Molecular evolution in the CREB1 signal pathway and a rare haplotype in CREB1 with genetic predisposition to schizophrenia

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ABSTRACT

CREB1 is a cAMP responsive transcriptional factor which plays a key role in neural development. CREB1 signal pathway (CSP) has been implicated repeatedly in studies of predisposition for schizophrenia. We speculated that CSP has undergone positive selection during evolution of modern human and some genes that have undergone natural selection in the past may predispose to schizophrenia (SCZ) in modern time. Positive selection and association analysis were employed to explore the molecular evolution of CSP and association with schizophrenia. Our results showed a pan-ethnic selection event on CSP. Positive selection and association analysis were employed to explore the molecular evolution of CSP and association with schizophrenia. Our results showed a pan-ethnic selection event on CREB1 and the CSP, as confirmed in all 14 ethnic populations studied, which also suggested a selection scenario. Analysis of 62 SNPs covering 6 CSP genes in 2019 Han Chinese (976 SCZ patients and 1043 healthy individuals) showed an association of two SNPs (rs4379857, P = 0.009, OR [95% CI]: 1.200 [1.379–1.046]; rs2238751, P = 0.023, OR [95% CI]: 1.253 [1.522–1.032]) with SCZ. However, none of these significances survived after multiple testing corrections. Nonetheless, we observed an association of a rare CREB1 haplotype CCGGC (Bonferroni corrected P = 1.74 × 10−5) with SCZ. Our study showed that there was substantial population heterogeneity in genetic predisposition to SCZ, and different genes in the CSP pathway may predispose to SCZ in different populations.

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1. Introduction

Schizophrenia (SCZ) [OMIM: 181500] is a severe and chronic neuropsychiatric disorder, with a lifetime prevalence of approximately 1%. The heritability was estimated to be 81% (Sullivan et al., 2003). Despite of a consistent prevalence and high heritability globally, the genetic underpinnings of schizophrenia have not been sufficiently resolved. Most genetic association studies were not successfully replicated in different ethnic population. For example, despite the strong support of putative mechanism, some studies did not replicate the association between NRG1 (Neuregulin 1) and SCZ (Doi et al., 2009; Gong et al., 2009; Li et al., 2006; Munafò et al., 2006). Previous association studies of DGCR2 (DiGeorge syndrome critical region gene 2) with SCZ had yielded conflicting results (Betcheva et al., 2009; Georgi et al., 2009; Ishiguro et al., 2008; Shifman et al., 2006). We failed to replicate the positive signals in two genome-wide association studies (GWAS) of Han Chinese patients with SCZ (Shi et al., 2011; Yue et al., 2011) in an independent Han Chinese population from Hunan Province of China (Ma et al., 2013). SCZ might be caused by the disorder of neural circuits in brain and...
many predisposition genes reported so far play roles in neural development (Lewis and Sweet, 2009). Therefore, it will be worthy to analyze the key genes and their pathway regulating neural development, and CSP is a good candidate.

SCZ may be related to de-coupling of molecular components underlying intelligence and language, some of which might have an important role during the evolution of human brain, which favors a selective advantage for higher cognitive function (Keller and Miller, 2006). This situation of “advantageous” gene in ancient time turned into disease gene in modern time could be found in diabetes mellitus (Neel, 1962). Here, we hypothesized genes and pathways that have been subjected to adaptive evolution since the divergence of human populations might be involved in the pathogenesis of SCZ and other mental disorders. Therefore, we studied the evolutionary signals of CREB1 signaling pathway (CSP).

CREB1 (cAMP responsive element binding protein 1) is a transcriptional factor in response to cAMP level and, in central nervous system, it plays crucial roles in mediating neurotrophin-induced axon growth (Lonze et al., 2002). Lacking CREB and CREM (cAMP response element modulator) in neurons of the developing central nervous system could lead to apoptosis and neurodegeneration in hippocampus and dorsolateral striatum (Mantamadiotis et al., 2004). Animal and behavioral studies have demonstrated that CREB1 signaling pathway can influence mood, addiction, learning and memory by activating an array of proteins (Carlezon et al., 2005). Two large European studies found that multiple single nucleotide polymorphisms (SNPs) of CREB1 were significantly associated with SCZ and bipolar disorder (Li et al., 2014; Ripke et al., 2013). Previous studies of candidate genes in SCZ also focused on CSP genes (Kvajo et al., 2010). In addition, CREB1 could regulate gene expression of a group of neuronal proteins, and ultimately affect the function of neurons, brain circuits and complex behavior (Carlezon et al., 2005).

