Analysis of association between common SNPs in ErbB4 and bipolar affective disorder, major depressive disorder and schizophrenia in the Han Chinese population

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1. Introduction

Schizophrenia (SCZ, population prevalence 1%, heritability 70–85%), bipolar affective disorder (BPAD, population prevalence 2%, heritability 60–85%) and major depressive disorder (MDD, population prevalence 17%, heritability 40%) are three common and severe psychiatric disorders. (Burmeister et al., 2008; O'Donovan et al., 2009). In Han Chinese population, they have the prevalence of 0.66% for SCZ (1994, mainland, China), 4% for MDD (1993, Shanghai, China) and 0.7%–1.6% for BPAD (1982–1987, Taiwan and Hong Kong). These three psychiatric disorders share clinical phenotypes and genetic risks (Barnett and Smoller, 2009; Carroll and Owen, 2009; Kato, 2007; Yu et al., 2008). The number of common genes which was found in the Phenopedia module realized with the HuGe Navigator is about 272 between SCZ and BPAD, 33 between SCZ and MDD, 43 between BPAD and MDD, respectively (http://www.hugenavigator.net/HuGENavigator/). V-erb-a erythroblastic leukemia viral oncogene homolog 4 (ErbB4) has also been reported to be associated with schizophrenia. Since there can be shared genetic variants among bipolar affective disorder, major depressive disorder and schizophrenia, we tested the association between ErbB4 and these three major psychiatric disorders in the Han Chinese population. Five single nucleotide polymorphisms (SNPs) were selected based on previous positive reports and linkage disequilibrium information of the HapMap Han Chinese individuals from Beijing (CHB) + individuals from Tokyo, Japan (JPT) population. These SNPs were genotyped in 1140 bipolar affective disorder (BPAD) patients, 1140 schizophrenia (SCZ) patients, 1139 major depressive disorder (MDD) patients and 1140 normal controls. Two SNPs (rs707284 and rs839523) showed nominal significance in the BPAD patients but this was eliminated after permutation. No significant association between ErbB4 and the two other psychiatric disorders was observed, nor did haplotype analysis reveal any positive signal.

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2. Methods

2.1. Subjects

Our sample sets consisted of 1140 unrelated bipolar affective disorder patients (623 males and 517 females, with a mean age of 36.6 years, SD = 13.8), 1140 unrelated schizophrenia patients (635 males and 505 females, with a mean age of 35.4 years, SD = 7.2), 1139 unrelated major depressive disorder patients (487 males and 652 females, with a mean age of 35.1 years, SD = 11.6) and 1140 normal controls (374 males and 766 females, with a mean age of 58.7 years, SD = 9.9). All subjects were of Han Chinese origin in Shanghai, China. Patients were interviewed by two independent experienced psychiatrists. Diagnoses were strictly according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, the fourth edition) criteria based on SCID-I (Structured Clinical Interview for DSM-IV Axis I Disorders). Normal people were randomly selected from the Shanghai general population as control. The control subjects were also interviewed by two independent psychiatrists with SCID-I. The same set of patients was used for another study. We obtained written informed consent from all participants after the nature of the study had been explained. The protocol was reviewed and approved by the local Ethical Committee of Human Genetics Resources.

2.2. SNPs selecting and genotyping

ErbB4 genomic DNA located in chromosome 2 at 211,948,687–213,111,499, spans about 1.16 Mb of DNA with 28 exons and contains several SNPs as identified by the International SNP Consortium (http://www.ncbi.nlm.nih.gov=SNP), Ensembl Genome Browser (http://www.ensembl.org) and UCSC database (http://genome.ucsc.edu=cgi-bin=hgGateway). We chose the 5 common tagSNPs from the HapMap CHB + JPT population (The International HapMap Consortium, 2003). Among the five SNPs, rs839523 and rs707284 were strongly associated with expression change in splice variants (Law et al., 2007). The other 3 SNPs were chosen as tagsSNPs to cover the region around the 2 SNPs efficiently. The relationship between tagging SNPs and exons in ErbB4 gene was illustrated in Fig. 1.

Genomic DNA was extracted from peripheral blood using the standard phenol-chloroform method. Genotyping was performed on the ABI 7900 DNA detection system (Applied Biosystems, Foster City, California, USA) using TaqMan® probes designed by the Applied Biosystems service. The standard 5 μl PCR reaction was carried out using TaqMan® Universal PCR Master Mix reagent kits as directed by the guidelines.

