Evaluating gene × gene and gene × smoking interaction in rheumatoid arthritis using candidate genes in GAW15

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Abstract

We examined the potential gene × gene interactions and gene × smoking interactions in rheumatoid arthritis (RA) using the candidate gene data sets provided by Genetic Analysis Workshop 15 Problem 2. The multifactor dimensionality reduction (MDR) method was used to test gene × gene interactions among candidate genes. The case-only sample was used to test gene × smoking interactions. The best predictive model was the single-locus model with single-nucleotide polymorphism (SNP) rs2476601 in gene PTPN22. However, no clear gene × gene interaction was identified. Substantial departure from multiplicativity was observed between smoking and SNPs in genes CTLA4, PADI4, MIF, and SNPs on chromosome 5 and one haplotype of PTPN22. The strongest evidence of association was identified between the PTPN22 gene and RA status, which was consistently detected in single SNP association, gene × gene interaction and gene × smoking interaction analyses.

Background

Rheumatoid arthritis (RA) is a complex autoimmune disease. The etiology of the disease is not clearly understood. Risk factors of RA include genetic factors, race (Native American), female gender, obesity, old age, and smoking [1,2]. However, like most complex diseases, few studies of gene × gene interaction and gene × environmental interaction have been performed because a large sample size is required to identify such effects in traditional statistical paradigms. Logistic regression is commonly used in detecting interactive effects between genes or environmental factors in epidemiologic studies. However, the parameters cannot be accurately estimated when there are many independent variables while the sample size is not large enough [3]. Recently, Ritchie et al. [4] introduced a multifactor dimensionality reduction (MDR) method for identifying gene × gene interaction or gene × environmental interaction to overcome this limitation of traditional logistic regression [3-5]. This approach enumerates all possible combinations of genotype or environmental fac-
tors associated with high risk and low risk of disease, and it may enable us to find interactions between genes in the absence of main effects [3-5].

To detect potential epistasis in RA, we evaluated 1) disease associations using single SNPs (single-nucleotide polymorphisms) from 15 candidate genes and haplotypes of the PTPN22 gene, 2) gene × gene interactions among the candidate genes using the MDR method and logistic regression, and 3) gene × environmental (smoking) interactions using a case-only study design.

**Methods**

**Materials**
The data sets for the candidate gene studies of RA were provided by Genetic Analysis Workshop 15 (GAW15) Problem 2. There were two case-control data sets. The first one included 855 unrelated controls and 839 cases, as well as genotype data on 20 SNPs from 15 candidate genes, which were selected from previously published associations with RA or other autoimmune disorders by Plenge et al. [6]. The second data set included 1519 unrelated controls and 1393 cases, and genotype data on 14 SNPs from the PTPN22 gene. Additional phenotype data, including smoking history, age of onset, sex, and body mass index, were available for cases only in both data sets. There were 408 and 720 affected sibling pairs among cases in the two data sets, respectively.

**Statistical analysis**
Single SNP and haplotype (PTPN22 only) associations with disease status were first evaluated. To account for the dependency among family members, the generalized estimating equations methods (GEE1) [7] as implemented in the GENMOD procedure of SAS 9.0 was utilized in the association analysis by using family as the cluster factor, i.e., members from the same family were assumed to be correlated and those from different families were assumed to be independent. The haplotype block structure of PTPN22 was evaluated by Haploview [8]. Individual haplotypes were reconstructed using the PHASE 2.0 by assigning each haplotype with maximum probability [9]. Seventy-four percent of haplotype assignments had probabilities of 100% and 93% had probabilities of 80% or better. Individuals whose haplotype assignment had probability below 80% were excluded from subsequent analysis. Association analysis was carried out for each common haplotype in turn. For each haplotype, a dominant model was assumed, i.e., carriers of the particular haplotype versus non-carriers were compared for their RA status.

