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Population-dependent contribution of the major histocompatibility complex region to schizophrenia susceptibility

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ABSTRACT

There is consistent data from European cohorts suggesting a genetic contribution from the major histocompatibility complex (MHC) to the pathogenesis of schizophrenia. However, the genomic complexity and ethnicity-specific diversity found in the MHC cause difficulties in identifying causal variants or genes, and there is a need for studies encompassing the entire MHC region in multiple ethnic populations.

Here, we report on association signals in the MHC region, with schizophrenia in the Japanese population. We genotyped and imputed a total of 10,131 single nucleotide polymorphisms (SNPs), spanning the entire MHC interval. The analysis included 3302 participants (1518 schizophrenics and 1784 healthy controls) from the Japanese population. In this study, we present evidence for association at rs494620, located in the *SLC44A4* gene. The association survived after correction for multiple testing (unadjusted $P = 7.78 \times 10^{-5}$, empirical $P = 0.0357$). The imputation results detected the highest association at rs707937 in the *MSH5-SAPCD1* gene (imputed $P = 8.40 \times 10^{-5}$). In expression analysis using postmortem brains from schizophrenia and control samples, *MSH5-SAPCD1* showed marginally significant expression differences in Brodmann's area 46 ($P = 0.044$ by unpaired t test with Welch's correction, $P = 0.099$ by Mann–Whitney U test). Our study further strengthens evidence for the involvement of the MHC in schizophrenia across populations, and provides insight into population-specific mechanisms for the MHC region in schizophrenia susceptibility.

1. Introduction

Recent genome-wide association studies (GWASs) have provided evidence for the association of the major histocompatibility complex (MHC) region with schizophrenia (International Schizophrenia, C., et al., 2009; Shi et al., 2009; Stefansson et al., 2009). The MHC, located at chromosome 6p21–6p22, encodes human leukocyte antigen (HLA) genes, which are integral to immune and neural function. Although the MHC has been implicated in schizophrenia genetic studies even from pre-GWAS era (for review see Wang et al., 2006), identifying causal variants of specific genes has been hampered by the high levels of genomic variation and the extensive linkage disequilibrium (LD) patterns present in this region.

In addition to the genomic complexity found in the MHC, ethnicity-specific diversity also causes problems in identifying causal variants or genes. To date, most reported genetic studies of schizophrenia focusing on the MHC region, including GWAS, have been conducted in populations of European ancestry. Prior studies reported signals associated with schizophrenia distributed in a broad region from 6p21.3 to 6p22.1, spanning a genomic interval of 27.2 Mb to 32.7 Mb, and containing more than 250 genes. The strongest evidence for association is observed in and near a cluster of histone-coding genes (International Schizophrenia, C., et al., 2009; Shi et al., 2009; Stefansson et al., 2009) in populations consisting of mainly Caucasian subjects. In the Japanese population, GWAS and a follow-up study show genetic association of a SNP (rs2071287) in the *NOTCH4* gene, located in the HLA class II region (32.16 to 32.19 Mb) with schizophrenia (Ikeda et al., 2011, 2013). However, no study has addressed MHC signals across the region and their association with this disease.

In this study, to refine the complex association signals from the MHC region in Japanese subjects, we genotyped and imputed a total of 10,131 SNPs spanning the MHC loci.

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2. Methods

2.1. Subjects

This study examined samples from 3302 participants whose SNP data passed quality control filters. The dataset consisted of Japanese cohorts comprising 1518 subjects with schizophrenia (827 men, 691 women; mean age 50.6 ± 13.8 years) and 1784 healthy controls (641 men, 1143 women; mean age 43.5 ± 14.1 years).

The schizophrenia subjects were recruited from the mid-area of Honshu, the main island of Japan. All samples were of Japanese origin. Our previous studies using a subset of the same participants showed that populations in the area belong to a single genetic cluster (Hattori et al., 2009; Yamada et al., 2006, 2004). These data indicate that the effect of population stratification is negligible in our Japanese samples. All participants were interviewed by two board-certified psychiatrists. Diagnoses were made using DSM-IV criteria, with all available information.

The present experiments on our participants were conducted in accordance with the Declaration of Helsinki. After the study procedures had been fully explained, written informed consent was obtained from all participants. The study was approved by the Ethics Committees of RIKEN and all participating institutes.

2.2. Selection of SNPs and genotyping

Genomic DNA was extracted from whole blood using standard methods. All samples were genotyped on the Illumina BeadXpress platform (http://www.illumina.com). The custom SNP chips for the Illumina Golden Gate assay were designed using the following criteria: 1) region-wide tagSNPs were chosen using the LDSelect program developed by Carlson et al. (2004). In this tagging procedure, the r^2 threshold was set to 0.80, 2) HLA tagSNPs were derived from phased-haplotype data by de Bakker et al. (2006) (http://www.inflammgen.org/inflammgen/files/data/JPT_phased.emppp.txt), and 3) significant SNPs from a meta-analysis of previous studies (International Schizophrenia, C., et al., 2009; Shi et al., 2009; Stefansson et al., 2009).

