Nucks1 gene polymorphism rs823114 is associated with the positive symptoms and neurocognitive function of patients with schizophrenia in parts of southern China

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Nuclear casein kinase and cyclin-dependent kinase substrate 1 (nucks1) are considered a potential susceptibility gene for certain neurological diseases, such as Parkinson's disease (PD). In our study, we genotyped three single nucleotide polymorphisms (SNPs) (rs4951261, rs823114 and rs951366) of the nucks1 gene in 774 schizophrenic patients and 819 healthy controls using the improved multiplex ligation detection reaction (imLDR) technique. Furthermore, we also studied the relationship between the above SNPs and the clinical psychiatric symptoms and neurocognitive function of the patients. Genotype distributions and allele frequencies of these SNPs showed no significant differences and were found between patients and healthy controls. However, in an analysis of the positive symptom score of rs823114 among male patients, we found that the score of the A/A genotype was lower than that of the G/A+G/G genotypes ($P = 0.001$, P(corr) $= 0.003$]. Additionally, we also found that among the female patients, G allele carriers with rs823114 had lower semantic fluency scores than subjects with the A/A genotype ($P = 0.010$, P(corr) $= 0.030$]. Our data show for the first time that rs823114 polymorphism of nucks1 may affect positive symptoms and neurocognitive function in patients with schizophrenia in parts of southern China. ***Psychiatr Genet*** **31: 119–125**

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Introduction

Schizophrenia is a serious disabling mental illness that affects approximately 1% of the world's population (Watkins and Andrews, 2016; Feigenson *et al*., 2014). The disease is characterized by a suite of positive, negative and cognitive symptoms (Khandaker *et al*., 2015). The positive symptoms of schizophrenia include visual and/or auditory hallucinations, delusions, delusions and major thought disorders. Its cognitive symptoms are mainly manifested as working memory impairment, executive dysfunction and inability to sustain attention (Meyer *et al*., 2011).

Studies have shown the role of oxidative stress and subsequent DNA damage among the development of schizophrenia (Boskovic *et al*., 2011; Yao and Reddy, 2011; Nordholm *et al*., 2016). The mechanisms of oxidative stress in schizophrenia include impaired inflammatory responses, oligodendrocyte abnormalities, mitochondrial dysfunction and other pathophysiological processes (Markkanen *et al*., 2016). DNA damage may destroy cell function, cause neuronal damage and induce cell death and apoptosis, which may lead to the occurrence and development of schizophrenia (Sertan Copoglu *et al*., 2015). In addition, it has been hypothesized that inflammation and immunoreaction lead to clinical psychiatric symptoms in schizophrenia (Kirkpatrick and Miller, 2013; Leza *et al*., 2015; Feigenson *et al*., 2014; Lucas *et al*., 2006). Studies have shown that an increase in proinflammatory cytokines [including interleukin 6 (IL-6), interleukin 1β (IL-β) and tumor necrosis factor-alpha (TNF-α)] and a

decrease in anti-inflammatory cytokines, which can lead to the activation of microglia, were related to the symptoms of schizophrenia (Khandaker *et al.*, 2015). For instance, studies have found that the serum levels of TNF-α and IL-2 are negatively correlated with the severity of positive symptoms in schizophrenia patients (Lv *et al.*, 2015; Zhang *et al.*, 2002). These results indicate that the inflammatory pathway participates in the pathogenesis of schizophrenia. Nucks1 maps to schizophrenia susceptibility loci in the human chromosome 1q32.1 (Grundt *et al.*, 2004; Drosos *et al.*, 2014) and is involved in inflammation and the immune response. Inhibition of nucks1 can weaken the expression of NF-κB–mediated cytokines (including IL-6 and TNF-α), which means that nucks1 can enhance the expression of IL-6 and TNF-α and other inflammatory factors (Poon *et al.*, 2017). Furthermore, studies have shown that nucks1 play a part in the DNA damage response and can also be used for DNA repair (Huang *et al.*, 2018; Yue *et al.*, 2016; De Angelis *et al.*, 2018). Nucks1 has recently been confirmed to be important for homologous recombination (HR) and genomic stability (Parplys *et al.*, 2015). Additionally, nucks1 has also been determined to be a potential susceptibility gene for certain neurodevelopmental and neurodegenerative diseases, such as bipolar disorder (BD) (Fries *et al.*, 2017), Alzheimer's disease (AD) (Cruz-Rivera *et al.*, 2018) and Parkinson's disease (PD) (Vacic *et al.*, 2014; Liu *et al.*, 2011; Zhu *et al.*, 2018). Recently, there has been robust and replicable evidence for genetic variation near the miR-137 gene in schizophrenia (Yin *et al.*, 2014; Ma *et al.*, 2014; Xu *et al.*, 2020). Interestingly, Giunti *et al.* (2019) demonstrated that nucks1 was a direct target gene of miR-137. Based on the information above, we hypothesize that the nucks1 gene may be involved in the etiology of schizophrenia.

