Association between single nucleotide polymorphisms in MiR219-1 and MiR137 and susceptibility to schizophrenia in a Chinese population

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1. Introduction

Schizophrenia is one of the most common mental disorders to severely affect human health worldwide. Single nucleotide polymorphisms (SNPs) within related genes are candidate susceptible factors for the disorder. Rs107822 within MiR219-1 and rs1625579 within MiR137 were genotyped in 589 cases and 622 controls to investigate the possible association between the loci and schizophrenia in a Chinese population. Our results showed significant association between rs107822 and the disorder in allele (C vs. T: adjusted OR = 0.773, 95%CI = 0.655–0.912), co-dominant (TC vs. TT: adjusted OR = 0.734, 95%CI = 0.571–0.943; CC vs. TT: adjusted OR = 0.655, 95%CI = 0.459–0.936), dominant (TC + CC vs. TT: adjusted OR = 0.707, 95%CI = 0.559–0.895), and recessive (CC vs. TC + TT: adjusted OR = 0.724, 95%CI = 0.524–0.999) models, respectively. Meanwhile, negative associations were also observed between rs107822 and the disorder in male and female subgroups, and genotype CC of the locus was significantly associated with a lower positive symptom score of PANSS compared to genotype TT carrier in the cases group. However, we didn’t observe a significant association between rs1625579 and the disorder. These findings indicate that rs107822 within MiR219-1 might be involved in pathogenesis of schizophrenia and that genotypes TC, CC and allele C of the locus are protective factors for schizophrenia in a Chinese population.

Abstract

Schizophrenia is one of the most common mental disorders to severely affect human health worldwide. Single nucleotide polymorphisms (SNPs) within related genes are candidate susceptible factors for the disorder. Rs107822 within MiR219-1 and rs1625579 within MiR137 were genotyped in 589 cases and 622 controls to investigate the possible association between the loci and schizophrenia in a Chinese population. Our results showed significant association between rs107822 and the disorder in allele (C vs. T: adjusted OR = 0.773, 95%CI = 0.655–0.912), co-dominant (TC vs. TT: adjusted OR = 0.734, 95%CI = 0.571–0.943; CC vs. TT: adjusted OR = 0.655, 95%CI = 0.459–0.936), dominant (TC + CC vs. TT: adjusted OR = 0.707, 95%CI = 0.559–0.895), and recessive (CC vs. TC + TT: adjusted OR = 0.724, 95%CI = 0.524–0.999) models, respectively. Meanwhile, negative associations were also observed between rs107822 and the disorder in male and female subgroups, and genotype CC of the locus was significantly associated with a lower positive symptom score of PANSS compared to genotype TT carrier in the cases group. However, we didn’t observe a significant association between rs1625579 and the disorder. These findings indicate that rs107822 within MiR219-1 might be involved in pathogenesis of schizophrenia and that genotypes TC, CC and allele C of the locus are protective factors for schizophrenia in a Chinese population.

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schizophrenia in a genome wide association study recently [24]. However, emerging case-control studies showed an inconsistent result in Chinese population [25–28].

MiR-219-1 is an important regulator in n-methyl-D-aspartate glutamate receptors (NMDAR)-mediated glutamate signal pathway [29]. The pathway is involved in synaptic plasticity and fast neurotransmission in brain, and disruption of its signal pathway can lead to schizophrenia [30,31]. Calcium/calmodulin-dependent protein kinase II gamma subunit (CAMKII) is a component of the signal pathway, and it is a targeted gene of miR-219-1. Inhibition of miR-219-1 in the murine brain can significantly modulate behavioral responses by disrupting NMDAR signal transmission in vivo [29]. Rs107822 is an allele T/C alternated polymorphism which is located in flanking sequence-37 bp of pre-miR-219-1. Since emerging studies showed that SNP in miRNA or 3′-UTR of its targeted gene could affect the transcription process or interaction between hsa-miR-219-1 and mRNA 3′-UTR of its targeted gene. So we hypothesized that rs107822 within pri-miR-219-1 would affect its structure or expression, leading to neuropsychiatric disorders such as schizophrenia.

In order to investigate the association between rs 107822 within MiR-219-1 and rs1625579 of MiR-137 and susceptibility to schizophrenia, a hospital-based case-control study including 589 clinical confirmed schizophrenia and 622 healthy checking-up individuals was carried out in present study.

