) and A allele (p = 4.868 × 10^{-5}, OR = 1.163, 95% CI 1.081-1.251).

When it comes to the receptor CD44, AR patients had a significantly increase in the frequencies of AA, TT genotype (p = 8.217 × 10^{-3}, OR = 1.289, 95% CI 1.070-1.552; p = 3.618 × 10^{-4}, OR = 1.594, 95% CI 1.376-2.066) and T allele (p = 1.831 × 10^{-4}, OR = 1.149, 95% CI 1.068-1.235) in rs187116. However, all other SNPs of OPN receptors did not display significant associations with AR (Table 4).

### Haplotype analysis of OPN allelic variants

A haplo type analysis was performed using the Haplview V3.32 program and the SHE siss online software platform. The eight possible haplotype frequencies are shown in Table 5. The ATG and CCG haplotypes accounted for 70% and 7.6% of the AR patients respectively, and these were significantly higher in the patients than in controls (P = 6.69 × 10^{-5}, OR = 1.700, 95% CI 1.307-2.213; P = 2.16 × 10^{-4}, OR = 1.594, 95% CI = 1.243-2.044 respectively). We also found that the CTA haplotypes were significantly less prevalent in AR patients (P = 5.00 × 10^{-15}, OR = 0.593, 95% CI = 0.523-0.673).

### Discussion

OPN, which was originally identified as a bone matrix protein, was subsequently identified as a cytokine (Eta-1) produced by activated T cells and transformed cells [20,21,22]. As a matrix cellular protein, Osteopontin (OPN) is expressed in many different cell types, including dendritic cells, T cells, B cells and so on [23]. As a cytokine, OPN can stimulate T cell proliferation, interfere with the differentiation of T cells into Th1 or Th2 and regulate immunoreactions [24,25,26]. Receptors of OPN include certain integrins [27,28,29] and CD44 variants [30,31,32]. With the potential to interact with multiple ubiquitously expressed cell surface receptors, OPN plays active roles in many physiological and pathological processes including wound healing, inflammation, immune response and so on.

So far, OPN has been identified as a biomarker for various types of inflammatory diseases and cancers [33,34] dysregulated and excessive expression of OPN have also been linked to some of the autoimmune disorders such as SLE [35], MS [36], RA [37], chronic obstructive pulmonary disease [38], and asthma [39]. It has been reported that OPN can stimulate the production of the Th1 cytokine interferon-γ and the Th17 cytokine IL-17 and inhibited the Th2 cytokine IL-10 [23,40].

In the present study, to decrease the influence of confounding factors on the results, we selected the AR patients and controls using strict guidelines and confirmed the genotyping results by direct sequencing. And we observed a novel association between SNPs of OPN and AR in the Chinese Han population. However, it is worth mentioning that there are some limitations to our study. First, we did not detect the protein level of OPN and receptors in the peripheral blood and perform a functional analysis study, so we could not draw a certain conclusion about the influence of these polymorphisms on the cytokine levels. Second, as it is well known that environmental factors are critical for the development of AR, further studies will be needed to clarify the genetic influence of OPN in AR pathogenesis, including gene-gene and gene-environment interactions.
In conclusion, this study provides further evidence of the association of OPN and receptors with AR, suggesting the possible involvement of these genes in the susceptibility to AR. This suggests that combining information from common risk polymorphisms could improve disease prediction. Further functional studies of genetic variants could contribute to an increased understanding of the roles of the studied genes in the pathogenesis of AR.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (81300811) and National Key Clinical Specialties Construction Program of China.

Author Contributions

Z.Z. and X.K. devised the concept, designed the experiment, and wrote the manuscript. Y.S. and H.K. performed the experiments and analyzed the data. S.H. supervised the project. All authors discussed the results and contributed in this manuscript.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

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