Association between HLA-DQA1 gene copy number polymorphisms and susceptibility to rheumatoid arthritis in Chinese Han population

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Introduction

Rheumatoid arthritis (RA) is a multifactorial and systemic autoimmune disease that can lead to progressive joint destruction and disability. In addition to the contribution of infectious, hormonal and environmental factors, several lines of evidence have suggested that the disease has a genetic basis. The concordance rates for RA in monozygotic twins (15%) were higher than those in dizygotic twins (3.6%), and the heritability of RA was estimated to be around 50–60% (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000). Several susceptibility loci have been suggested, including the HLA-DRB1, PTPN22 and PADI4 genes (MacGregor et al. 2000).

So far, some CNVs of genes, such as chemokine ligand 3-like1 (CCL3L1) (Townson et al. 2002), Fc gamma receptor 3B (FCGR3B) (McKinney et al. 2008), pre-B lymphocyte1 (VPREB1) (Yim et al. 2011) and late cornified envelope (LCE) (Docampo et al. 2010) have been reported to be associated with RA. Human leucocyte antigen (HLA) is an important part of human immune system, participating in the process such as antigen processing and immune regulating. It is reported that the polymorphisms of HLA genes are associated with RA (Ruyssen-Witrand et al. 2012). HLA-DQA1 gene is a member of HLA system and has CNV but it has not been reported that CNV of HLA-DQA1 gene is associated with RA. The aim of this study was to explore HLA-DQA1 CNVs that potentially contribute to genetic susceptibility to RA.

Keywords. HLA-DQA1 gene; copy number polymorphism; rheumatoid arthritis.
Materials and methods

Subjects

For CNV analysis, 138 Chinese patients (30 men, 108 women and mean age 53.36 ± 13.74 years) were recruited from Shanghai and Taizhou Hospital, who were diagnosed with RA according to criteria of the American College of Rheumatology (http://www.rheumatology.org/). We also recruited 191 normal control subjects (111 men, 80 women and mean age 66.99 ± 5.208 years) who were free of RA based on the interviewer-administered questionnaires and clinical tests. All participants signed informed consent. The studies were approved by Ethics Committee of Fudan University.

Locating CNV regions with comparative genomic hybridization microarrays

Ten RA patients were examined by Agilent Human CGH Microarrays following the manufacturer’s protocol (http://www.geneskies.com/). Commercial genomic DNA (Promega, Madison, USA) was used as the internal controls. The data was extracted by Agilent Feature Extraction 10.7.3.1 and analysed by Agilent Workbench 7.0. Copy number (CN) gains or losses of at least five consecutive oligomers on the array were selected for further public data via UCSC Genome Browser (Iafrate et al. 2004) and our private Chinese CNV data (Lou et al. 2011). Genes present in these common aberrations regions (refer to as CNV regions, CNVRs) were identified using human genome browser at UCSC. The Database of Genomic Variants (http://projects.tcag.ca/variation/) was used to determine whether the highlighted CNVRs have been previously reported in the normal population.

Accucopy™ technology for CNV validation

Accucopy™ technology was used to validate the specific CNVs identified from the array-based CGH. Briefly, genomic DNA of each subject was mixed with fluorescently-labelled specific primers, PCR Master mix and a competitive DNA with known CN for a multiple competitive real-time PCR reaction. The PCR products were diluted and then were loaded on an ABI3730XL sequencer for quantification analysis (http://www.home.agilent.com/agilent/home.jspx?&cc=CN&lc=chi). Raw data were analysed by GeneMapper 4.0 (http://www.appliedbiosystems.com/absite/us/en/home/support/software/dna-sequencing/genermapper.html). The peak ratio between sample DNA and corresponding competitive DNA (S/C) was calculated and normalized to the median of four preset two-copy reference genes, respectively. Two normalized S/C ratios were further normalized to the median value in all samples for each reference gene and the averaged. The CN of each target fragment was determined by the average S/C ratio times two. Cases and controls were examined and read at the same time to minimize nonrandom errors.

