INTRODUCTION

As a common and severe mental disease, schizophrenia affects approximately 1% of the worldwide population and continues to be one of the least understood psychiatric disorders.\(^1\) Family, twin, and adoption studies have strongly indicated schizophrenia with a high heritability estimate of approximately 80%. Recent genome-wide association studies suggested that schizophrenia has a substantial polygenic component involving numerous common alleles of very small effects.\(^2\) During the last decades, the idea of schizophrenia as a neurodevelopmental disorder has been an attractive and most popular hypothesis.\(^3\) Patients with schizophrenia display various neurodevelopmental abnormalities. Numerous identified schizophrenia susceptibility genes are indispensable in the regulation of neurodevelopment.\(^4\) It has been well documented that cytoarchitectural anomalies are present in the brains of schizophrenia patients which may reflect synaptic organization perturbations during neurodevelopment.\(^5\) Synaptic pathology of schizophrenia suggests that alterations of synaptic transmission and neuronal connectivity may be one of the core features of...
schizophrenia. It has been reported that numerous proteins affecting synaptic functions change in schizophrenia. One of these proteins is the neuronal growth-associated protein GAP43, a membrane phosphoprotein with high expression level in the developing brains, and acts fundamentally in the alteration of the brain structure and function. GAP43 is involved in the initial establishment and reorganization of synaptic connections. Even though GAP43 expression sharply decreases after the maturation of synaptic connections in most neurons, synapses located in the limbic system and the neocortex remain in high GAP43 level throughout life. The enrichment of GAP43 in higher integrative regions, such as association cortices and hippocampus, indicates that GAP43 functions fundamentally in specialized functions in these areas, for example, acquisition, processing, and storing of new information. With dysfunctional learning and memory, animals characterized with cognitive impairment, following contextual fear conditioning or exposed to alcohol, displayed abnormal GAP43 expression and phosphorylation in the hippocampus. Due to the alteration of these crucial processes in schizophrenia and expression difference of GAP43 between schizophrenia patients and normal controls, GAP43 has been related to schizophrenia since two decades ago.

Genetic studies have demonstrated that schizophrenia is a complex disorder with a considerable high degree of genetic contribution to its etiology with conservative estimates of heritability of ~80%. The human GAP43 gene is mapped to chromosome 3q13.1–2, a potential susceptibility region for schizophrenia. In the current study, an independent case–control study was applied to investigate the genetic association of the human GAP43 gene with schizophrenia in a Northeast Chinese Han population with 741 schizophrenia patients and 1330 healthy controls.

**METHODS**

**Subjects**
Participants were all recruited in the Northeast China from the Department of Psychiatry, Dalian Seventh People’s Hospital, P. R. China and unrelated Chinese Han nationality born. The sample set included 741 schizophrenia patients (347 males and 394 females; mean age: 31.7 ± 7.9 years) and 1330 healthy controls (649 males and 681 females; mean age: 30.2 ± 13.4 years). The consensus diagnoses were made by at least two experienced psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition criteria (American Psychiatric Association, 2000) on the basis of clinical observation, medical records, and family information. All healthy controls in this study were without any Diagnostic and Statistical Manual of Mental Disorders, fourth edition axis I disorder and matched for age, sex, education, and ethnicity to the patients. The objectives and procedures of the study were explained to all participants, and written informed consent was obtained. The study was approved by the Ethics Committee of the Dalian Seventh People’s Hospital, P. R. China.

**Genotyping**
Eleven single-nucleotide polymorphisms (SNPs) in the human GAP43 gene region were selected. Genomic DNA was extracted from venous blood using a commercially QiAamp DNA Blood Mini Kit (QIAGEN, German). All the SNPs were genotyped by either polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis or direct DNA sequencing. All pairs of PCR primers were designed by software Primer Premier 5.0 (Premier, Canada). PCR products were either completely digested with 4U of restriction enzyme overnight and then separated by agarose gel electrophoresis (2%–3% gel) stained with ethidium bromide or sequenced on an ABI PRISM 377–96 DNA Sequencer (Applied Biosystems, Foster City, California, USA) after purifying them using a BigDye terminator cycle sequencing ready reaction kit. All the results were read by two experienced technicians independently.