Recently, the catalog of downstream genes regulated by transactivation of CREB1 was systematically characterized (Zhang et al., 2005). Among the gene list, NRGI, CNNM2 (cyclin M2), NTSC2 (5′-nucleotidase, cytosolic II), DGCR2 and OPCML (opioid binding protein/cell adhesion molecule-like) are related to neural development and have been implicated in SCZ (Aberg et al., 2013; Bergen et al., 2012; Law et al., 2012; Mistry et al., 2013; Ripke et al., 2011; Xu et al., 2011). This downstream gene set of CSP play essential roles in neural development and synaptic plasticity (Fazzari et al., 2010; Kato et al., 2011; Li et al., 2007). Disease-associated SNPs in NRGI could affect expression of specific NRGI isoforms in human brain (Law et al., 2006; Tan et al., 2007), and this abnormal expression was associated with human brain activation and psychiatric symptoms (Hall et al., 2006; Hatzimanolis et al., 2013; Law et al., 2012). CNNM2-NTSC2 was reported to reach a genome-wide significance in a large European SCZ-GWAS (Ripke et al., 2011) and in a meta-analysis with a large Swedish sample (Bergen et al., 2012). This positive association was consistently validated in a family-based study (Aberg et al., 2013) and a comprehensive replication study (Bergen et al., 2012). DGCR2 encodes a potential adhesion receptor protein with unclear function (Kajiwara et al., 1996). Deletion of this region accounted for ~2% of sporadic schizophrenia (Xu et al., 2011). OPCML is highly expressed in hippocampus and cortex and was implicated in neuronal connection (Struyk et al., 1995). Genome-wide expression study of a large SCZ cohort showed that OPCML was significantly down-regulated in prefrontal cortex of SCZ patients (Mistry et al., 2013). Recent association studies supported its genetic effects on SCZ (O'Donovan et al., 2008).

In light of the above findings, we speculated that historical molecular evolution of CSP genes may contribute to emergence of SCZ pathogenesis in modern society (Fig. 1). Functional adaptations that drive species differentiation could be found in genes that have undergone positive selection (Vamathevan et al., 2008). Therefore we investigated whether there were signatures of selection effect on the six candidates CSP genes related to neural development during the origin of modern human and determined whether CSP genetic variants contribute to SCZ risk in Han Chinese.

2. Materials and methods

2.1. Molecular evolution analysis of CSP genes using the 1000 genome project data

Imputation data for CREB1 and its target genes (NRGI, CNNM2, NTSC2, DGCR2 and OPCML) in the 1000 Genomes Project were obtained from web (http://mathgen.stats.ox.ac.uk/impute/). This data set includes 97 Han Chinese in Beijing [CHB], 100 Han Chinese South [CHS] and 89 Japanese in Tokyo [JPT], 85 Utah residents (CEPH) with Northern and Western European ancestry [CEU], 93 Finnish from Finland [FIN], 89 British from England and Scotland [GBR], 14 Iberian populations in Spain [IBS] and 98 Toscani in Italy [TSI], 61 African Ancestry in Southwest US [ASW], 88 Yoruba in Ibadan, Nigeria [YRI] and 97 Luhya in Webuye, Kenya [LWK], 60 Colombian in Medellin, Colombia [CLM], 66 Mexican Ancestry in Los Angeles, CA [MXL] and 55 Puerto Rican in Puerto Rico [PUR]. Nucleotide diversity (π) and Tajima’s D (Tajima, 1989) were used to measure the deviation from neutrality in each population. These two statistics were estimated on the basis of 10 kb windows. In addition, integrated haplotype score (IHS) were used to detect long haplotypes that have not been broken by recombination (Voight et al., 2006).
2.2. Genetic analysis of CSP genes in a Han Chinese SCZ patient cohort

A total of 976 unrelated SCZ patients (mean age ± SD 24.92 ± 8.33) and 1043 matched healthy controls (mean age ± SD 37.41 ± 14.22), all of Han Chinese, were recruited from Hunan Province in South Central China, which were described in our previous studies (Ma et al., 2013). In brief, patients were diagnosed independently by two experienced psychiatrists as SCZ according to DSM-IV criteria. The controls were clinically diagnosed as no family history of psychiatric disorders. All participants gave written informed consent. This study was approved by the ethics committee of the Kunming Institute of Zoology.

Genomic DNA was extracted from whole blood by using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen, USA). 68 SNPs of six CSP genes (CREB1, NRG1, CNNM2-NT5C2, DGCR2 and OPCLM) were selected for genotyping. These SNPs included susceptible SNPs reported in previous genome-wide association studies for SCZ (Aberg et al., 2013; Bergen et al., 2012; Ripke et al., 2011) and tagSNPs that covering the entire gene region. SNP location, gene structure and linkage disequilibrium pattern of these SNPs were shown in Supplementary Fig. 1. SNPs were genotyped using SNPEX (Supplementary Table 1). GeneMarker software (Holland and Parson, 2011) was used to read the genotyping results. The detailed procedures were described in the supplementary file (Supplementary methods).