To date, multiple single nucleotide polymorphisms (SNPs) of nucks1 have been identified, including rs4951261, rs823114 and rs951366, which may have a significant impact on the expression or function of nucks1. Rs4951261 is located in the intron of nucks1 (Cui *et al.*, 2013). Rs823114 is located in an intergenic region proximal to nucks1, which has been found to be related to the transcript level of the nucks1 (Liu *et al.*, 2011; Zhu *et al.*, 2018; Lv *et al.*, 2017). Bryzgalov proposed that the two polymorphisms rs4951261 and rs823114 are considered to be related to the risk of cognitive impairment (Bryzgalov *et al.*, 2018). Rs951366 is located in the 3'-untranslated region of nucks1 and may be involved in regulating its transcriptional activity (Xu *et al.*, 2017). Therefore, this study chose these loci to examine the potential relationship between the nucks1 gene and schizophrenia in the Chinese population.

Materials and methods

Subjects

A total of 774 unrelated schizophrenic patients (503 men and 271 women, with an average age of 35.34 ± 13.68 years) and 819 well-matched healthy controls (474 men and 345 women, with an average age of 38.94 ± 13.97 years)) from the Department of Psychology, Affiliated Hospital of Guangdong Medical University were enrolled in this study. According to the diagnostic criteria for schizophrenia, namely the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V), at least two experienced senior psychiatrists are required to diagnose schizophrenic patients. Moreover, the positive and negative symptom scale (PANSS) was used to evaluate the clinical psychiatric symptoms of schizophrenic patients. All patients with schizophrenia have no neurological history, intellectual disability, organic brain injury, autoimmune disease, drug abuse or alcoholism or metabolic disease. In addition, all healthy controls did not suffer from serious physical illness, family histories of mental illness (first-degree relatives) or diagnosed with drug abuse (except for nicotine dependence). This study was given official approval by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University. All participants signed an informed consent form to agree to participate in the study.

Genetic analysis

Genomic DNA was extracted from peripheral blood treated with EDTA by a TIA Namp Blood DNA Kit and genotyping of the nucks1 rs4951261, rs823114 and rs951366 polymorphisms was performed for 1593 individuals using the imLDR technique (Genesky Biotech, Shanghai, China) as described previously (Xu *et al.*, 2018; Xu *et al.*, 2020; Yin *et al.*, 2019). The primer information is described in Table 1.

Neurocognitive function assessment

In this study, the Brief Assessment of Cognition in Schizophrenia (BACS) was used to evaluate the neurocognitive function of the patients with schizophrenia (Xu *et al.*, 2018), which includes a brief assessment of the

Table 1 Primer sequences and allele-specific probes for PCR amplification on the rs4951261, rs823114 and rs951366 loci

