Research article

An evaluation of association between common variants in C4BPB/C4BPA genes and schizophrenia

Shuichong Wang a, Houquan Lu b, Jianliang Ni a, Jiatao Zhang a, Wenxin Tang c, Wei Hong Lu d, Jun Cai d, Chen Zhang d,a

a Tongde Hospital of Zhejiang Province, Zhejiang, China
b The People’s Hospital of Changxing County, Zhejiang, China
c Hangzhou Seventh People’s Hospital, Zhejiang, China
d Schizophrenia Program, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China

HIGHLIGHTS

• This is the first genetic study to address the association of C4BPB and C4BPA with schizophrenia.
• Nine C4BPB/C4BPA polymorphisms were analyzed in Han Chinese with and without schizophrenia.
• Our results provided preliminary evidence that C4BPB/C4BPA may not confer susceptibility to schizophrenia among Han Chinese.

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ABSTRACT

Epidemiological studies have indicated that both maternal bacterial and viral infections during pregnancy increase the risk of schizophrenia among offspring, but to date there is not clear explanation for this increased risk. Previously, the decreased C4b-binding protein (C4BP), a potent circulating soluble inhibitor of the classical and lectin pathways of complement, was reported to be associated with risk of schizophrenia. Here, we analyzed 4 common single nucleotide polymorphisms (SNPs) of C4BPB and 5 SNPs of C4BPA in a group of 556 schizophrenia patients and a matched group of 610 healthy controls to see if the genes C4BPB and C4BPA, which encode C4BP, may confer a susceptibility to schizophrenia. Comparing the genotype and allele frequencies of those SNPs between cases and controls, we found no association between the C4BPB/C4BPA variants and schizophrenia. Our results provided preliminary evidence that C4BPB/C4BPA may not confer susceptibility to schizophrenia among Han Chinese. Further genetic studies from large-scale population are required to obtain more conclusive results.

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1. Introduction

Schizophrenia is a severe and chronic mental disorder that affects approximately 1% of the worldwide population. However, its etiology remains largely unknown despite decades of search. To date, several epidemiological studies have indicated that both maternal bacterial and viral infections during pregnancy increase an offspring’s risk of schizophrenia [2], but precisely why these infections seem to act as a risk factor are unclear. However, a growing body of evidence suggests that schizophrenia and certain autoimmune diseases may share some key clinical, epidemiological and genetic features [1]. Early genetic studies reported an association between schizophrenia and chromosome 6p22–24, which includes the human major histocompatibility complex (MHC) region [17], while recent genome-wide association studies (GWASs) have identified several genes within the extended MHC region as a susceptibility locus for schizophrenia in individuals from European [15,18,22], Japanese [6] and Chinese [29,34] populations. These different lines of evidence suggest that, potentially, immune abnormalities play some unknown role in the etiology of schizophrenia [25], increasing the susceptibility for schizophrenia in individuals with genetically altered immune functions [13].

Complement is an integral part of the human immune system that serves to protecting the body against the invasion and proliferation of various microorganisms [24]. Accordingly, some researchers have speculated that deficiencies in the complement classical pathway may be involved in the autoimmune pathomechanisms and aberrant programmed cell death that possibly
contribute to progression of schizophrenia [5]. Within the complement system, the complement protein C4 is a non-enzymatic component that is proteolytically activated when complement is activated via the classical or lectin activation pathways. The products of this activation are C4b, an opsonin that binds covalently to the complement activating target. Some evidence has indicated that dysfunction of C4b may be involved in the alterations of the innate and adaptive immune systems in schizophrenia [10,14].

The C4b-binding protein (C4BP)—a large plasma protein consisting of seven identical α-chains and a unique β-chain encoded by two separate genes, C4BPA and C4BPA—controls the classical and lectin pathways and acts as a cofactor for the serine proteinase factor I in degradation of the activated complement components C4b/C3b and enhances decay of complement convertases [12]. In human, C4BP acts as an important regulator of the complement system, strongly binding to apoptotic and necrotic cells, and limiting complement activation on the cells [26,27]. Both C4BPA and C4BPA are located at chromosome 1q32 [16], which has been reported to be a genetic schizophrenia-susceptibility region in East Asian population [7]. Building on this premise, we hypothesized that C4BPA and C4BPA may be promising candidate genes for schizophrenia susceptibility in East Asians. To the best of our knowledge, no genetic study to date has addressed the association of C4BPA and C4BPA with schizophrenia, so here we aimed to explore whether or not common variants with C4BPA and C4BPA confer some risk of susceptibility to schizophrenia among a Han Chinese population.