**Statistics**
The deviation of the genotypes from Hardy–Weinberg equilibrium was tested using a χ² goodness-of-fit test. Statistical differences in allelic distribution between patients and controls were evaluated by Pearson’s Chi-square test. Pairwise linkage disequilibrium (LD) between any two alleles was evaluated using the D’ value. Odds ratios (ORs) and their 95% confidence intervals (95%) were calculated to evaluate the effect of different alleles. The Bonferroni correction was applied for multiple tests analyzing the independent variables to control inflation of the Type I error rate according to an effective number of independent marker loci. The statistic power of our sample size was calculated by the genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). The sample had approximately 80% power to detect allele frequency differences assuming an OR of 1.5 with a minor allele frequency of 0.1. These analyses were performed using SHEsis software (http://analysis.bio-x.cn/SHEsisMain.htm). Results were considered statistically significant at two-tailed P < 0.05.

**RESULTS**
Eleven SNPs of the human GAP43 were genotyped in a Northeast Chinese Han population. Genotype distributions of the 11 SNPs in case and control groups showed no significant deviations from Hardy–Weinberg equilibrium. There were no significant differences in age, sex, or education distributions between the case and control groups. The genetic association results are shown in Table 1. Of the 11 SNPs, three showed significant differences in allele and genotype frequencies between cases and controls (rs2028248, allelic P = 0.0413, genotypic P = 0.466; rs6790048, allelic P = 0.0276, genotypic P = 0.0330;
Table 1: Genotype and allele frequencies of 11 single-nucleotide polymorphisms in the GAP43 gene between schizophrenia patients and controls

