Genetic association of LMAN2L gene in schizophrenia and bipolar disorder and its interaction with ANK3 gene polymorphism

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A B S T R A C T
Recent studies have shown that bipolar disorder (BPD) and schizophrenia (SZ) share some common genetic risk factors. This study aimed to examine the association between candidate single nucleotide polymorphisms (SNPs) identified from genome-wide association studies (GWAS) and risk of BPD and SZ. A total of 715 patients (244 BPD and 471 SZ) and 593 controls were genotyped using the Sequenom MassARRAY platform. We showed a positive association between LMAN2L (rs6746896) and risk of both BPD and SZ in a pooled population (P-value = 0.001 and 0.009, respectively). Following stratification by ethnicity, variants of the ANK3 gene (rs1938516 and rs10994336) were found to be associated with BPD in Malays (P-value = 0.001 and 0.006, respectively). Furthermore, an association exists between another variant of LMAN2L (rs2271893) and SZ in the Malay and Indian ethnic groups (P-value = 0.003 and 0.002, respectively). Gene–gene interaction analysis revealed a significant interaction between the ANK3 and LMAN2L genes (empirical P = 0.0107). Significant differences were shown between patients and controls for two haplotype frequencies of LMAN2L: GA (P = 0.015 and P = 0.010, for BPD and SZ, respectively) and GG (P = 0.013 for BPD). Our study showed a significant association between LMAN2L and risk of both BPD and SZ.

1. Introduction

Bipolar disorder (BPD) is a severe mental disease with profound social and economic impacts. BPD accounted for 7% of disability-adjusted life years worldwide, as indicated by the Global Burden of Diseases 2010 report (Whiteford et al., 2013). The etiology of BPD has yet to be fully understood, although one hypothesis is that gaining much support is the involvement of ion channelopathy (Ferreira et al., 2008). Genomewide association studies (GWAS) have identified susceptibility genes associated with BPD, a few of which are in a class of genes related to the structure and regulation of ion channel (Ferreira et al., 2008; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). The notion of ion channel involvement is further supported by findings of increased intracellular calcium in the lymphocytes of BPD patients (Brotman et al., 1986; Goodnick, 2000) and the effectiveness of calcium channel blockers in the treatment of BPD (Hough et al., 1999). These findings add weight to the evidence for cellular calcium imbalance and calcium ion channelopathy in the etiology of BPD.

In considering a possible association of BPD with genetic variants, their interplay with schizophrenia (SZ) cannot be overlooked. Although the two disorders may present distinct symptoms, growing evidence has shown that they share some common genetic variations (Bertettoni et al., 2000; Lichtenstein et al., 2009; Maier et al., 2005, 2006). Confirmation of results from GWAS in a well-characterized population is an important approach to identify the susceptibility loci for BPD and SZ. In this study, we aimed to examine the association between BPD and several single nucleotide polymorphisms (SNPs) from candidate genes identified in the GWAS. Among these genes are those involved in ion channel transport (BTF3L1/KCTD12 and ANK3) and endoplasmic reticulum (ER) transport (LMAN2L).

Considering the possible genetic overlap between BPD and SZ, we set out to examine the association between variants within these genes and these disorders. We hypothesized the root genetic cause of BPD and SZ by highlighting genetic variants that overlap in both disorders, as well as the genetic variants that are distinctly different between the two conditions. The BPD and SZ patients in our study were of three
major ethnic groups in Malaysia: Malays, Chinese, and Indians; each of which are presumably from a different genetic pool. Hence, this sample provided a good opportunity to study ethnic differences in susceptibility to BPD and SZ in association with gene polymorphisms.

2. Materials and methods

2.1. Study population

A case control study was conducted at the University Malaya Medical Centre (UMMC). A total of 244 unrelated BPD patients and 471 SZ patients were recruited, together with 593 healthy controls. The patients were from the psychiatry outpatient clinic of UMMC, whereas healthy controls were recruited from healthy volunteers from the University of Malaya, as well as from blood donors at UMMC. All patients were diagnosed through consensus by at least two experienced psychiatrists, according to the criteria for BPD and SZ, as set out by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Subjects were excluded from the study if they had a history of substance abuse or alcoholism, or other chronic diseases, including hypertension and diabetes. Most of the BPD patients (90%) were BPD Type I patients and all were in euthymic state during the period of sample collection. The controls were interviewed by a psychiatrist to confirm that they were free from any mental disorders and had no family history of mental illness (at least for one generation). Ethnicity of the cases and controls was confirmed by the absence of mixed marriage for at least three generations through self-report by the subjects. Subjects provided written informed consent after they were given a full explanation of the research outline. The study protocol was reviewed and approved by the Medical Ethics Committee of UMMC.

