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Neurogenic locus notch homolog protein 4 and brain-derived neurotrophic factor variants combined effect on schizophrenia susceptibility

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Yang X, Zhu A, Li F, Zhang Z, Li M. Neurogenic locus notch homolog protein 4 and brain-derived neurotrophic factor variants combined effect on schizophrenia susceptibility

Objectives: To investigate the relationships between single-nucleotide polymorphisms (SNPs) in *NOTCH4* and brain-derived neurotrophic factor (*BDNF*) with schizophrenia among Han Chinese in Southern China.
Methods: Two *NOTCH4* SNPs (rs520688 and rs415929) and two *BDNF* SNPs (rs2030324 and rs12273539) were examined in 464 schizophrenics and 464 healthy controls from Hunan province in South China, using the Sequenom MassARRAY® iPLEX System.
Results: In the study population, rs520688 and rs2030324 were significantly associated with schizophrenia. A decreased risk of schizophrenia was associated with the rs520688 GA genotype ($p = 0.035$), whereas an increased risk of schizophrenia was associated with the rs2030324 CC/CT genotype ($p = 0.044$). The genotype distributions of rs415929 in *NOTCH4* and rs12273539 in *BDNF* did not differ significantly between the case and control groups. Although no allele–allele interactions were detected between rs520688 and rs2030324, recombination analysis revealed a combined effect of the two on the susceptibility to schizophrenia, with GA-TT decreasing and CT/CC-GG/GA increasing the risk of schizophrenia.
Conclusion: In conclusion, rs520688 in *NOTCH4* and rs2030324 in *BDNF* are significantly associated with schizophrenia among Han Chinese in Southern China. The two had a combined effect on the susceptibility to schizophrenia among Han Chinese in Southern China, but this may not be caused by an allele–allele interaction.

Keywords: BDNF, NOTCH4, schizophrenia, SNP, susceptibility

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Significant outcomes

- rs520688 in *NOTCH4* is associated with the susceptibility of schizophrenia.
- rs2030324 in brain-derived neurotrophic factor (*BDNF*) is associated with the susceptibility of schizophrenia.
- rs520688 and rs203032 have combined effects on the susceptibility to schizophrenia but this is not caused by allele–allele interaction.

Limitations

- To further validate these results, more case and control samples are needed.
- Further investigations into the functional consequence of *NOTCH4* and *BDNF* on schizophrenia would be very interesting.
- The single-nucleotide polymorphisms (SNPs) may act in haplotypes, and analyses of more SNPs are needed.

Introduction

Schizophrenia is a serious mental disorder with a lifetime prevalence rate of 1% in the general population worldwide (1,2). As the illness places heavy economic and social burdens on families and society, it is important to establish ways to treat and prevent schizophrenia. However, the pathogenesis of schizophrenia is unclear. Although there are clearly environmental contributors to the disease, genetic predisposition is the major determinant of who develops schizophrenia, with heritability estimates as high as 80% (3,4), placing schizophrenia among the most heritable of common diseases. Genome-wide association studies (GWAS) and candidate gene approaches to schizophrenia have produced many positive results (5), but most of these have poor reproducibility. Several studies have shown that *NOTCH4* and *BDNF* are associated with the pathophysiology of schizophrenia (6–11). In the first association study (6), we found that *NOTCH4* polymorphisms were associated with the risk of schizophrenia in British patients. Associations between *NOTCH4* and schizophrenia were replicated in other studies (7–9); however, the results of these studies are inconsistent across geographic regions. Furthermore, GWAS and meta-analyses revealed that *NOTCH4* SNPs are associated with schizophrenia (12).

BDNF is another well-defined schizophrenia-related gene. Decreased BDNF concentrations have been found in the cortical areas and the hippocampus of schizophrenics (13), and the concentration is also associated with the treatment of schizophrenia (14). SNPs in *BDNF* have been shown to play an important role in structural and functional plasticity in schizophrenia and are potential markers for schizophrenia prevention and target therapy (15). Both BDNF and NOTCH4 are involved in neural development; however, there is no report of a direct relationship between the two proteins.