SNP	Direction	Sequence (5′-3′)
rs4951261	Forward	CCACCACCAGTTGGAGGATGAT
	Reverse	GCCATTTATGGAGGGGTGTGGT
rs4951261RC		TTCCGCGTTCGGACTGATAT GACCCTGG-CAAGGGTTTTGTACG
rs4951261RA		TACGGTTATTCGGGCTCCTGT GACCCTGG-CAAGGGTTTTGTGCT
rs4951261RP		ACACCGTTCTGTCTCCAGACTAGATC T
rs823114	Forward	GGATGAATCAAACCAGCCGAAAA
	Reverse	CCCCAGACGAATCTGCAACTCT
rs823114FG		TTCCGCGTTCGGACTGATAT CAAGATGCTCT-GAGAGCGCTCG
rs823114FA		TACGGTTATTCGGGCTCCTGT CAA-GATGCTCTGAGAGCGCTCA
rs823114FP		GCCGCAAAACAAAAATGGTCG TTTT
rs951366	Forward	GAAGCCCAATCAAATGCACACA
	Reverse	ATTTGGGGGGAGGGCTCTTTTA
rs951366RC		TCTCTCGGGTCAATTCGTCCTT GGGGGAG-GGCTCTTTTAAAACTACG
rs951366RT		TGTTCGTGGGCCGGATTAGT GGGGGAG-GGCTCTTTTAAAACTGCA
rs951366RP		GCCTCAGTTGTTTAACAGTCTCTTTAATATAT-TAATC TT

following six cognitive aspects: working memory (digit sequencing task), motor speed (token motor task), semantic and letter fluency (category instances and controlled oral word association test), verbal memory (list learning), reasoning and problem solving (Tower of London) and attention and processing speed (symbol coding) (Keefe *et al.*, 2008).

Table 2 Genotype and allele frequency of the nucks1 SNPs in patients with schizophrenia and controls

dbSNP ID	Patient $n = 774$ (%)	Control $n = 819$ (%)	*P* value	OR (95% CI)
rs4951261				
Genotype				
A/A	371 (47.9%)	387 (47.3%)	0.667	
C/A	341 (44.1%)	356 (43.5%)		
C/C	62 (8.0%)	76 (9.3%)		
C/C+C/A	403 (52.1%)	432 (52.7%)	0.786	0.973 (0.80–1.19)
Allele				
C	465 (30.0%)	508 (31.0%)		1.000 (reference)
A	1083 (70.0%)	1130 (69.0%)	0.551	1.047 (0.90–1.22)
rs823114				
Genotype				
A/A	241 (31.1%)	263 (32.1%)	0.813	
G/A	399 (51.6%)	409 (49.9%)		
G/G	134 (17.3%)	147 (17.9%)		
G/A+G/G	533 (68.9%)	556 (67.9%)	0.676	1.046(0.85-1.29)
Allele				
G	667 (43.1%)	703 (42.9%)		1.000 (reference)
A	881 (56.9%)	935 (57.1%)	0.923	0.993 (0.86–1.14)
rs951366				
Genotype				
T/T	372 (48.1%)	386 (47.1%)	0.494	
C/T	343 (44.3%)	357 (43.6%)		
C/C	59 (7.6%)	76 (9.3%)		
C/T+C/C	402 (51.9%)	433 (52.9%)	0.71	0.963 (0.79–1.17)
Allele				
C	461 (29.8%)	509 (31.1%)		1.000 (reference)
T	1087 (70.2%)	1129 (68.9%)	0.428	1.063 (0.0.91–1.24)

OR, odds ratio; 95% CI, 95% confidence interval.

Statistical analyses

Normally distributed quantitative data are represented as the mean ± SD. Pearson's chi-square test was used to assess deviations from the Hardy–Weinberg equilibrium (HWE) and to compare haplotype, genotype and allele distributions. Our study also calculated the odds ratios (ORs) with 95% confidence intervals (CIs) of the alleles. And then, haploview 4.2 software was applied to calculate the linkage disequilibrium coefficient D' (normalized D), r^2 (squared correlation coefficient) and frequency distribution of haplotypes. To make a calculation of the power of the sample size, our study has applied Power and sample size calculation software (William D. DuPont, USA). In this study, SPSS 23.0 software was used to perform all statistical analyses. Statistical significance was established for $P < 0.05$. Bonferroni correction was applied in counteracting the problem of multiple comparisons.

Results

Association study for SNPs (rs4951261, rs823114 and rs951366) and schizophrenia

The genotype distribution of the three SNPs (rs4951261, rs823114 and rs951366) in schizophrenic patients and controls reached HWE (all $P > 0.05$).