2.2. Genotyping

Genomic DNA was extracted from peripheral white blood cells using the QiAamp DNA Mini Kit (Qiagen, Hilden, Germany). LMAN2L gene variants (rs6746896 and rs2271893), a BTF3L1/KCTD12 gene variant (rs2073831), and ANK3 gene variants (rs10994336 and rs1938526) were genotyped at the University of Hong Kong Genome Research Centre using the Sequenom MassARRAY technology platform with iPLEX Gold chemistry (Sequenom, San Diego, CA, USA) according to the manufacturer’s protocols. Briefly, the MassARRAY Assay Design software package (v4.0) was used to design the specific assays with proximal SNP filtering. The quality of PCR fragment amplification and extension primer specificity was checked prior to running the reaction. Residual nucleotides were dephosphorylated prior to the iPLEX Gold reaction. Following a single-base extension, reaction products were desalted with SpectroClean resin (Sequenom), and 10 nL was spotted onto the SpectroCHIP using the MassARRAY Nanodispenser. A MassARRAY Analyzer Compact MALDI-TOF mass spectrometer was used to determine product mass. For proper data acquisition and analysis, MassARRAY Typer 4.0 software was used. Genotypes were called after cluster analysis by using the default setting of the Gaussian mixture model. The clusters were inspected to ensure a clear cluster separation with good signal-to-noise cut-off. A manual review was done to further clarify uncertain genotype calls. An assay with a call rate of less than 80% within the same SpectroCHIP was considered to have failed. A blank and five duplicates were introduced as quality controls. A SpectroChip with a call rate of more than 25% in the blank control or concordance of less than 99.5% in the duplicate checks, along with a call rate of more than 10% in the blank check, was considered to have failed and would need to be repeated.

2.3. Statistical analyses

The genotype distribution was assessed for Hardy–Weinberg equilibrium (HWE) using a χ² test. A P-value of more than 0.05 indicates agreement with HWE. Determination of allele associations was performed by using logistic regression. In order to avoid false discoveries due to population difference, we performed the association analysis for each SNP marker separately by ethnicity. Linkage disequilibrium (LD) and haplotype analysis were performed with the Haploview 4.2 program. Pairwise LD was used to investigate the inter-marker relationship through D’ values in case and control subjects. A permutation test with 5000 replications was used to obtain empirical levels of significance.

To investigate the influence of gene–gene interaction on BPD and SZ, the Generalized Multifactor Dimensionality Reduction (GMDR) method (Lou et al., 2007) was employed. All possible interactions were tested by using 10-fold cross-validation with an exhaustive search that considers all possible variable combinations. GMDR provides a cross-validation consistency score, which is a measure of the degree of consistency with which the selected interaction is identified as the best model among all possibilities considered. The testing-balanced accuracy generated is a measure of the degree to which the interaction accurately predicts case–control status. Testing accuracy is a measure of the strength of gene–gene interaction with a power of 80% with accuracy of 0.58–0.60, given a sample of 500 (Chen et al., 2011). Ethnicity was used as the covariate in the gene–gene interaction analysis.

Power analysis was carried out by assuming a gene-only effect. A calculated sample size of 244 bipolar cases and 593 controls was used to provide a desired power of 80% at a P-value of 0.05 with the following assumptions: the allele frequency range was 0.20–0.80, the baseline risk for the Malaysian population was 0.16, and the minimum detectable odds ratio (OR) was 1.5. As for SZ, a sample size of 471 SZ cases and 593 controls would provide 80% power at a P-value of 0.05 with the following assumptions: the allele frequency range was 0.31–0.41, the baseline risk for the Malaysian population was 0.15, and the minimum detectable OR was 1.5.