To investigate the relationship between polymorphisms in *NOTCH4* and *BDNF* and schizophrenia, four SNPs (rs520688, rs415929, rs2030324, and rs12273539) in *NOTCH4* and *BDNF* were examined in a Han Chinese population from southern China, using the MassARRAY iPLEX platform. Gene–gene interactions were explored and a genotype recombination analysis was performed.

Materials and methods

Subjects

The subjects who participated in our study were recruited between July 2008 and September 2011 from Xiangya Hospital, Xiangya, Hunan Province, China. All participants were permanent residents of Hunan from the Han Chinese population. Clinical information on each subject was collected from medical records.

The study recruited 470 patients with schizophrenia diagnosed by psychiatrists using the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10), and the Chinese Classification and Diagnostic Criteria of Mental Disorders, 2nd Revision (CCMD-II-R). Blood samples were collected from the subjects. As genotyping failed for six samples, 464 case subjects were included in the final analysis. Their ages ranged from 16 to 62 years. In addition, blood samples were collected from 464 healthy controls selected randomly from outpatients ranging in age from 18 to 68 years, during the same period with no history of schizophrenia or other mental disease.

The study protocol was approved by the Clinical Research Ethics Committee of Xiangya Hospital. Written informed consent was obtained from the guardians of the participants.

DNA extraction

Peripheral blood samples were drawn from the participants at Xiangya Hospital, Xiangya, Hunan Province, China. The samples were delivered frozen by express mail to the School of Biotechnology, Southern Medical University, Guangzhou, Guangdong Province, China, and stored at −70°C. Genomic DNA was extracted from 200 μl of peripheral blood using a Genomic DNA Purification kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions and stored at −70°C until use.

Genotyping

All SNPs were genotyped using the Sequenom MassARRAY matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry platform (Sequenom, San Diego, CA, USA). Primers were designed using a semi-automated method (Assay Design 3.1, Sequenom). The primer sequences are listed in Table 1.

Statistical analyses

Hardy–Weinberg equilibrium (HWE) for the four SNPs in the control group was assessed using Haploview 4.2 (Daly Lab, Cambridge, MA, USA). Differences in genotype distributions between the cases and controls were evaluated by χ^2 analysis. Associations between the polymorphisms and risk of schizophrenia were estimated using odds ratios (ORs) and 95% confidence intervals (95% CIs) with binary logistic regression analyses, controlling for

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age and sex as covariates. Their associations with schizophrenia were analysed using the web-based tool SNPStats (http://bioinfo.iconcologia.net/SNPstats). $p < 0.05$ was considered statistically significant. Allele–allele interactions were tested by logic regression cross-validation and combination analyses between rs520688 and rs2030324 with an overall χ^2 test in a logistic regression.

Results

After adjusting for age and gender, all SNPs were in HWE, except rs520688, which was out of HWE for both the patients and controls. The distributions of the rs415929, rs520688, rs2030324, and rs12273539 polymorphisms in the schizophrenia and control groups are shown in Table 2. For *BDNF* rs2030324, the case group was 33.3% TT and 66.7% CT-CC, which differed significantly from the controls (39.7% TT and 60.3% CT-CC). The CC/CT genotype in rs2030324 increased the risk of schizophrenia with an OR of 1.34 (95% CI, 1.01–1.78, $p = 0.044$). For *NOTCH4* rs520688, the case group was 79.9% AA-GG and 20.1% GA, which differed significantly from the controls (72.8% AA-GG and 27.2% GA). The GA genotype of rs520688 ($p = 0.035$) decreased the risk of schizophrenia with an OR of 0.71 (95% CI, 0.51–0.98, $p = 0.035$). The genotype distributions of rs415929 in *NOTCH4* and rs12273539 in *BDNF* did not differ significantly between the case and control groups.