Our current study showed that for the rs4951261, rs823114 and rs951361 polymorphisms, no significant difference was found in the genotype and allele frequencies between the schizophrenic patients and healthy controls

Table 3 Genotype and allele frequency of the nucks1 SNPs in the patients with schizophrenia and controls (according to sex)

dbSNP ID	Men $n = 503$(%)	Controls $n = 474$(%)	*P* value	OR (95% CI)	Women $n = 271$(%)	Controls $n = 345$(%)	*P* value	OR (95% CI)
rs4951261								
Genotype								
A/A	238 (47.3%)	230 (48.5%)	0.369		133 (49.1%)	157 (45.5%)	0.663	
C/A	224 (44.5%)	195 (41.1%)			117 (43.2%)	161 (46.7%)		
C/C	41 (8.2%)	49 (10.3%)			21 (7.7%)	27 (7.8%)		
C/C+C/A	265 (52.7%)	244 (51.5%	0.706		138 (50.9%)	188 (54.5%)	0.378	
Allele								
C	306 (30.4%)	293 (30.9%)		1.000 (reference)	159 (29.3%)	215 (31.2%)		1.000 (reference)
A	700 (69.6%)	655 (69.1%)	0.814	1.023 (0.84–1.24)	383 (70.7%)	475 (68.8%)	0.49	1.090 (0.85–1.39)
rs823114								
Genotype								
A/A	152 (30.2%)	157 (33.1%)	0.295		89 (32.8%)	106 (30.7%)	0.648	
G/A	267 (53.1%)	228 (48.1%)			132 (48.7%)	181 (52.5%)		
G/G	84 (16.7%)	89 (18.8%)			50 (18.5%)	58 (16.8%)		
G/A+G/G	351 (69.8%)	317 (66.9%)	0.329		182 (67.2%)	239 (69.3%)	0.575	
Allele								
G	435 (43.2%)	406 (42.8%)		1.000 (reference)	232 (42.8%)	297 (43.0%)		1.000 (reference)
A	571 (56.8%)	542 (57.2%)	0.854	0.983 (0.82–1.18)	310 (57.2%)	393 (57.0%)	0.933	1.010 (0.81–1.27)
rs951366								
Genotype								
T/T	237 (47.1%)	230 (48.5%)	0.203		135 (49.8%)	156 (45.2%)	0.51	
C/T	227 (45.1%)	194 (40.9%)			116 (42.8%)	163 (47.2%)		
C/C	39 (7.8%)	50 (10.5%)			20 (7.4%)	26 (7.5%)		
C/T+C/C	266 (52.9%)	244 (51.5%)	0.66		136 (50.2%)	189 (54.8%)	0.257	
Allele								
C	305 (30.3%)	294 (31.0%)		1.000 (reference)	156 (28.8%)	215 (31.2%)		1.000 (reference)
T	701 (69.7%)	654 (69.0%)	0.739	1.033 (0.85–1.25)	386 (71.2%)	475 (68.8%)	0.367	1.120 (0.88–1.43)

OR, odds ratio; 95%CI, 95%confidence interval.

Table 4 Clinical characteristics of the patients with schizophrenia and distribution by genotypes of the three SNPs

	rs4951261			rs823114			rs951366		
Parameters	A/A ($n = 312$)	C/A+C/C ($n = 327$)	*P* value	A/A ($n = 209$)	G/A+G/G ($n = 430$)	*P* value	T/T ($n = 313$)	C/T+C/C ($n = 326$)	*P* value
Age of educations	9.5 ± 3.3	9.4 ± 3.4	0.748	9.4 ± 3.4	9.4 ± 3.4	0.98	9.5 ± 3.3	9.3 ± 3.4	0.552
Age at onset (years)	25.8 ± 10.7	25.2 ± 9.4	0.454	25.4 ± 10.1	25.5 ± 10.0	0.937	25.8 ± 10.6	25.2 ± 9.4	0.481
Duration of illness (months)	90.2 ± 104.8	93.7 ± 97.8	0.665	91.0 ± 108.1	92.5 ± 97.8	0.861	89.8 ± 104.5	94.11 ± 98.0	0.599
Family psychotic history	50 (16.0%)	39 (11.9%)	0.135	32 (15.3%)	57 (13.3%)	0.481	51 (16.3%)	38 (11.7%)	0.091
Age at onset (years)									
<18	56 (17.9%)	58 (17.7%)	0.944	33 (15.8%)	81 (18.8%)	0.345	56 (17.9%)	58 (17.8%)	0.974
≥18	256 (82.1%)	269 (82.3%)		176 (84.2%)	349 (81.2%)		257 (82.1%)	268 (82.2%)	

Values are the mean ± SD.