3. Results

Table 1 shows the demographic data of the patients and controls. The 244 BPD patients consisted of 73 Malays, 98 Chinese, and 73 Indians, while the 471 SZ patients were made up of 132 Malays, 222 Chinese, and 117 Indians. Of the 593 controls, 173 were Malays, 286 Chinese, and 134 Indians. There is a significant difference in the mean age between BPD patients and healthy controls (P = 0.027). Likelihood tests indicated a significant effect of ethnicity, but no significant effect of age and gender. None of the genotypes for the tested SNPs deviated from Hardy–Weinberg equilibrium for BPD patients, SZ patients, and controls.

As indicated in Table 2, of the five SNPs tested, only one (LMAN2L rs6746896) shows significant differences in allelic distribution between both BPD and SZ patients and controls for the pooled subjects (P = 0.009 and 0.001, respectively). However, when the subjects were stratified according to ethnicity, significant differences were observed for some SNPs. The ANK3 rs1938526 and rs10994336 were significantly different between BPD and SZ patients and controls of Malay ethnicity.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>BPD (N = 244)</th>
<th>SZ (N = 471)</th>
<th>Controls (N = 593)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>43.0 ± 12.0</td>
<td>40.5 ± 12.1</td>
<td>41.1 ± 10.3</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>128 (53%)</td>
<td>270 (57%)</td>
<td>375 (63%)</td>
</tr>
<tr>
<td>Female</td>
<td>116 (48%)</td>
<td>201 (43%)</td>
<td>218 (37%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>73 (30%)</td>
<td>132 (28%)</td>
<td>173 (29%)</td>
</tr>
<tr>
<td>Chinese</td>
<td>98 (40%)</td>
<td>222 (47%)</td>
<td>286 (48%)</td>
</tr>
<tr>
<td>Indian</td>
<td>73 (30%)</td>
<td>117 (25%)</td>
<td>134 (23%)</td>
</tr>
</tbody>
</table>

Table 1: Demographic data of BPD and SZ patients and healthy controls.
Table 2
Association tests of LMAN2L, ANK3, and KCTD12 SNPs with BPD and SZ.

<table>
<thead>
<tr>
<th>LMAN2L</th>
<th>Malay</th>
<th>BPD OR (CI) P-value</th>
<th>Malay SZ OR (CI) P-value</th>
<th>BPD OR (CI) P-value</th>
<th>Malay SZ OR (CI) P-value</th>
<th>BPD OR (CI) P-value</th>
<th>Malay SZ OR (CI) P-value</th>
<th>BPD OR (CI) P-value</th>
<th>Malay SZ OR (CI) P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs67406896</td>
<td>A</td>
<td>1.36 (1.08–1.71)</td>
<td>0.009</td>
<td>1.34 (1.12–1.61)</td>
<td>0.001</td>
<td>1.09 (0.74–1.60)</td>
<td>0.678</td>
<td>1.50 (1.07–2.10)</td>
<td>0.020</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rs2271893</td>
<td>A</td>
<td>1.12 (0.85–1.48)</td>
<td>0.429</td>
<td>1.24 (0.99–1.56)</td>
<td>0.060</td>
<td>0.83 (0.49–1.41)</td>
<td>0.490</td>
<td>2.63 (1.40–4.97)</td>
<td>0.003</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rs1938526</td>
<td>T</td>
<td>0.92 (0.72–1.17)</td>
<td>0.483</td>
<td>0.86 (0.71–1.04)</td>
<td>0.123</td>
<td>0.47 (0.30–0.74)</td>
<td>0.001</td>
<td>0.60 (0.41–0.89)</td>
<td>0.012</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rs10994336</td>
<td>T</td>
<td>1.01 (0.76–1.33)</td>
<td>0.976</td>
<td>0.98 (0.78–1.23)</td>
<td>0.840</td>
<td>0.49 (0.30–0.81)</td>
<td>0.006</td>
<td>0.62 (0.40–0.95)</td>
<td>0.029</td>
</tr>
<tr>
<td>C</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rs2073831</td>
<td>T</td>
<td>0.99 (0.80–1.23)</td>
<td>0.94</td>
<td>1.07 (0.90–1.28)</td>
<td>0.445</td>
<td>0.90 (0.40–1.35)</td>
<td>0.602</td>
<td>0.68 (0.40–0.95)</td>
<td>0.025</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ANK3</td>
<td>T</td>
<td>1.23 (0.65–2.32)</td>
<td>0.518</td>
<td>0.87 (0.67–1.22)</td>
<td>0.273</td>
<td>0.59 (0.33–1.08)</td>
<td>0.085</td>
<td>0.71 (0.44–1.15)</td>
<td>0.166</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>KCTD12</td>
<td>T</td>
<td>1.11 (0.43–2.87)</td>
<td>0.825</td>
<td>0.85 (0.43–1.68)</td>
<td>0.643</td>
<td>0.24 (0.05–1.16)</td>
<td>0.076</td>
<td>0.27 (0.07–1.09)</td>
<td>0.066</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