Association analysis

Distribution of CNs among patients and controls after CN assignment according to the predefined threshold were compared using chi-squared test for trend in proportions, using R (Wang et al. 2013). Logistic regression models were constructed to determine the odds ratio (OR) and confidence intervals (CI) in the condition of adjusting for gender using SPSS (ver. 17.0 SPSS Inc., 2008). Thresholds for deletions and duplications were set at below 0.75 and above 1.25, respectively, in above CNV validation assays according to the manufacture’s instruction. All samples were tested in duplicate.

Results

Locating CNV regions of HLA-DQA1 with comparative genomic hybridization microarrays

Ten RA healthy controls were examined by Agilent Human CGH Microarrays. CN gains or losses of at least five consecutive oligomers on the array were selected (see figure 1). So there exist CNVs of HLA-DQA1 in Chinese population, located between 32608277–32620568 kb of chromosome 6 (6p21.3).

Analysis of CNV and RA

Specific probes were designed for validation assays with the AccuCopy™ technology. The cohort was examined in validation assays and showed that the CN distributions of

Figure 1. Results of CNVs of HLA-DQA1 with a CGH. Each dot represented a probe, green dot means deletions of CNV.
**Table 1.** CN at HLA-DQA1 in RA patients and controls.

<table>
<thead>
<tr>
<th>HLA-DQA1 CN</th>
<th>Control</th>
<th>Case</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>26</td>
<td>11</td>
<td>0.009</td>
<td>0.135 (0.035–0.516)</td>
</tr>
<tr>
<td>=2</td>
<td>159</td>
<td>115</td>
<td></td>
<td>0.337 (0.131–0.867)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

CNV has been recently acknowledged as a rich source of human genetic diversity, including disease susceptibility (Estivill and Armengol 2007). Although not all the potential associations have been successfully replicated, the evidence suggests that the CNV of dosage-sensitive genes can contribute to diverse complex diseases, including RA (Estivill and Armengol 2007; McCarron and Altshuler 2007; Schaschel et al. 2009). In RA, CNVs in the CCL3LI, FCGR3B, LCE3C and LCE3B genes were reported to have an influence on susceptibility, and several lines of evidence suggest the existence of additional CNVs associated with the susceptibility and/or pathogenesis of RA (Docampo et al. 2010; Mamtani et al. 2010; McKinney and Merriman 2012).

The HLA-DQA1 is a HLA class II gene encoding an alpha chain of HLA-DQ molecule, along with a beta chain (DQB) to form a heterodimer anchored in membrane of antigen presenting cells (APC). Like other HLA class II molecules, HLA-DQ plays central role in immune response to foreign antigens by presenting specific antigenic peptides to T cells. Genetic variations including sequence and CN of HLA genes contribute to enhance the recognition repertoire of the immune system, as well as to wide range of disease susceptibility. Specific alleles and gene-dosage of HLA-DQA1 have been associated with celiac disease (Docampo et al. 2010) and type 1 diabetes (Britten et al. 2009).

In this study, we explored the association between the RA and CNV of the HLA-DQA1 gene located at 6p21.3, a region that has been suggested to be associated with several immunologic disorders (Murray et al. 2007; Chai et al. 2013; LeishGEN Consortium et al. 2013). We found that the proportion of the individuals with >2 copies of the HLA-DQA1 gene was significantly higher in the RA patients, but that with ≤2 copies was significantly lower in the patients than in the control group, suggesting that >2 copies of the HLA-DQA1 gene may be a risk factor to RA.

**In conclusion,** our findings clearly support that CNV of the HLA-DQA1 are associated with susceptibility to RA in Chinese Han population. To our knowledge, this is first report in studies of CNVs in RA in Chinese Han population. More studies based on larger sample size, case–control design, and stratification by ethnic and clinical outcomes are still needed for future research, and further functional studies of the CNV of the HLA-DQA1 will be necessary.

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**References**


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