2. Material and methods

2.1. Subjects

For the current study, 556 unrelated schizophrenia patients (291 males and 265 females; mean age of 31.4, SD = 5.7) were recruited. All patients were either inpatients and outpatients from Shanghai Mental Health Center, and included on the basis of previously established criteria [23,28,31]: (1) patients met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for schizophrenia and the diagnoses were made on the Structured Clinical Interview for DSM-IV-TR Axis I Disorders; (2) patients were not first-episode patients, because initial diagnoses are often unreliable; and (3) patients had no evidence of any physical disease or any psychiatric disorder aside from schizophrenia. Prior to analysis, all diagnosis and review of psychiatric case records were independently checked and verified by two senior psychiatrists. The schizophrenia patients were matched with 610 control subjects (324 males and 286 females; mean age of 32.9, SD = 6.1) enrolled from the hospital staff and students of the School of Medicine in Shanghai, all of which were interviewed by a specialized psychiatrist using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Patient Edition (SCID-P) to determine that they had no psychiatric disorders [30,32]. Any healthy controls found to have any psychiatric disorder or chronic physical disease were excluded from this analysis. There was no significant difference between the case and control groups with respect to the gender and age. All subjects in both the patient and control group were of Han Chinese origin.

All procedures for this study were reviewed and approved by the Institutional Review Boards of the Shanghai Mental Health Center and other participating institutions. This study was performed in strict accordance with the Declaration of Helsinki and other relevant national and international regulations. Written informed consent was obtained from each participant prior to any procedures related to this study being performed.

2.2. SNP selection and genotyping

The principal hypothesis underlying this study was that common single nucleotide polymorphisms (SNPs) in C4BPA and C4BPA may potentially confer susceptibility to schizophrenia. To set inclusion criteria for tagging SNPs, we retrieved CHB data from the HapMap database (http://www.hapmap.org) and defined linkage disequilibrium (LD) blocks using Haploview 4.2 (Broad Institute, Cambridge, MA, USA). Haplotype-tagging SNPs (hSNPs) were selected with r^2 cutoff > 0.8, minor allele frequency (MAF) > 0.1. In total, three tag SNPs of C4BPA and three tag SNPs of C4BPA were selected for genotyping; three C4BPA/C4BPA protein-associated variants [3] were also examined. Genomic DNA of all participants was extracted from peripheral blood using a Tangen DNA Isolation Kit (Tiangen Biotech, Beijing, China). All nine SNPs were amplified independently via polymerase chain reaction (PCR) and then genotyped via direct sequencing on an ABI PRISM 3730 Genetic Analyzer (PerkinElmer Applied Biosystems). Genotyping was carried out according to the methods described in our previous studies [33,35]. PCR amplification was performed in a volume of 25 μL containing primer pair for each SNP. PCR primers were also used for sequencing. Sequencing results were handled using DNASTar package (DNA Star Inc, USA), and the original sequencing chromatograms of each sample were then manually checked.

2.3. Statistical analysis

Hardy–Weinberg equilibrium testing, and allele and genotype frequency analyses were conducted using SHeSi (http://analysis.bio-x.cn). Pairwise linkage disequilibrium of all pairs of hSNPs was performed using Haploview 4.2 (Broad Institute, Cambridge, MA, USA), and the extent of linkage disequilibrium (LD) was measured by the standardized D' and r^2. To adjust for multiple testing, the level of significance was corrected via Bonferroni correction. Power calculations were carried out using Quanto 1.2.3 (http://hydra.usc.edu/GxE).

### Table 1

<table>
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<th>SNP ID</th>
<th>Genotype</th>
<th>Number of samples</th>
<th>P-value</th>
<th>P-value</th>
<th>Allele</th>
<th>Number of samples</th>
<th>P-value</th>
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* Raw P-values.