CI = confidence interval; OR = odds ratio. Genotype distribution can be found in Supplementary Table 1. CI = confidence interval; OR = odds ratio. Genotype distribution can be found in Supplementary Table 1.

* Results based on combining results across ethnicities.
In Table 3, we derived one best model with perfect cross-validation consistency between the two risk genes on the occurrence of BPD and SZ. As indicated to add strength to these results, we further investigated interaction between these genes. ANK3 rs1938526 and LMAN2L rs6746896 were significantly associated with BPD and SZ in our study population. The ANK3 gene variants and BPD has gained support from various studies (Chen et al., 2013). Nevertheless, our results suggest that the A allele of rs2271893 confers an over 2.5-fold increased risk of SZ in ethnic Malays and Indians.

The variants ANK3 rs1938516 and rs10994336 were not significantly associated with the occurrence of BPD and SZ in the pooled subjects. However, after ethnic stratification, significant differences were observed between BPD patients and controls and between SZ patients and controls among ethnic Malays. The rs1938526 variant was also significantly associated with BPD in Indians. The association between ANK3 gene variants and BPD has gained support from various studies (Chen et al., 2013; Gonzalez et al., 2011; Schulze et al., 2009; Tesli et al., 2011), while other studies have failed to report any significant association (Gella et al., 2011; Gonzalez et al., 2013; Kondo et al., 2013; Lett et al., 2011; Takata et al., 2011), suggesting that ethnicity contributes to the risk of these disorders. Among supporting studies were those by Takata et al. (2011), who showed an association in the East Asian population, including Han Chinese, Japanese, and Koreans, and by Gella et al. (2011) and Kondo et al. (2013), who reported an association of rs1938526 and rs10994336 with SZ. These studies are in contrast, however, with the work of Tesli et al. (2011), who reported no association. We failed to show any association between rs1938526 and rs10994336 with BPD and SZ in our Chinese subgroup. Variant rs1938526 was found to be associated with BPD and not with both BPD and SZ in the Malaysian population. We observed an overlapping association between the positive significant finding in the GWAS of the Han Chinese BPD (Yuan et al., 2012). There are, however, no reports of association with both BPD and SZ in other ethnic groups.

Results from this study suggest that variants of the LMAN2L and ANK3 genes confer increased risk to BPD and SZ in our study population. To add strength to these results, we further investigated interaction between the two risk genes on the occurrence of BPD and SZ. As indicated in Table 3, we derived one best model with perfect cross-validation consistency: a two-locus model (ANK3 rs10994336, LMAN2L rs6746896) that would fit best for SZ (empirical P = 0.0107). Interaction is however not observed in BPD.

4. Discussion

In this study, we investigated the association of gene variants involved in ion channel transport and ER transport with BPD and SZ. We showed a significant association between LMAN2L rs6746896 and both BPD and SZ in the pooled population. However, following ethnic stratification, this association was modified, with a strong and significant association that was apparent in the Chinese for BPD and in the Malays for SZ. Our pooled result on BPD is consistent with two previous GWAS: one by Chen et al. (2013) on the European and Asian ancestry samples and another by the Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011). However, to date no study has investigated the association between LMAN2L rs6746896 and SZ; ours is the first to report a significant positive association. LMAN2L rs2271893 was also found to be associated with SZ but not with BPD in Malays and Indians. These findings are partially consistent with those of Andreassen et al. (2013) in that the authors reported an overlap of rs2271893 with BPD and SZ, whereas we found an association with SZ but not BPD. Our finding is inconsistent, however, with the significant association with BPD reported by Chen et al. (2013). Nevertheless, our results suggest that the A allele of rs2271893 confers an over 2.5-fold increased risk of SZ in ethnic Malays and Indians.