Calculations of Hardy–Weinberg equilibrium (HWE) test, allele and genotype comparisons were performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). Haplotype test was calculated by R Haplo Stats Package (http://www.r-project.org). P-value was calculated by logistic model in which gender was used as the covariate in allele and genotype tests. We allocated the SNPs and reconstructed the haplotypes according to the chromosome that they were located. There were five blocks in chromosome 2 (CREB1), chromosome 8 (NRG1), chromosome 10 (CNNM2-NT5C2), chromosome 11 (OPCLM) and chromosome 22 (DGCR2), respectively. Linkage disequilibrium patterns (r² algorithm) of these five blocks were performed by using Haplovew (Barrett et al., 2005). Power calculations were performed using the G-power software (http://www.gpower.hhu.de/).

3. Results

3.1. Positive selection on CSP during the divergence of modern human populations

We used three detection methods with different estimates of time scale (π and Tajima’s D, <250,000 years; iHS, <30,000 years) (Sabeti et al., 2006) to properly indicate the different ages of selection events. We first detected whether genetic diversity was reduced in human populations during their dispersal across the world by using π and Tajima’s D (Tajima, 1989). The sliding window fashion calculations showed that π and Tajima’s D values of CREB1 region were significantly lower than those of its upper and lower region in all 14 populations of 1000 Genomes Project (Fig. 2). This finding implied that CREB1 might have undergone positive selection in human populations and the selection might appear before the divergence of human populations, as all populations showed the signal. The positive selection was unlikely caused by population history such as population expansion because it should have the same effect on the genome. Note that negative selection might also lead to the deviation from the neutral test. We failed to detect any significant signal in other CSP genes.

Large positive iHS (iHS > 3) indicates that haplotypes on the ancestral allele background are longer than derived allele background (Voight et al., 2006). In contrast, an extreme negative iHS score (iHS < −3) indicates that haplotypes on the derived allele background are longer than haplotypes containing the ancestral allele. We found a total of 22 SNPs within the NRG1 region had iHS values either > 3 or < −3 in all 14 populations of 1000 Genomes Project (Supplementary Table 2).

3.2. CSP genetic variants and SCZ susceptibility in Han Chinese

To detect whether CSP is associated with SCZ, we genotyped 68 SNPs of CSP genes in a total of 976 SCZ patients and 1043 normal controls from South Central China. This sample had a statistical power of 98.59% to detect a genetic effect with an effect size index of 0.1 under an assumption of weak gene effect. Our recent analysis of the matrilineal components of the patient and control populations showed no evidence of population stratification (Zhang et al., 2014). We excluded five SNPs with a successful calling rate less than 95%, and one SNP that was failed to be genotyped from the subsequent analyses. The genotype and allele frequencies of the remaining 62 SNPs were shown in Fig. 3 and Supplementary Tables S3 and S4. The strongest signal was located in intron 1 of OPCML (rs4379857, P = 0.008, odds ratio (OR) [95% confidence interval (CI)]: 1.200 [1.379–1.046]) and intron 2 of DGCR2 (rs2238751, P = 0.023, OR [95% CI]: 1.525 [1.522–1.532]) at the allele frequency level (Fig. 3 and Supplementary Table 3).
We further evaluated potential haplotype association between SCZ patients and healthy controls. Haplotype test identified a strong association signal: a rare CREB1 haplotype CCGGC surpassed stringent Bonferroni correction and showed an association with SCZ \((P = 1.74 \times 10^{-5})\) (Table 1). To further confirm this result, we downloaded and re-analyzed genotype data of these SNPs for CHB from HapMap database (http://hapmap.ncbi.nlm.nih.gov/). The result showed that haplotype frequencies of the four common haplotypes in our data were similar to those of the CHB population from HapMap (Table 1). Linkage disequilibrium pattern of CREB1 SNPs composing the significant haplotype was also similar between our control sample and CHB population (Supplementary Fig. 2).

