RESEARCH NOTE

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 *HLADRB1*, *PTPN22* and *PADI4* genes (Yamada and Yamamoto 2007; Imboden 2009). Recent genomewide association studies (GWAS) have also led to the putative association of genetic variants including *TRAF1-C5*, *CD28*, *PRDM1*, *D2/CD58*, *PTPRC* and *FCGR2A* with RA susceptibility (Plenge *et al.* 2007; Raychaudhuri *et al.* 2009; Sirota *et al.* 2009).

Recently, copy number variation (CNV) in the human genome is increasingly recognized to also play important roles in trait heritability. CNV is another class of genetic variation that alters quantity of gene rather than the DNA sequence. CNVs whose allele frequency is greater than 1% are regarded as copy number polymorphisms (CNPs). Common CNVs include deletion, duplication and insertion that occur in about 15% of the human genome (MacGregor *et al.* 2000). Specific CNVs may explain a proportion of disease risk in addition to sequence variations, which has been reported in a number of human complex diseases, such as systemic lupus erythematosus (SLE) (Fanciulli *et al.* 2007), type 1 diabetes (T1D) (Grayson *et al.* 2010), human immunodeficiency virus (HIV) acquisition and progression (Shostakovich-Koretskaya *et al.* 2009) and Alzheimer disease (Swaminathan *et al.* 2011).

So far, some CNVs of genes, such as chemokine ligand 3-like-1 (*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.

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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 CNVs 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^{TM} technology for CNV validation

Accucopy^{TM} technology was used to validate the specific CNVs identified from the array-based CGH. Briefly, genomic DNA of each subject was mixed with fluorescence-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 Gene Mapper 4.0 (http://www.appliedbiosystems.com/absite/us/en/home/support/software/dna-sequencing/genemapper.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^{TM} technology. The cohort was examined in validation assays and showed that the CN distributions of

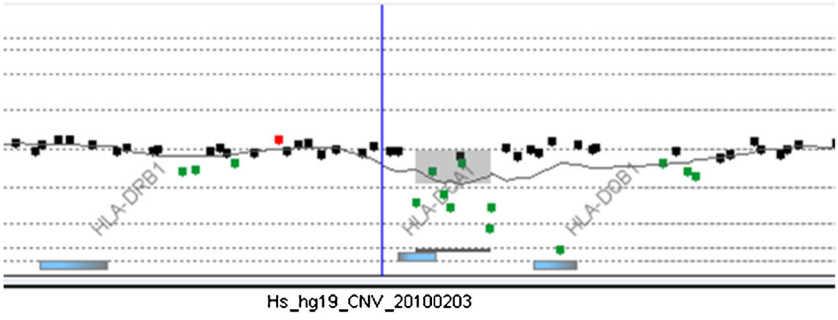

Figure 1. Results of CNVs of *HLA-DQA1* with a CGH. Each dot represented a probe, green dot means deletions of CNV.

Table 1. CN at HLA-DQAl in RA patients and controls.

HLA-DQA1 CN	Control	Case	*P*	OR (95% CI)
<2	26	11	0.009	0.135 (0.035–0.516)
=2	159	115		0.337 (0.131–0.867)
>2	6	12		1

HLA-DQA1 alleles among the RA patients and controls are significant by chi-squared test for trend proportions with *P* values of 0.009. In particular, higher CN (>2) of the *HLA-DQA1* allele were presented more in RA patients compared to the controls (table 1). Logistic regression analysis with gender as covariates showed that low CN (<2) and normal CN (=2) of *HLA-DQA1* appeared to be protective from RA (OR = 0.135, 95% CI = 0.035–0.516; OR = 0.337, 95%CI = 0.131–0.867, respectively), on the other hand, CN higher than 2 may be a potential risk for RA.

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; McCarroll and Altshuler 2007; Schaschl *et al.* 2009). In RA, CNVs in the *CCL3L1*, *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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