Table 5 Clinical psychiatric symptoms of the patients with schizophrenia and distribution by genotypes of the three SNPs

rs4951261 Parameters	A/A ($n = 312$)	Total C/A+C/C ($n = 327$)	*P* value	A/A ($n = 194$)	Men C/A+C/C ($n = 208$)	*P* value	A/A ($n = 118$)	Women C/A+C/C ($n = 119$)	*P* value
PASS total score	74.0 ± 18.5	75.1 ± 18.7	0.449	71.8 ± 19.3	73.5 ± 19.7	0.378	77.8 ± 16.6	78.1 ± 16.3	0.89
Positive score	21.3 ± 7.8	21.9 ± 7.4	0.382	20.6 ± 7.8	21.6 ± 7.7	0.2	22.6 ± 7.6	22.4 ± 6.9	0.819
Negative score	16.9 ± 8.8	17.5 ± 8.7	0.416	16.4 ± 8.1	17.0 ± 7.3	0.412	17.8 ± 9.8	18.3 ± 10.7	0.709
Pathological score	35.8 ± 10.0	35.8 ± 9.7	0.974	34.8 ± 10.5	34.9 ± 10.3	0.93	37.4 ± 8.9	37.4 ± 8.4	0.991
rs823114 Parameters	A/A (n = 209)	Total G/A+G/G (n = 430)	P value	A/A (n = 126)	Men G/A+G/G (n = 276)	P value	A/A (n = 83)	Women G/A+G/G (n = 154)	P value
PASS total score	73.7 ± 18.9	75.0 ± 18.4	0.399	70.7 ± 19.5	73.5 ± 19.5	0.18	78.3 ± 17.2	77.7 ± 16.1	0.815
Positive score	20.8 ± 7.6	22.0 ± 7.6	0.053	19.4 ± 7.3	21.9 ± 7.9	0.003	22.9 ± 7.6	22.3 ± 7.0	0.541
Negative score	17.3 ± 8.9	17.1 ± 8.7	0.769	16.9 ± 8.3	16.6 ± 7.5	0.755	18.0 ± 9.8	18.0 ± 10.5	0.99
Pathological score	35.6 ± 10.3	35.9 ± 9.6	0.716	34.4 ± 10.9	35.0 ± 10.2	0.594	37.4 ± 9.1	37.5 ± 8.4	0.937
rs951366 Parameters	T/T (n = 313)	Total C/T+C/C ($n = 326$)	*P* value	T/T ($n = 193$)	Men C/T+C/C ($n = 209$)	*P* value	T/T ($n = 120$)	Women C/T+C/C ($n = 117$)	*P* value
PASS total score	74.0 ± 18.5	75.2 ± 18.7	0.42	71.8 ± 19.3	73.4 ± 19.7	0.403	77.5 ± 16.6	78.3 ± 16.3	0.714
Positive score	21.3 ± 7.8	21.9 ± 7.4	0.362	20.6 ± 7.8	21.6 ± 7.7	0.205	22.5 ± 7.5	22.4 ± 6.9	0.917
Negative score	16.9 ± 8.8	17.5 ± 8.7	0.38	16.4 ± 8.1	17.0 ± 7.3	0.44	17.7 ± 9.8	18.4 ± 10.7	0.59
Pathological score	35.8 ± 10.0	35.8 ± 9.7	0.969	34.8 ± 10.6	34.9 ± 10.3	0.959	37.3 ± 8.8	37.5 ± 8.4	0.885

Values are the mean ± SD.
PANSS, positive and negative syndrome scale.

($P > 0.05$) (Table 2). Besides, when the distributions were further stratified by sex, there were no significant differences can be found ($P > 0.05$) (Table 3).