2.3. Statistical analysis

The differences in distribution of allele frequencies between cases and controls were calculated on SHEsis (http://analysis.bio-x.cn/) (Shi and He, 2005). The deviation from Hardy–Weinberg equilibrium (HWE), haplotype analysis and permutation tests were calculated on Haploview4.2 (Barrett et al., 2005). Only haplotypes with an estimated frequency ≥3% were evaluated. 10,000 permutations were carried out to correct the p-values of the multiple tests.

2.4. Population stratification analysis

We performed the stratification analysis using STRUCTURE software (version 2.3.1, http://pritch.bsd.uchicago.edu/structure.html) (Pritchard et al., 2000). This software assumes that there were K populations (K is the number of assumed populations) in the data set, and then it tries to find the distinct populations using the genotype data. Taking the immigration and geographical genetic isolation...
into consideration, the admixture model will suit for our samples. The correlated frequencies model is selected because the samples of the three disorders were all recruited from the sample population. We applied the admixture model and correlated frequencies model, with a burn-in length of 10,000 and MCMC (Markov chain Monte Carlo) repeats of 10,000. We ran the program several times at each K (number of assumed populations) from 2 to 5 to make sure the results were consistent. We chose the genotype data of 52 negative SNPs from previous genotyping experiments as the input for population stratification analysis. To test whether the 52 SNPs employed would reflect the stratification status, we first analyzed a combined population of 162 HapMap U.S. residents of northern and western European ancestry (CEU) individuals, 163 HapMap Yoruba people in Ibadan, Nigeria (YRI) individuals (The International HapMap Consortium, 2003) and 180 individuals randomly selected from our samples. STRUCTURE software triangle charts illustrate the analysis results (Fig. 3a). Then we analyzed our 4 cohorts of SCZ, MDD, BPAD and normal controls by the same procedure (Fig. 3b).

3. Results

3.1. Linkage disequilibrium

The pairwise linkage disequilibrium (LD) among the 5 investigated SNPs was different in the different sample sets. There was relatively higher LD in the BPAD sample set than in the other two. SNPs with LD was different in the different sample sets. There was relatively obvious signiﬁcance stratiﬁcation in the population (Fig. 3b). The results were consistent with each other when K ranged from 2 to 5. We can therefore conclude that our positive results before permutation were unlikely to have been caused by population stratification.

3.2. Single site association

Two SNPs, rs839523 and rs707284, were signiﬁcantly associated with BPAD in both allele and genotype distributions, but the association was eliminated after permutation (Tables 1 and 2). No positive signal was observed for SCZ and MDD from any of the 5 SNPs. All the SNPs were in Hardy–Weinberg equilibrium in both cases and controls.

3.3. Population stratification analysis

Fig. 3 shows the triangle chart of K = 3, in which each angle represents a possible independent ancestry and the dots with different colors represent the individuals in assumed population components. The results are best described by the triangle chart when K = 3. The combined population of CEU, YRI and our samples displayed a clear stratiﬁed pattern (Fig. 3a), and our samples of the three disorders and controls distributed evenly in the triangle indicating that there was no obvious signiﬁcant stratification in the population (Fig. 3b). The results were consistent with each other when K ranged from 2 to 5. We can therefore conclude that our positive results before permutation were unlikely to have been caused by population stratification.

4. Discussion

Genome-wide association studies (GWAS) on these three psychiatric disorders have yielded several risk genes, though different from previous candidate gene studies (Baum et al., 2008; Lencz et al., 2007; O’Donovan et al., 2008). In 2008, ankyrin 3 (ANK3) and calcium channel voltage-dependent L type alpha 1C subunit (CACNA1C) surpassed the genome-wide signiﬁcance in a meta-analysis of GWAS studies including 4387 cases and 6209 controls (Ferreira et al., 2008). The promising susceptibility gene shared by SCZ and BPAD was highlighted in a cross-disorder meta-analysis of the two psychiatric disorders. It was found that zinc ﬁnger protein 804A (ZNF804A) was associated with SCZ at a 10−7 level in a GWAS. Moreover, when they broadened the phenotype to include BPAD, the signiﬁcance level reached 10−8 with an odds ratio of 1.09 (O’Donovan et al., 2008). The meta-analyses of GWAS of MDD indicated two probable risk genes, Sp4 transcription factor (SP4) and glutamate receptor metabotropic 7 (GRM7), though both of them didn’t surpass the genome-wide signiﬁcance level (Muglia et al., 2010; Shi et al., 2011). The major problem of psychiatric disorders GWAS may be that they’re not consistent with each other. Despite the complex genetic background, there is no overlap between risk genes found in separate studies. One of the reasons for this phenomenon should be the insufﬁcient power of these studies. When we assume the odds ratio as 1.10 and the