2. Materials and methods

A total of 589 clinical confirmed and unrelated schizophrenia patients (275 males: mean age = 39.51 ± 14.15; 314 females: mean age = 43.33 ± 14.82 years) and 622 healthy checking-up individuals (291 males: mean age = 39.70 ± 13.3 years; 331 females: 41.08 ± 14.3 years) with free of clinical symptom and without any other disease from January of 2014 to March of 2015 in the Nantong Fourth People’s Hospital were recruited in present study. The psychiatric evaluation of each patients were performed by three psychiatrists according to the DSM-IV criteria for schizophrenia and psychotic symptoms were evaluated according to structured interviews for the Positive and Negative Symptom Scale (PANSS). The controls were healthy checking-up individuals without personal or family history of neuropsychiatric disorder or any abuse of addictive drug. All included individuals are of Chinese Han nationality, which consists of more than 95% of the general population. 1 ml heparin-anticoagulated peripheral blood sample of each participant was collected and stored at –80 °C till extraction. The baseline characteristics such as sex, age, smoking and drinking were retrieved from medical record. This study was approved by the Ethical Committee of Nantong Fourth People’s Hospital and all written informed consents were obtained from all participants.

Human genomic DNA was extracted from 200 μl heparin-anticoagulated peripheral blood using Tiangen human blood genome isolation Kit (Tiangen, Beijing, China) according to the manufacturer’s protocol. DNA concentration and purity were detected by Ultraviolet spectrophotometer (Eppendorf, Hambrug, German). DNA concentration of all eligible sample should be higher than 200 ng/μl and A260/A280 ratio should be in the interval of 1.8–2.1.

Genotypes of rs1625579 and rs107822 were detected by TaqMan genotyping discrimination assay using ABI7500 PCR system (Applied Biosystems, Foster City, USA). The detection was performed in a total volume of 5 μl which contained 10 ng genomic DNA template, 2.5 μl 2′ TaqMan PCR MasterMix, 2.5 μl 20′ SNP Assay (including primer and FAM/VIC probe). After initial denaturation at 95 °C for 10 min, samples were amplified through 40 cycles (92 °C for 15 s, 60 °C for 1 min). Primer and probe sequences of the two loci were used according to the description by Guella et al. [32] and Cheong et al. [33], respectively. In order to validate the result, 5% PCR products were randomly selected to DNA sequencing.

Genotype frequencies of the two loci in case and control groups were obtained by counting. Hardy–Weinberg equilibrium (HWE) software was used to evaluate the genotypes of the loci in controls whether or not was fit for HWE. Two-sided student T test was selected to compare the difference in quantitative data and personal chi-square test was used to examine distribution difference of allele or genotype in two groups. Odds ratios (ORs) and 95% confidence intervals (95%CIs) were selected to estimate the strength between the two loci and risk of schizophrenia. All statistics were performed using SPSS software 17.0 (SPSS Inc., Chicago, IL, USA) and p < 0.05 was considered as statistical significance.

3. Results

DNA concentration of all samples was higher than 200 ng/μl, and purity was in the interval of 1.8–2.1, thus all samples were included in our study. The baseline characteristics of two groups were described in Table 1. Clinical global impression rating scales (CGI) in cases was 5.31 ± 0.60, and there was no significant difference in age, sex, status of smoking and drinking, BMI, Glu, TG, CHO, LDL, and HDL in two groups.

The genotype and allele distributions of rs107822 and rs1625579 in cases and controls were described in Table 2. p-Value of HWE in two SNPs were larger than 0.05 (χ² = 0.002, p-value = 0.988 for rs107822; χ² = 0.004, p-value = 0.952 for rs1625579), suggesting that genotype distributions of the loci were conformed to HWE. Genotype TT, TC, CC and allele T, C frequencies of rs107822 within MiR-219-1 in case and control groups were 43.1% and 35.2%, 44.3% and 48.2%, 12.6% and 16.6%, 65.3% and 59.3%, 34.7% and 40.7%, respectively. Comparing to genotype TT of the locus, frequencies of genotype TC (p = 0.022, adjusted OR = 0.734, 95%CI = 0.571–0.943) and CC (p = 0.007, adjusted OR = 0.655, 95%CI = 0.459–0.936) were significantly decreased in cases, and a negative association was examined between allele C of the locus and risk of schizophrenia (p = 0.003, adjusted OR = 0.773, 95%CI = 0.655–0.912). The significant association was also observed in dominant (p = 0.005, adjusted OR = 0.707, 95% CI = 0.559–0.885), recessive (p = 0.049, adjusted OR = 0.724, 95% CI = 0.524–0.999) models. However, we did not observe any significant difference of genotype and allele frequencies of MiR-137 rs1625579 between two groups in co-dominant, dominant, recessive, over-dominant and allele models.