** P-values were calculated after Bonferroni correction.

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3. Results

Two non-polymorphic SNPs (rs8942 and rs3813948) that deviated from Hardy–Weinberg equilibrium were excluded for further analysis. Among the remaining 7 SNPs, no deviation from the Hardy–Weinberg equilibrium was observed in genotype distribution. Comparing the genotype and allele frequencies of the 7 SNPs between schizophrenia patients and control subjects showed no significant difference (Table 1). After calculating LD for all pairs of SNPs, we found a low LD in either C4BPB or C4BPA (Supplementary Figure S1), indicating that no specific haplotype block could be identified. With false positive rate controlled at 0.05, the statistical power to detect the odds ratio (OR) value as 1.5 for risk allele was expected to be more than 90% in our samples under a log additive model.

4. Discussion

Previously, abnormalities in the complement cascade or biosynthesis have been frequently observed in schizophrenia [11]. Mayilayan et al. [10] found that the level of C4BP—which controls classical and lectin pathways and activates apoptotic and necrotic cells clearance after injury—was significantly decreased among Armenian schizophrenia patients as compared with matched healthy controls. More recently, a proteomics analysis of an Asian population observed a similarly decreased expression of C4BPB among patients with schizophrenia [8]. Alongside several other lines of evidence, these findings have suggested that C4BPB may be involved in the alternation of innate and adaptive immune systems in schizophrenia. However, there was no available data to investigate whether C4BP is an etiological factor for the development of schizophrenia.

In this case-control study, our genetic data did not support the involvement of C4BP in the pathogenesis of this disorder, at least in Han Chinese population. Here, we examined 9 SNPs evenly distributed across C4BPB and C4BPA in 1166 samples taken from 556 schizophrenia patients and 610 matched healthy controls. In total, 7 of the 9 tested SNPs (rs12711513 and rs6969037 within C4BPB, rs4844573, rs11120218, rs2808470, rs2842704 and rs1126618 within C4BPA) were in Hardy–Weinberg equilibrium. Following analyses of these SNPs, we did not observe any evidence suggesting a positive association between the SNPs within C4BPB/C4BPA and schizophrenia.

Our present findings are consistent with two independent GWAS analyses in Chinese Han populations, in which both indicated that there was no observable association between the C4BP and schizophrenia [19,29]. Curiously though, the extended MHC region was reported to be associated with schizophrenia in Chinese Han population, and a number of schizophrenia–related genes including MICB [20], HLA-A and HLA-B [21] are classic MHC molecules that play central roles in the development of host defense and immunity. Since multiple genes—especially in the same pathway or having similar functions—may interact in a non-linear mode to influence susceptibility [9], better understanding of gene–gene interactions may be a more sensitive method of characterizing the effects of candidate genes on schizophrenia [4]. That said, addressing interactive effect of C4BP gene with SNPs in the MHC region may help to clarify the role of C4BP in the development of schizophrenia.

When interpreting the present results, we would be remiss in not noting several limitations. First, we did not examine the schizophrenia–related SNPs in the MHC region observed in GWAS. Second, the lack of a significant association may be caused by the modest sample size, possibly resulting in a type II error. Third, this study was designed based on the “Common Disease–Common Variant” hypothesis, and we did not sequence the genes to assess the influence of more rare variant(s) on schizophrenia. Future targeted deep sequencing may help to uncover fundamental characteristics of pathogenic C4BPB/C4BPA mutations and any potential association with schizophrenia. Lastly, case-control association analyses always have the potential for population stratification. Although the subjects were all of Chinese origin, we could not completely exclude the possibility of a population structure effect in our sample. On the whole, follow-up studies with a larger sample size or using a different methodology may help to find somewhat different results, again prompting further investigations into the connection between the C4BPB/C4BPA and schizophrenia.

In conclusion, our results provided preliminary evidence that C4BPB/C4BPA may not confer susceptibility to schizophrenia among Han Chinese. Further genetic studies from large-scale population are required to obtain more conclusive results.

Conflict of interest

The authors have no conflict of interest to disclose.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2015.02.005.

References