| Marker     | Participants | Genotype and frequency* | $\chi^2$ | Allele and frequency* | $\chi^2$ | OR (95% CI) |
|------------|--------------|--------------------------|---------|-----------------------|---------|-------------|
| rs13059137 | Cases        | GG (304 (0.411)) GT (350 (0.473)) TT (86 (0.116)) | 2.2357  | G (958 (0.647)) T (522 (0.353)) | 1.4240  | 1.0840 (0.9495-1.2376) |
|            | Controls     | AA (527 (0.397)) AG (617 (0.464)) GG (185 (0.139)) | 0.3270  | A (1671 (0.629)) G (987 (0.371)) | 0.2237  |             |
| rs1837206  | Cases        | GG (191 (0.258)) GT (359 (0.484)) TT (191 (0.258)) | 1.3594  | G (741 (0.500)) T (741 (0.500)) | 0.4224  | 0.9587 (0.8443-1.0887) |
|            | Controls     | AA (342 (0.257)) AG (673 (0.506)) GG (314 (0.236)) | 0.5068  | A (1357 (0.511)) G (1301 (0.489)) | 0.5157  |             |
| rs2028248  | Cases        | GG (151 (0.204)) GT (375 (0.506)) TT (215 (0.290)) | 6.1310  | G (677 (0.457)) T (805 (0.543)) | 4.1625  | 0.8758 (0.7711-0.9948) |
|            | Controls     | AA (335 (0.252)) AG (633 (0.476)) GG (362 (0.272)) | 0.0466  | A (1303 (0.490)) G (1357 (0.510)) | 0.0413  |             |
| rs10934301 | Cases        | GG (62 (0.084)) GT (314 (0.424)) TT (365 (0.493)) | 1.8805  | G (438 (0.296)) T (1044 (0.704)) | 0.0095  | 0.9931 (0.8641-1.1414) |
|            | Controls     | AA (130 (0.098)) AG (530 (0.398)) GG (670 (0.504)) | 0.3905  | A (790 (0.297)) G (1870 (0.703)) | 0.9222  |             |
| rs6790048  | Cases        | GG (291 (0.393)) GT (350 (0.472)) TT (100 (0.135)) | 6.8275  | G (932 (0.629)) T (550 (0.371)) | 4.8558  | 1.1583 (1.0163-1.3201) |
|            | Controls     | AA (488 (0.367)) AG (604 (0.454)) GG (238 (0.179)) | 0.0330  | A (1580 (0.594)) G (1080 (0.406)) | 0.0276  |             |
| rs2164930  | Cases        | GG (582 (0.785)) GT (151 (0.204)) TT (8 (0.011)) | 11.0442 | G (1315 (0.887)) T (167 (0.113)) | 9.8064  | 1.3610 (1.1216-1.6514) |
|            | Controls     | AA (977 (0.735)) AG (314 (0.236)) GG (39 (0.029)) | 0.0400  | A (2268 (0.853)) G (392 (0.147)) | 0.0017  | 0.9996 (0.9185-1.0861) |
| rs11926976 | Cases        | GG (77 (0.104)) GT (331 (0.447)) TT (333 (0.449)) | 3.7272  | G (485 (0.327)) T (997 (0.673)) | 3.6608  | 0.8771 (0.7668-1.0032) |
|            | Controls     | AA (168 (0.126)) AG (613 (0.461)) GG (549 (0.413)) | 0.1551  | A (949 (0.357)) G (1711 (0.643)) | 0.0557  |             |
| rs16823991 | Cases        | GG (79 (0.107)) GT (322 (0.435)) TT (340 (0.459)) | 0.5392  | G (480 (0.324)) T (1002 (0.676)) | 0.5423  | 1.0525 (0.9185-1.2061) |
|            | Controls     | AA (132 (0.099)) AG (568 (0.427)) GG (630 (0.474)) | 0.7637  | A (832 (0.313)) G (1828 (0.687)) | 0.4615  |             |
| rs13065936 | Cases        | GG (440 (0.594)) GT (269 (0.363)) TT (32 (0.043)) | 3.3918  | G (1149 (0.775)) T (333 (0.225)) | 2.5160  | 1.1295 (0.9717-1.3129) |
|            | Controls     | AA (755 (0.568)) AG (494 (0.371)) GG (81 (0.061)) | 0.1834  | A (2004 (0.753)) G (656 (0.247)) | 0.1128  |             |
| rs3772934  | Cases        | GG (434 (0.586)) GT (252 (0.340)) TT (55 (0.074)) | 2.8491  | G (1120 (0.756)) T (362 (0.244)) | 1.5739  | 0.9089 (0.7829-1.0552) |
|            | Controls     | AA (800 (0.602)) AG (456 (0.343)) GG (74 (0.056)) | 0.2406  | A (2056 (0.773)) G (604 (0.247)) | 1.2096  |             |

Significant $P (<0.05)$ are in boldface. *Frequencies are shown in parenthesis; $^*$P value after the strict Bonferroni correction. OR – Odds ratio; CI – Confidence interval

rs2164930, allelic $P = 0.001747$, genotypic $P = 0.0040$.

After the Bonferroni correction, some differences in the allele frequencies remained significant which are also shown in Table 1. To further analyze the haplotype structure, we computed pairwise D’ for the 11 SNPs of GAP43 in our sample set using standardized measure D’. D’ ranging between 0.8 and 1.0 indicated strong LD between each other. There was a strong LD between three SNPs (rs2164930, rs11926976, and rs16823991); thus, the haplotype analyses were carried out. Eight different allele combinations of the three SNPs were found in the case and control groups. However, there were only four predominant allele combinations (distribution frequencies >0.01). Haplotype consisting of the three SNPs showed a significant association with schizophrenia regarding the global $\chi^2$ value or individual haplotypic $\chi^2$ values (global: $\chi^2 = 8.6915, P = 0.0037$; G-C-G: $\chi^2 = 8.638, P = 0.0033$).