Table 3 Best fitted gene–gene interaction model.

<table>
<thead>
<tr>
<th>Locus number</th>
<th>Model</th>
<th>Cross-validation consistency</th>
<th>Testing accuracy (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>LMAN2L (rs6746896), ANK3 (rs10994336)</td>
<td>8/10</td>
<td>56.80</td>
<td>0.0107</td>
</tr>
</tbody>
</table>

P-values are based on 1000 permutations. Analysis of GMDR is with adjustment of ethnicity.
An ion channel gene, potassium channel tetramerization domain containing 12 (KCTD12), is involved in potassium ion transport. KCN2, another potassium channel, was previously identified in the Wellcome Trust Case Control Consortium Study as being associated with BPD (Baum et al., 2008). The role of potassium channel mutations has also been found to be particularly prominent in human channelopathies (Ryan and Ptacek, 2010). In addition, an association between altered gene function related to potassium ion transport and BPD was reported in a study by Goldstein et al. (2009). Sodium- and potassium-activated adenosine triphosphatases (Na+, K+-ATPase), major plasma membrane transporters for sodium and potassium, were found to be associated with BPD (Goldstein et al., 2009). These studies have clearly suggested a role for potassium ion transport in the etiology of BPD.

The role of the lectin, mannosone-binding 2-like (LMAN2L) gene, also known as the VIPL (VIP36-Like) gene, as an ER export receptor has been demonstrated in a study by Neve et al. (2003). Studies have shown that LMAN2L may act as a regulator for the ERGIC-53 (LMAN 1) gene. Mutation of the latter gene has been associated with a genetic bleeding disorder with a combined deficiency of coagulation factors FV and FVIII (FSFB8D) (Nichols et al., 1999). However, the functional role of LMAN2L in the pathophysiology of BPD has yet to be discovered. Nonetheless, two large-scale GWAS have shown that this gene is associated with BPD (Chen et al., 2013; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011).

Genetic association studies have provided clues on possible risk genes associated with psychiatric disorders including BPD and SZ (Berrettini et al., 2000). These genetic variants are responsible for the pathophysiology of the disorders mainly by altering the protein functions, thereby regulating brain activity (Bigos et al., 2010; Meyer-Lindenberg et al., 2006). Expression studies have shown that the activities of the BPX2Z genes were altered following treatments (Pandey et al., 2008; Rueckert et al., 2013). These observations have strongly hinted that the genetic factor may play a significant role in etiology of these two disorders. A limitation of our study is the small sample size compared with earlier GWAS samples, which could have contributed to the small effect size of the associations. Taking into consideration that BPD and SZ develop as a result of the interplay of genetic and environmental factors, the lack of environmental background information of the subjects such as exposure to nicotine, advanced paternal age and stressful or traumatic events could be a further limitation of the study (de Leon and Diaz, 2005; Goldberg and Gorno, 2005; Torrey et al., 2009). On the other hand, its strength lies in the genotyping of polymorphisms across the BPD and SZ population simultaneously, which enabled us to study common genetic variants involved in the pathophysiology of both BPD and SZ. The results of our study favor the hypothesis that both BPD and SZ share some common genetic risk factors. Moreover, comparison of ethnicities in relation to BPD and SZ allowed us to investigate how ethnicity modifies the association between gene variants and BPD and SZ.

5. Conclusion

To our knowledge, this study is the first to report an association between LMAN2L rs6746896 and SZ. Further evidence is needed to confirm this interaction in a larger population. We found a positive association between LMAN2L rs6746896 (with both BPD and SZ). We also showed that variants of the ANK3 gene (rs1938516 and rs10994336) are associated with BPD in Malays and that LMAN2L rs2271893 is associated with SZ in Malays and Indians. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pnpbp.2014.05.017.

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