When stratifying cases into male and female subgroups, genotype CC (13.4% vs. 20.6%, p = 0.022, adjusted OR = 0.571, 95% CI = 0.352–0.928), and allele C (35.1% vs. 41.4%, p = 0.028, adjusted OR = 0.765, 95%CI = 0.600–0.975) of rs107822 were significantly associated with a decreased risk of schizophrenia in male subgroup. Meanwhile, negative associations were also observed between rs107822 and the disorder in co-dominant (45.2% vs. 54.1%, p = 0.010, adjusted OR = 0.640, 95%CI = 0.460–0.897 for TC vs. TT), dominant (57.0% vs. 67.1%, p = 0.009, adjusted OR = 0.650, 95%CI = 0.440–0.901), over-dominant (45.2% vs. 54.1%, p = 0.027, adjusted OR = 0.700, 95%CI = 0.516–0.960) and allele (34.4% vs. 40.0%, p = 0.038, adjusted OR = 0.780, 95%CI = 0.623–0.989) genetic models in female subgroups (Table 3).

Results of association between rs107822, rs1625579 and PANSS scores were showed in Table 4. As shown in Table 4, rs1625579 was not associated with total, positive symptom, negative symptom and general symptom scores in case group. Whereas, only positive symptom score in patient harbored genotype CC of rs107822 was significantly lower than those carrying genotype TT.
Genotype and allele distributions of rs107822 within miR-219-1 and rs1625579 of miR-137 in cases and controls.

Clinical features of case and control groups in present study.

In our study, we conducted a case-control study including 589 clinical confirmed schizophrenia patients and 622 healthy checking-up controls to investigate the association between rs107822 within MiR-219-1 and rs1625579 within MiR-137 and risk of the disorder. Our results showed that rs107822 were significantly associated with a decreased risk of schizophrenia in co-dominant, dominant, recessive and allele models, there was a negative association between the locus and the disorder in male and female subgroups, and genotype CC of the locus were significantly associated with a lower positive symptom score of PANSS comparing to genotype TT in cases group, indicating that rs107822 within MiR-219-1 was involved in pathogenesis of schizophrenia, it might be a susceptible locus and genotype TT and allele C of the locus might be protective factors for this disorder.

4. Discussion

Data from emerging evidence supports the general consensus that schizophrenia is a heterogeneous and complex disorder with genetic, environmental, psychological and social components [34]. The disorder is leaded by dysfunction of related protein encoding and non-coding genes that are involved in initiation and progression of the disease. MiR-137 and MiR-219-1 are involved in regulation of neuron development and maturation [20–22] as well as NMDAR-signal pathway in synaptic plasticity and brain fast neurotransmission [30,31], respectively. Genetic variations of the two miRNAs may affect transcription process or binding ability between the miRNA and its targeted gene and contribute to aberrant expression of them, eventually triggering onset of neuropsychiatric disorders including schizophrenia.

In our study, we conducted a case-control study including 589 clinical confirmed schizophrenia patients and 622 healthy checking-up controls to investigate the association between rs107822 within MiR-219-1 and rs1625579 within MiR-137 and risk of the disorder. Our results showed that rs107822 were significantly associated with a decreased risk of schizophrenia in co-dominant, dominant, recessive and allele models, there was a negative association between the locus and the disorder in male and female subgroups, and genotype CC of the locus were significantly associated with a lower positive symptom score of PANSS comparing to genotype TT in cases group, indicating that rs107822 within MiR-219-1 was involved in pathogenesis of schizophrenia, it might be a susceptible locus and genotype TT and allele C of the locus might be protective factors for this disorder. Rs107822 has been reported to affect the biological process from pri-miRNA–3’-UTR to regulate transcription and translation of the targeted gene [38]. Since CAMKIIG is an important downstream signal molecular in NMDAR signal pathway [39] and aberrant expression of CAMKIIG contributed to dysfunction of NMDAR signal pathway [40], leading to occurrence of schizophrenia.

However, we did not observe the significant association between rs1625579 within MiR-137 and susceptibility to schizophrenia in our study. The results showed that rs1625579
was unlikely to have a major relevance to this disorder and genotype and allele of the locus couldn't be susceptible factors for the disease. Yuan et al reported that either allele or genotype of the locus couldn't be susceptible factors for the disease and geno-

type TT, TC, and allele C of the locus were protective factors.
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