For further analysis, the clinical characteristics of patients with schizophrenia by genotypic distribution were described. There were no significant differences among the three SNP genotypes in terms of age of onset, age of education, duration of illness, family history of psychosis, PANSS total score, positive symptom score, negative symptom score and pathological score. However, in men patients, the AA genotype had lower positive symptom scores than the G allele carrier subjects [Average + SD of genotype A/A and G/A+G/G were 19.4 ± 7.3 and 21.9 ± 7.9, respectively; $P = 0.003$, P(corr) = 0.009] (Tables 4,5). Moreover, among men patients, we further found that those with the GA+GG genotypes exhibited marginally higher delusion, disorganization and hallucination scores than those with the AA genotype ($P = 0.016$, 0.002 and 0.002, respectively) (Table 6).

Neurocognitive function analysis

The genotypes of the three polymorphisms of rs4951261, rs823114 and rs951366 were not found any significant differences in the scores of the seven BACS items (Table 6). However, the results revealed that the semantic fluency score of rs823114 polymorphism G allele carriers was significantly lower than that of the AA genotype in the sex-stratified analysis ($P = 0.010$, P(corr) = 0.030) (Table 7).

Linkage disequilibrium analysis and haplotype analysis

For these three SNPs (rs4951261, rs823114, rs951366), we also performed a haplotype analysis. According to the analysis of haplotype frequencies, we found no significant difference between schizophrenic patients and healthy controls (Table 8).

Discussion

Schizophrenia is a complex neurological disease with a variety of etiologies. It has been shown to be related to inflammatory immune responses and DNA damage (Markkanen *et al.*, 2016; Howes and McCutcheon, 2017; Watkins and Andrews, 2016). Nucks1 is a vertebrate-specific gene (Bai *et al.*, 2017) that has been indicated that the response to DNA damage by HR is important and plays a role in inflammatory immune response (Cruz-Rivera *et*

al., 2018). It has been shown that nucks1 is related to several nervous system diseases, including BD (Fries *et al.*, 2017), AD (Cruz-Rivera *et al.*, 2018) and PD (Zhu *et al.*, 2018, Liu *et al.*, 2011, Vacic *et al.*, 2014). To date, the association between the nucks1 gene and schizophrenia has not been reported. In addition, genome-wide association study (GWAS) data has identified 14 unique SNPs associated with the risk of cognitive impairment, including the rs4951261 and rs823114 polymorphisms of the nucks1 gene (Bryzgalov *et al.*, 2018). Based on the above, we reasonably speculate that the nucks1 gene may be a logical candidate gene for the potential causes of schizophrenia.

In this study, we assessed the potential link of the rs4951261, rs823114 and rs951366 polymorphisms of the nucks1 gene with schizophrenia. However, the results showed that even after sex stratification analysis, we did not find any significant differences in the genotype and allele distribution of these three SNPs between patients and healthy controls ($P > 0.05$).

The nucks1 gene may affect some characteristics of schizophrenia. Therefore, for the clinical manifestations and neurocognitive function distribution of patients with different genotypes, we have done further analysis. As far as we know, this is the first research on the relationship between nucks1 gene polymorphism and clinical manifestations and neurocognitive function in schizophrenia. Regarding the rs4951261 and rs951366 polymorphisms, no significant differences were found among the age of onset, duration of illness, PANSS score, family history of psychosis or neurocognitive function scores. However, with regard to rs823114, the positive symptom score of men G allele carriers was higher than that of men with the A/A genotype, mainly reflected in the symptoms of delusion, confusion and hallucination, which may be used as a more detailed description of schizophrenia; furthermore, the semantic fluency score of women G allele carriers was significantly lower than that of women patients with the A/A genotype. Therefore, we speculate that the nucks1 gene is related to the clinical manifestations and neurocognitive function in schizophrenia and that the G allele of its rs823114 polymorphism may be a harmful factor in schizophrenia, which may affect the positive symptoms

Table 6 Positive symptoms of male patients with schizophrenia and genotype distribution of rs823114