<table>
<thead>
<tr>
<th>Marker</th>
<th>Position</th>
<th>Functional</th>
<th>Polymorphism</th>
<th>BPAD OR</th>
<th>P</th>
<th>MAF(allele)</th>
<th>SCZ OR</th>
<th>P</th>
<th>MAF(allele)</th>
<th>MDD OR</th>
<th>P</th>
<th>MAF(allele)</th>
<th>Control OR</th>
<th>P</th>
<th>MAF(allele)</th>
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<tr>
<td>rs10207288</td>
<td>212541377</td>
<td>Intron 13</td>
<td>C/T</td>
<td>0.991</td>
<td>0.929</td>
<td>0.100 (T)</td>
<td>1.014</td>
<td>0.889</td>
<td>0.098 (T)</td>
<td>0.946</td>
<td>0.578</td>
<td>0.105 (T)</td>
<td>0.100 (T)</td>
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<td>rs2371276</td>
<td>212555905</td>
<td>Intron 12</td>
<td>C/T</td>
<td>1.048</td>
<td>0.547</td>
<td>0.140 (C)</td>
<td>1.086</td>
<td>0.470</td>
<td>0.142 (C)</td>
<td>1.174</td>
<td>0.062</td>
<td>0.155 (C)</td>
<td>0.135 (C)</td>
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<tr>
<td>rs839523</td>
<td>212816889</td>
<td>Intron 2</td>
<td>C/T</td>
<td>1.156</td>
<td>0.019</td>
<td>0.373 (T)</td>
<td>1.072</td>
<td>0.264</td>
<td>0.391 (T)</td>
<td>1.037</td>
<td>0.555</td>
<td>0.329 (T)</td>
<td>0.408 (T)</td>
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<td>0.690</td>
<td>0.101 (T)</td>
<td>0.992</td>
<td>0.937</td>
<td>0.099 (T)</td>
<td>0.938</td>
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<td>0.104 (T)</td>
<td>0.098 (T)</td>
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<td>rs707284</td>
<td>212839046</td>
<td>Intron 2</td>
<td>C/T</td>
<td>1.162</td>
<td>0.015</td>
<td>0.361 (T)</td>
<td>1.080</td>
<td>0.220</td>
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<td>0.799</td>
<td>0.400 (T)</td>
<td>0.396 (T)</td>
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</table>

Abbreviations: HWE P, Hardy–Weinberg equilibrium P-value; OR, odds ratio; P, P-value; BPAD, bipolar affective disorder; SCZ, schizophrenia; MDD, major depressive disorder. P-values ≤0.05 are in bold.