The resulting set of 767 SNPs covering the MHC region from 25.9 Mb to 33.8 Mb (UCSC Genome Database, GRCh37/hg19) was used in genotyping. Three SNPs were genotyped using the TaqMan SNP Genotyping Assay on a 7900HT Sequence Detection System (Applied Biosystems), as duplicates for the QC procedure.

2.3. Real-time quantitative reverse transcription PCR (real-time qRT-PCR)

Postmortem brain tissue from dorsolateral prefrontal cortex (Brodmann's area 46, BA46) and hippocampal CA1 regions was obtained from the Maryland Brain Collection (http://www.mprc.umaryland.edu/mbc.asp). Schizophrenia ($n = 35$ for BA46; $n = 20$ for CA1) and control ($n = 35$ for BA46; $n = 20$ for CA1) samples were age-matched, and there were no significant demographic differences in terms of postmortem interval and sample pH. Details of sample information are presented elsewhere (Balan et al., 2014; Maekawa et al., 2014). Total RNA was extracted using a miRNeasy Mini kit (QIAGEN GmbH, Hilden, Germany). Single stranded cDNA was synthesized using a SuperScript VILO cDNA synthesis kit. TaqMan probes were obtained from TaqMan™ Gene Expression Assays products (Applied Biosystems) (Supplementary Table S2). Real-time qRT-PCR analysis was conducted using an ABI7900HT Fast Real-Time PCR System (Applied Biosystems, Grand Island, NY, USA). Real-time qRT-PCR data was captured using SDS v2.4 (Applied Biosystems). The concentration of target molecules was calculated relative to the *GAPDH* gene. We previously confirmed that the level of *GAPDH* was linearly correlated with those of other house-keeping genes, *ACTB* and *PKG1* in human postmortem brain samples (unpublished data). All reactions were performed in triplicate, based on a standard curve method.

2.4. Statistical analysis

We performed case/control association analysis implemented in the PLINK program (http://pngu.mgh.harvard.edu/~purcell/plink/) for samples and SNPs that passed quality control filtering. Conditional analysis was conducted using PLINK to test for the independence of top SNPs. GWAMA (Genome-Wide Association Meta Analysis) software was used to perform meta-analysis of the previous GWASs and the present study (http://www.well.ox.ac.uk/gwama/) (Magi and Morris, 2010). The confidence interval (CI) was calculated using statistical procedure when it was not given (Altman and Bland, 2011).

In real-time qRT-PCR experiments, after removing outliers greater than 1.5 times the inter-quartile range (IQR), unpaired *t* test with Welch's correction (two-tailed) and the Mann–Whitney *U* test (two-tailed) were used to detect significant changes in transcript expression levels for each gene.

2.5. SNP imputation

The genotypes were imputed using HapMap 2 (release 23) data for Japanese and Chinese populations as a reference (http://hapmap.ncbi.nlm.nih.gov/), and using the PLINK program. Allele-based association tests were performed on the 10,131 imputed SNPs. LocusZoom (http://csg.sph.umich.edu/locuszoom/) was used to plot regional association results.

3. Results

3.1. Genotyping

We genotyped 767 SNPs from the MHC region. In our genotyping process, 602 SNPs remained after applying quality control (QC) criteria (SNP call rate > 90%, sample call rate > 95%). A total of 165 SNPs failed to pass QC. It is likely that the highly polymorphic nature of the MHC region may be responsible for the high genotyping failure rate.

3.2. SNP associations

Of the 602 properly genotyped SNPs, the highest association with schizophrenia was obtained for rs494620 (unadjusted $P = 7.78 \times 10^{-5}$, empirical $P = 0.0357$ by 100,000 permutations) (Table 1 and Fig. 1). This SNP is a synonymous polymorphism in the *SLC44A5* (choline transporter-like protein) gene (Tyr→Tyr). Testing 10,131 imputed SNPs, rs707937 in the *MSH5-SAPCD1* gene showed the strongest association (imputed $P = 8.40 \times 10^{-5}$) (Table 2 and Fig. 1) with disease.

3.3. HLA tagSNPs

The tagSNPs for HLA haplotyping were selected according to a previous report (de Bakker et al., 2006). We were unable to obtain enough information to positively identify common HLA alleles associated with schizophrenia, due to a relatively high proportion of genotyping fails for HLA tagSNPs (Supplementary Table S1). In the evaluation of successfully genotyped HLA tagSNPs, corrections for multiple testing were not applied. We found significant associations (unadjusted $P < 0.01$) for the following SNPs: rs660550 from the *SLC44A4* gene located in HLA-C*0103 ($P = 0.00221$), rs2523653 from HLA-C*1403 ($P = 0.00574$), rs4569 from the *CSNK2B* gene and rs2294881 from the *BTNL2* gene located in HLA-DRB*1201 ($P = 0.00671$ and $P = 0.000468$, respectively), and rs4151657 from the *CFB* gene in HLA-DRB*1401 ($P = 0.00124$) (Supplementary Table S1).