To test gene × gene interactions, MDR was used to determine the genetic model that could most successfully predict the disease status or phenotype from several loci. SNP rs2240340 on the PADI4 gene was excluded from analysis due to its large amount of missing data. One thousand three hundred and thirty case-control samples with completed marker data on 19 SNPs from 14 candidate genes were utilized in the MDR analysis. Cross-validation (CV) consistency and balanced accuracy estimates were calculated for each combination of a pool of genetic polymorphisms. The model with the highest accuracy and maximal CV was considered to be the best [5]. We determined statistical significance by comparing the accuracy of the observed data with the distribution of accuracy under the null hypothesis of no associations derived empirically from 1000 replicates of permutations [10]. The null hypothesis was rejected when the p-value derived from the permutation test was 0.05 or less. As a follow-up, logistic regression analysis was conducted if there was suggestive interaction.

We also examined the interaction between SNPs and smoking history in RA cases. The logistic function in the GENMOD procedure was used to quantify departure from multiplicativity. Odds ratios and 95% CIs were estimated. To adjust for multiple tests, empirical p-values were obtained from 1000 permutations. For the PTPN22 gene, interaction effects between PTPN22 haplotypes and smoking among cases were evaluated for RA status.

**Results**

1. **Single SNP and PTPN22 haplotype association**
Table 1 lists the association analysis results between disease and individual markers. One SNP from each of the five genes, HAVCRI, CTLA4, SUMO4, MAP3K7IP2, and PTPN22, were found significantly associated with RA.

Five common haplotypes of the PTPN22 with frequency >10% were constructed. Of the two haplotypes with significant associations with RA, one was a risk haplotype (11222221122221; 1: minor allele, 2: major allele; frequency: 11.6%), with a higher carrier frequency in cases than in controls (30.0% vs. 14.9%, p < 0.0001); whereas the other was protective (22122222222222; frequency: 10.9%), with a lower carrier frequency in cases than in controls (16.4% vs. 24.7%, p < 0.0001).

2. **Gene × gene interaction**
Table 2 lists the results from MDR. The one-locus model with SNP rs2476601 on gene PTPN22 had a maximum test accuracy (p = 0.004) and a maximum CV consistency of 10 out of 10, indicating that this was the best model. The second-best model was a two-locus model consisting of rs1248696 on the DLG5 gene and rs2476601 on PTPN22 (p = 0.013). The combination of rs1248696_22 and rs2476601_22 was associated with being in the low-risk group when compared to others (OR = 0.46, 95%CI: 0.36, 0.60). However, we could not confirm the interac-
Table 1: Association between SNPs and RA

| Candidate gene | SNP         | p-value (11 vs. 12 vs. 22) | p-value (11/12 vs. 22) |
|----------------|-------------|----------------------------|------------------------|
| HAVCRI        | 5509_5511delCAA | 0.066                      | 0.034*                 |
| HAVCRI        | rs6149307    | 0.189                      | 0.068                  |
| CTLA4         | CT60        | 0.016                      | 0.005                  |
| CARD15        | HugotSNP12ms3 |                           | 0.754                  |
| CARD15        | HugotSNP8ms2 | 0.838                      | 0.553                  |
| CARD15        | Hugot_SN13ms2 |                           | 0.695                  |
| Chr 5         | IGR2096ms1  | 0.473                      | 0.243                  |
| Chr 5         | IGR3084ms1  | 0.819                      | 0.713                  |
| Chr 5         | IGR3138ms1  | 0.861                      | 0.732                  |
| IL3           | rs31480     | 0.618                      | 0.384                  |
| SUMO4         | rs237025    | 0.0003                     | <0.0001                |
| MAP3K7IP2     | rs577001    | 0.002                      | 0.001                  |
| MIF           | rs755622    | 0.842                      | 0.979                  |
| TNFRFF1/b     | rs1061622   | 0.704                      | 0.684                  |
| DLG5          | rs1248696   | 0.269                      | 0.129                  |
| SLC22A4       | rs2073838   | 0.904                      | 0.771                  |
| PADI4         | rs2240340   | 0.574                      | 0.330                  |
| IL4           | rs2243250   | 0.311                      | 0.147                  |
| RUNX1         | rs2268277   | 0.55                       | 0.583                  |
| PTPN22        | rs2476601   | <0.0001                    | <0.0001                |

Allele “1” is the putative susceptibility allele, and allele “2” is the non-susceptibility allele.