Gene–gene combinations can be tested using an overall χ^2-test in a logistic regression (16). Recombination of rs520688 and rs2030324 was analysed using this method. There was a significant difference ($\chi^2 = 12.461$, $p = 0.006$); AA/GG-CT/CC increased the risk of schizophrenia with an OR of 1.424 ($p = 0.022$, 95% CI = 1.051–1.398; Table 3).

If two SNPs have a combined effect, it might arise because they have a biological interaction, which

Table 1. PCR primers designed using Sequenom MassARRAY Assay Design 3.1

Primer	Forward	Reverse	Extension
rs12273539	ACGTTGGATGCCTCACCCTTCTTAGCTAAC	ACGTTGGATGAATGTGTACGGCACTCCAAG	GCTTCATCACTTCTGCTC
rs520688	ACGTTGGATGATTGGTGGCTGACACAGTTG	ACGTTGGATGACAGCTGATCCATGACCCTG	TGGATTCACCTCACAGCC
rs2030324	ACGTTGGATGACTCCAAACATCACACAGCC	ACGTTGGATGGGGTTTCAGGACATTGAATC	TGAGTCTCAAAAATAAGCTAA
rs415929	ACGTTGGATGAACCCATGTGTTAATGGAGG	ACGTTGGATGCATCACGTTCACAGGCATGG	CCGCGGGGTGTGTCTGGCCAC

PCR, polymerase chain reaction.

Table 2. Distribution of the rs415929, rs520688, rs2030324, and rs12273539 polymorphisms in schizophrenia and control groups

rsID	Gene	Model	Genotype	Case (%)	Control (%)	OR (95% CI)	*p*-value
415929	*NOTCH4*	Codominant	A/A	288 (67%)	285 (61.6%)	1	0.45
			A/G	126 (29.3%)	160 (34.6%)	0.83 (0.62–1.11)	
			G/G	16 (3.7%)	18 (3.9%)	0.88 (0.43–1.80)	
520688	*NOTCH4*	Codominant	A/A-G/G	342 (79.9%)	335 (72.8%)	1	**0.035**
			G/A	86 (20.1%)	125 (27.2%)	0.71 (0.51–0.98)	
2030324	*BDNF*	Dominant	T/T	143 (33.3%)	184 (39.7%)	1	**0.044**
			C/T-C/C	287 (66.7%)	279 (60.3%)	1.34 (1.01–1.78)	
12273539	*BDNF*	Codominant	C/C	299 (70.3%)	319 (68.9%)	1	0.74
			C/T	111 (26.1%)	130 (28.1%)	0.91 (0.67–1.23)	
			T/T	15 (3.5%)	14 (3%)	1.16 (0.54–2.49)	

OR, odds ratio.
Bold values indicate $p < 0.05$.

Table 3. Combination analysis of rs520688 and rs2030324 between the case and control groups

Genotype	GA-TT	GA-CT/CC	AA/GG-CT/CC	AA/GG-TT
Case	26 (5.6%)	65 (14.1%)	242 (52.4%)	129 (27.9%)
Control	39 (8.5%)	86 (18.6%)	191 (41.4%)	145 (31.5%)
OR (95% CI)[a]	0.749 (0.432–1.298)	0.850 (0.570–1.268)	1.424 (1.051–1.398)	1
p-value	0.303	0.424	**0.022**	/

OR, odds ratio.
[a] OR (95% CI) was adjusted for age.
Bold value indicate $p < 0.05$.