**DISCUSSION**

In the present study, 11 SNPs of human GAP43 gene were investigated to establish their roles in susceptibility to schizophrenia in a Northeast Chinese Han population. Our data showed that three SNPs (rs2028248, rs6790048, and rs2164930) were significantly associated with schizophrenia. Haplotype analysis indicated a significant difference in strong LD block (rs2164930-rs11926976-rs16823991) between case and control groups. Combining our results, it is suggested that the GAP43 gene might be involved in susceptibility to schizophrenia.

The synaptic hypothesis of schizophrenia has been proposed for decades, indicating that abnormal synaptic sprouting and reorganization with subsequent misconnecting might be a core feature of schizophrenia.[11] The human GAP43 gene
encodes a protein mainly present in the synaptic plasma membranes playing a crucial role in axons specifically associated with growth cones and immature synaptic terminals.[10] GAP43 exclusively locates in particulate fractions of the brain tissue with high level in neocortical association areas and limbic system rich in synaptic contacts. GAP43 is involved in regenerated synaptic sprouting after injury. Upregulated levels of GAP43 have been found in association cortices in schizophrenia, indicating a role of GAP43 in preparing the synaptic connection perturbations in associative brain areas of schizophrenia. The comparison of the GAP43 protein level in postmortem samples between schizophrenia patients and healthy controls revealed that GAP43 expression increased in specific associative regions in schizophrenic brains. GAP43 expression levels altered in the frontal cortex, hippocampus, dorsolateral prefrontal cortex, primary visual cortex, and anterior cingulate gyrus in schizophrenia.[14-17,23] GAP43 has also been related to various other neural and mental diseases, such as multiple sclerosis, Alzheimer’s disease, and Parkinson’s disease.[24-27]

A previous genetic and functional study focusing on GAP43 gene and schizophrenia found no association between selected GAP43 polymorphisms and schizophrenia in a Southern Chinese Han population from Taiwan.[18] However, some rare variants of the GAP43 gene have been indicated with possible damaging effects may be involved in their sample. In this previous study, Shen et al. mainly focused on the functional exonic regions of GAP43 without considering conserved intronic regions which may include regulatory elements, and the sample size of their sample set was relatively limited. Moreover, even though the population in their study was also Chinese Han descents, the genetic difference between populations from Northern and Southern parts of China has been scientifically verified and declared. Usually, genotype and genotype frequencies showed a remarkable difference between different ethnic groups, and the genetic polymorphisms appear on different positions along the same gene location. In consequence, a specific SNP may be associated with schizophrenia in one population, whereas showed no difference in another ethnic population or even with no polymorphism exists on the allele. Nevertheless, there are various genes associated with schizophrenia in different ethnic populations, even though significant differences revealed at alternative alleles along the gene location. A number of studies showed genes associated with schizophrenia in both Chinese and Indian populations, such as NRG1, DISC1, PDLIM5, GRM3, and DRD2; however, different positive alleles were reported in these studies for one same gene. Therefore, the genetic involvement of the GAP43 gene requires further intensive studies in other ethnic groups or some subtypes of schizophrenia.

**Limitations**

Association analysis of more markers in the GAP43 gene in other independent samples and family-based association studies in more ethnic populations will be required to further strengthen that the GAP43 gene exerts in genetic susceptibility to schizophrenia. In addition, biological and functional analyses are needed to investigate the impact of the risk variants on the pathogenesis of schizophrenia.

**CONCLUSION**

Our study indicated an association of the human GAP43 gene with schizophrenia in a Northeast Chinese Han population, which may provide genetic clues and promote further biophysical studies for the function of GAP43 protein in schizophrenia and other mental diseases.

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**Conflict of interest**

There are no conflicts of interest.

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