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Genotype frequency</th>
<th>Chi²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10207288</td>
<td>SCZ 863(0.815)</td>
<td>844(0.144)</td>
<td>0.011</td>
</tr>
<tr>
<td>BPAD 916(0.809)</td>
<td>205(0.191)</td>
<td>0.019</td>
<td>0.995</td>
</tr>
<tr>
<td>MDD 865(0.800)</td>
<td>206(0.199)</td>
<td>0.025</td>
<td>0.678</td>
</tr>
<tr>
<td>Control 910(0.808)</td>
<td>208(0.185)</td>
<td>80(0.007)</td>
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</tr>
<tr>
<td>rs2371276</td>
<td>SCZ 23(0.022)</td>
<td>251(0.240)</td>
<td>770(0.738)</td>
</tr>
<tr>
<td>BPAD 19(0.017)</td>
<td>277(0.247)</td>
<td>827(0.736)</td>
<td>1.401</td>
</tr>
<tr>
<td>MDD 31(0.029)</td>
<td>268(0.251)</td>
<td>769(0.720)</td>
<td>3.472</td>
</tr>
<tr>
<td>Control 23(0.021)</td>
<td>254(0.228)</td>
<td>837(0.751)</td>
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<tr>
<td>rs839523</td>
<td>SCZ 398(0.380)</td>
<td>480(0.458)</td>
<td>170(0.162)</td>
</tr>
<tr>
<td>BPAD 433(0.387)</td>
<td>535(0.479)</td>
<td>150(0.134)</td>
<td>6.263</td>
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<tr>
<td>MDD 390(0.363)</td>
<td>519(0.476)</td>
<td>176(0.161)</td>
<td>0.357</td>
</tr>
<tr>
<td>Control 388(0.354)</td>
<td>523(0.477)</td>
<td>186(0.170)</td>
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<tr>
<td>rs839511</td>
<td>SCZ 862(0.816)</td>
<td>180(0.170)</td>
<td>14(0.013)</td>
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<td>BPAD 906(0.805)</td>
<td>210(0.187)</td>
<td>9(0.008)</td>
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<td>MDD 867(0.805)</td>
<td>197(0.183)</td>
<td>11(0.012)</td>
<td>0.433</td>
</tr>
<tr>
<td>Control 918(0.816)</td>
<td>194(0.172)</td>
<td>13(0.012)</td>
<td></td>
</tr>
<tr>
<td>rs707284</td>
<td>SCZ 405(0.387)</td>
<td>492(0.470)</td>
<td>149(0.142)</td>
</tr>
<tr>
<td>BPAD 456(0.410)</td>
<td>510(0.459)</td>
<td>146(0.131)</td>
<td>6.189</td>
</tr>
<tr>
<td>MDD 392(0.368)</td>
<td>496(0.465)</td>
<td>178(0.167)</td>
<td>0.074</td>
</tr>
<tr>
<td>Control 414(0.373)</td>
<td>512(0.462)</td>
<td>183(0.165)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BPAD, bipolar affective disorder; SCZ, schizophrenia; MDD, major depressive disorder. P-values ≤0.05 are in bold.
significance level as $7.2 \times 10^{-8}$, we need about 14,000 each of cases and controls to achieve power of 0.8. In our case, about 1140 each of cases and controls at a nominal significance level of 0.05, we achieved power of 0.43 to detect the risk factors with odds ratio of 1.10.

Although several other lines of evidence indicated that there might be an association between ErbB4 and schizophrenia (Hahn et al., 2006; Harrison and Law, 2006; Norton et al., 2006; Silberberg et al., 2006), we found no significant difference of allele or genotype frequencies in our Chinese population sample (after permutation). Silberberg and his colleagues reported that statistically significant differences were noted between schizophrenia and controls for rs707284 and rs839523 (allele, $p=0.014$ and 0.0045 respectively, not corrected for multiple testing) (Benzel et al., 2007; Silberberg et al., 2006). While in our case, the most significant signal is 0.015 for rs707284 in BPAD.

Molecular genetic studies in separate populations have identified specific DNA variants in the ErbB4 gene that are directly linked with the etiology of schizophrenia (Norton et al., 2006). As for the shared clinical phenotypes and genetic risks of the three disorders under discussion, we have genotyped ErbB4 in different samples respectively. ErbB4 is a complicated gene which can produce several different isoforms through alternative splicing (Elenius et al., 1997; Junttila et al., 2000). Evidence indicates that altered expression, splicing or function of the ErbB4 gene may underlie its association with schizophrenia. The intricate expression pattern of ErbB4 suggests that ErbB4 also has a complicated regulation mode. The observed up-expression of ErbB4 mRNA in schizophrenia might be explained by the altered expression of other genes such as NRG1 whose exact underlying mechanisms need further investigation (Bublil and Yarden, 2007).

We found that two SNPs, rs707284 and rs839523, were associated with BPAD before permutation. These two SNPs were reported to be strongly associated with the elevated expression of ErbB4 splice variants, CYT-1, in the brain tissue of schizophrenia patients (Law et al., 2007). The CYT-1 domain containing ErbB4 isoforms were also found to be overexpressed in Ashkenazi Jews (Silberberg et al., 2006). Since BPAD, SCZ and MDD may share a large number of risk genes, expression analysis in Han Chinese brain tissue may provide further evidence of association between BPAD and the ErbB4 gene.

In summary, we found no evidence that ErbB4 was associated with the three major psychiatric disorders in our Chinese population sample. Our results may provide a reference for further studies of ErbB4 in other populations. Its possible role in the etiology of psychiatric disorders will require replication with additional samples and combined meta-analysis across different studies.

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References