4. Discussion

SCZ is a complex neural development disorder and had an involvement of a series of genes, especially genes related to neural circuit development (Lewis and Sweet, 2009). The tremendous shifts experienced by human populations in diet, climate, population densities, social communications and mental pressure have surely led to direct selection pressures on medically relevant phenotypes (Vamathavan et al., 2008). Genes in certain pathway driven by selective pressure are required for functional convergence and mental diseases may be regarded as by-products of human brain evolution (Vamathavan et al., 2008). In this study, we focused on CREB1 and its five targeted genes, which have been reported as SCZ susceptibility genes in recent studies (Aberg et al., 2013; Bergen et al., 2011; Law et al., 2012; Li et al., 2014; Mistry et al., 2013; Ripke et al., 2011; Xu et al., 2011). We initially detected whether there are footprints of positive selection on these CSP genes in human populations by using a variety of evolutionary approaches, which are sensitive to show different age estimates of selection events.

Consistent with the finding that NRG1 fall into the top 1% of iHS signals in East Asians and the cross-population extended haplotype homozygosity (XP-EHH) signals observed in Africans (Pickrell et al., 2009), we found an abundance of extreme iHS signals (around 30,000 years ago) that were located within NRG1 (Supplementary Table 2). We also found that NRG1 and CREB1 had an adaptation pressure around 250,000–300,000 years ago. All 14 populations in 1000 Genomes Project were consistently experienced selection pressure at NRG1 and CREB1, which implied that these regions had been selected before the dispersal of modern human populations across the world. This selection advantage lasted for many generations in global populations and was hardly affected by inheritance (Voight et al., 2006). These results confirmed our speculation and previous findings that CREB1 signal pathway, especially NRG1 and CREB1, had undergone fast evolution in modern human evolution and this effect may affect brain development of our species.

To further determine whether genetic variants of the CSP genes contributed to SCZ risk, we analyzed 62 SNPs in six genes of CSP in Han Chinese. Unfortunately, we found no robust association between these SNPs and SCZ in Han Chinese populations at the allele and genotype levels, albeit we observed two SNPs showing a positive association with SCZ (Fig. 3), but the significance disappeared after multiple testing corrections. Nonetheless, a rare CREB1 haplotype CCGGC was found to be significantly associated with SCZ in our population. This association remained significant after multiple testing corrections, and this haplotype was not found in CHB data set from HapMap but was enriched in our SCZ patients (Table 1), which further supported that CREB1 is a risk for SCZ. This pattern seemed to support our initial speculation that the CSP genes (CREB1 in particular) that have undergone fast evolution during human evolution would harbor susceptible variants for SCZ.

There are several limitations in this study. First, our sample size is relatively modest, which might not be sufficient to detect SCZ

Table 1

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>HapMap</td>
</tr>
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<tr>
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</tr>
<tr>
<td>TCAGC</td>
<td>0.111</td>
<td>0.119</td>
<td>0.219</td>
</tr>
<tr>
<td>CCGGC</td>
<td>0.025</td>
<td>0.004</td>
<td>NA</td>
</tr>
</tbody>
</table>

The global P-value of the entire haplotype comparison between the case and control samples is 3.39 × 10^{-5}.

a SNP order: rs2300969, rs16839849, rs2253206, rs11904814 and rs4234080.

b P-value was obtained by comparing the frequency of certain haplotype in the case and control populations.

Fig. 3. Plot of P-values of 62 single nucleotide polymorphisms selected from CREB1 (red), NRG1 (blue), CNM2-NT5C2 (green), OPCML (black) and DGCC2 (orange) genes in Psychiatric Genomics Consortium (PGC) data (A) and Han Chinese from Hunan Province, China (B). P values in the case-control study are calculated on a –log_{10} scale. The risk SNP rs3016384 (marked with a triangle) from O’Donovan et al. (2008) was also included in the PGC plot for comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
susceptible SNPs with very small effects. Second, the significant haplotype found in this study should be viewed with caution, because only one homogenous Chinese population was used in this study. The result remains to be confirmed in independent samples. Finally, we did not perform functional assay to further characterize the association between the significant rare haplotype and SCZ.

In summary, we found that CREBI and NRG1 underwent fast evolution in recent human history and the selection appeared before the dispersal of modern human out of Africa. None of the 62 SNPs of the six CSP genes showed any significant association with SCZ after multiple testing corrections. However, we found a rare haplotype of CREBI confers susceptibility to SCZ. Taken all these results and previous reports together, we concluded that different genes in the CSP pathway may predispose to SCZ in different populations and substantial population heterogeneity in genetic predisposition to SCZ would account for the inconsistency. CREBI, the core component of CSP, might be a risk gene for SCZ in Han Chinese, albeit the risk effect was contributed by a rare haplotype.

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Contributors

LM and YGY designed the study; XC and LT collected the samples and clinical information; LM performed laboratory work; LM, DDW, SLM, NLST and YGY analyzed data; LM, NLST and YGY wrote the manuscript. All the authors read and revised the manuscript.

Conflict of interest

There is no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2014.06.008.

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