*Bold text indicates p < 0.05.

Table 2: Multilocus interaction model for RA selected from MDR

| Model                     | Balanced accuracy | CV consistency | p-value |
|---------------------------|-------------------|----------------|---------|
| rs2476601 (PTPN22)        | 0.574             | 10/10          | 0.004*  |
| rs2476601 (PTPN22) rs1248696 (DLG5) | 0.5705            | 8/10           | 0.013   |
| rs2476601 (PTPN22) rs6149307 (HAVCRI) rs2243250 (IL4) | 0.5534            | 7/10           | 0.09    |
| rs2476601 (PTPN22) IGR2096ms1 (chr 5) rs237025 (SUMO4) rs2268277 (RUNX1) | 0.5243            | 6/10           | 0.475   |

*Bold text indicates p < 0.05.

Discussion

We explored gene × gene and gene × smoking interactions using the candidate gene data set provided by GAW15. The best predictive model for RA status is the single-locus model containing rs2476601 on gene PTPN22. SNP rs2476601 is a well known functional SNP that is associated with increased risk of RA. The best combination model selected by MDR consisted of rs2476601 on PTPN22 and rs1248696 on DLG5. However, the susceptibility interaction was not confirmed in the following logistic regression analysis. The possible reason for the inconsistent results is that in MDR, we actually did not test statistical interaction which was defined as 'deviation from multiplicativity' as in logistic regression. The signifi-
Table 3: Gene × smoking interactions

| Marker       | Haplotype | No   | Yes  | OR_{int} | 95%CI       | p-Value | Empirical p |
|--------------|-----------|------|------|----------|-------------|---------|-------------|
| CT60 (CTLA4) | 11        | 67   | 58   | 1.69     | 1.11, 2.55  | 0.025   | 0.023       |
|              | 12/22     | 289  | 393  | 2.76     | 1.24, 6.16  | 0.026   | 0.019       |
| rs2240340 (PADI4) | 11 | 40   | 29   | 0.59     | 0.36, 0.97  | 0.039   | 0.042       |
|              | 12/22     | 122  | 162  | 0.62     | 0.40, 0.96  | 0.033   | 0.04        |
| IGR3084ms1 (chr 5) | 11 | 23   | 47   | 0.73     | 0.53, 0.99  | 0.046   | 0.052       |
| IGR313Bms1 (chr 5) | 11 | 36   | 69   | 0.73     | 0.53, 0.99  | 0.046   | 0.052       |
| rs755622 (MIF) | 22        | 234  | 329  | 0.73     | 0.53, 0.99  | 0.046   | 0.052       |
|              | 11/12     | 120  | 128  | 0.73     | 0.53, 0.99  | 0.046   | 0.052       |

PTPN22 has been reported to be associated with RA [6,12]. In this study, we tested single gene association, gene × gene interactions and gene × smoking interactions using three different methods. In single SNP analysis, PTPN22 showed the strongest association with RA status \( p < 0.0001 \). In the following gene × gene interaction analyses by MDR, both the best single and the best combined models included PTPN22 gene. Furthermore, haplotype analysis using the second data set identified two haplotypes of the PTPN22 associated with RA and more importantly, there was a trend toward interaction between this gene and smoking. Therefore, the consistent findings here provide further evidence of the genetic involvement of PTPN22 in the etiology of RA.

Conclusion
In conclusion, our analyses confirmed the role of genetic and environmental factors in rheumatoid arthritis. Strong evidence of association was identified for the PTPN22 gene, which was observed in all three analyses. Other genes (HAVCRI, CTLA4, SUMO4, MAP3K7IP2, PADI4, chromosome 5 locus, MIF) may also contribute to the development of rheumatoid arthritis directly or within the context of smoking.

Competing interests
The author(s) declare that they have no competing interests.

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