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Table 4. Allele–allele interaction analysis between rs520688 and rs2030324 ($\chi^2 = 12.461$, $p = 0.006$)

			Wald χ^2-test		OR estimates
Genotype	Estimate	Standard error	χ^2	Pr > χ^2	OR (95% CI)[a]
AA/GG-TT	−0.117	0.121	0.933	0.334	
GA-TT	−0.289	0.281	1.057	0.303	0.749 (0.432–1.298)
GA-CT/CC	−0.163	0.204	0.638	0.424	0.850 (0.570–1.268)
AA/GG-CT/CC	0.354	0.155	5.206	**0.022**	1.424 (1.051–1.398)

OR, odds ratio.
[a] OR (95% CI) was adjusted for age.
The bold values indicate $p < 0.05$.

can be tested using logic regression cross-validation (16). As both BDNF and NOTCH4 are involved in neural development, there may be an interaction between *NOTCH4* rs520688 and *BDNF* rs2030324. To confirm this hypothesis, the risk of rs520688 and rs2030324 and the interaction between rs520688 and rs2030324 were analysed using logic regression cross-validation; however, no significant result was obtained ($\chi^2 = 0.933$, $p = 0.334$; Table 4).

Discussion

NOTCH4, which is located on chromosome 6p21.3, is associated with neuronal development. The genetic knockout of *NOTCH4* and *NOTCH1* leads to abnormal vascular morphogenesis in mouse embryos (17). *BDNF*, which is located on chromosome 11p13, is expressed in neurons and endothelial cells during development (18). During the development of the cerebral cortex and hippocampus, BDNF induces the differentiation of neural stem cells into neurons and promotes the survival of newly generated neurons (19,20). BDNF expression in endothelial cells is involved in neurogenesis in the canary song system (21). *BDNF* also plays an important role in preventing the death of neurons during development, and it promotes cell survival under stressful conditions (22).

Studies show that schizophrenia is related to a reduction in the soma of prefrontal cortex neurons (23–25). In addition, the distribution of neurons in the prefrontal cortex is altered in schizophrenia, with fewer surface white matter and grey matter neurons and more deep white matter neurons (26,27). Numerous studies have shown that schizophrenia involves apoptosis via an abnormal pathway, changing the apoptotic activity of neurons and glial cells (28,29). BDNF and NOTCH4 are expressed in neurons and endothelial cells during development and are closely related to neuron development. *BDNF* and *NOTCH4* SNPs are associated with the susceptibility of individuals to schizophrenia (6–12). However, schizophrenia is caused by the interaction of many genes and environmental factors. To study the association between susceptibility genes and schizophrenia, it is insufficient to analyse just one SNP in a single gene, as this cannot describe the exact relationship between the disease and the gene. Analyses of SNP–SNP, gene–gene, and gene–environment interactions are also necessary. This study examined *NOTCH4* and *BDNF* as candidate genes and SNPs as genetic markers in a case–control study using correlation analysis to analyse the relationship between polymorphisms in *NOTCH4* and *BDNF* and schizophrenia. Four SNPs (rs520688 and rs415929 in *NOTCH4* and rs2030324 and rs12273539 in *BDNF*) were genotyped in 464 schizophrenics and 464 healthy controls from Southern China using the Sequenom MassARRAY® iPLEX platform. Of these four SNPs, rs520688 in *NOTCH4* and rs2030324 in *BDNF* were significantly associated, whereas rs415929 and rs12273539 had no associations with schizophrenia in our study population. Furthermore, there were significant differences in the combinations of genotypes and model testing. The AA/GG-CC/CT genotype was more frequent in the cases, indicating that it is a risk marker for schizophrenia. If two SNPs interact, this may be caused by a biological interaction; however, no significant allele–allele interaction was found between rs520688 and rs2030324. Consequently, the combined effect of rs520688 and rs2030324 may not be caused by a functional interaction between NOTCH4 and BDNF.

The combined effects of rs520688 and rs2030324 on the susceptibility to schizophrenia imply that *NOTCH4* and *BDNF* are closely linked to schizophrenia. These results may not only help to reveal the mechanism of action of NOTCH4 and BDNF in schizophrenia, but also provide data for developing individualised therapy for schizophrenia.

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