	Men		
rs823114 Parameters	A/A ($n = 126$)	G/A+G/G ($n = 276$)	*P* value
P1 (delusions)	3.7 ± 1.8	4.5 ± 1.7	0.016
P2 (disorganization)	2.7 ± 1.5	3.3 ± 1.7	0.002
P3 (hallucinations)	2.9 ± 1.8	3.6 ± 1.9	0.002
P4 (excitement)	2.4 ± 1.4	2.5 ± 1.6	0.565
P5 (grandiosity)	1.7 ± 1.1	1.8 ± 1.3	0.515
P6 (suspiciousness)	3.5 ± 1.8	3.9 ± 1.8	0.037
P7 (hostility)	2.5 ± 1.5	2.7 ± 1.6	0.187

Values are the mean ± SD

Table 7 BACS scores of patients with schizophrenia and distribution of the three SNPs by genotype

	Total			Men			Women		
rs4951261 Parameters	A/A ($n = 152$)	C/A+C/C ($n = 172$)	*P* value	A/A ($n = 110$)	C/A+C/C ($n = 123$)	*P* value	A/A ($n = 42$)	C/A+C/C ($n = 49$)	*P* value
Working memory	15.6 ± 9.0	16.0 ± 9.0	0.669	16.1 ± 9.0	17.3 ± 8.5	0.298	14.1 ± 8.9	12.7 ± 9.5	0.459
Semantic fluency	30.1 ± 12.0	29.1 ± 12.6	0.432	29.5 ± 11.5	29.0 ± 12.4	0.777	32.0 ± 13.1	29.2 ± 13.3	0.325
Letter fluency	9.9 ± 5.9	9.8 ± 5.7	0.95	9.6 ± 6.2	9.9 ± 5.4	0.729	10.6 ± 5.1	9.7 ± 6.4	0.496
Verbal memory	22.7 ± 13.8	22.5 ± 14.7	0.909	22.3 ± 13.7	22.2 ± 14.1	0.957	23.9 ± 14.1	23.5 ± 16.2	0.891
Motor speed	49.8 ± 17.9	50.9 ± 17.0	0.587	52.4 ± 19.3	51.9 ± 18.1	0.845	43.1 ± 11.3	48.3 ± 13.9	0.056
Reasoning and problem solving	7.7 ± 6.7	7.3 ± 6.2	0.609	7.6 ± 6.9	7.9 ± 6.0	0.693	7.9 ± 6.3	5.8 ± 6.5	0.117
Attention and processing speed	20.9 ± 18.9	20.7 ± 12.6	0.892	20.8 ± 13.1	20.6 ± 11.9	0.95	21.1 ± 12.2	20.7 ± 14.4	0.88
	Total			Men			Women		
rs823114 Parameters	A/A ($n = 98$)	G/A+G/G ($n = 226$)	*P* value	A/A ($n = 72$)	G/A+G/G ($n = 161$)	*P* value	A/A ($n = 26$)	G/A+G/G ($n = 65$)	*P* value
Working memory	15.9 ± 9.6	15.7 ± 9.0	0.867	16.3 ± 9.3	17.0 ± 8.6	0.575	15.0 ± 8.5	12.7 ± 9.5	0.291
Semantic fluency	30.7 ± 12.9	29.1 ± 12.1	0.264	28.8 ± 12.7	29.4 ± 11.7	0.731	36.1 ± 12.0	28.3 ± 13.1	0.01
Letter fluency	10.0 ± 6.2	9.7 ± 5.6	0.692	9.6 ± 6.5	9.8 ± 5.5	0.771	11.3 ± 5.2	9.6 ± 6.0	0.209
Verbal memory	22.8 ± 14.5	22.5 ± 14.2	0.867	21.8 ± 14.5	22.4 ± 13.6	0.745	25.8 ± 14.1	22.9 ± 15.6	0.412
Motor speed	50.3 ± 18.9	50.4 ± 16.8	0.964	53.2 ± 20.5	51.7 ± 17.8	0.576	42.5 ± 10.6	47.3 ± 13.6	0.108
Reasoning and problem solving	7.7 ± 7.3	7.4 ± 6.0	0.662	7.5 ± 7.7	7.8 ± 5.7	0.693	8.3 ± 6.1	6.2 ± 6.6	0.155
Attention and processing speed	20.3 ± 13.6	20.9 ± 12.3	0.691	20.0 ± 13.9	21.0 ± 11.8	0.565	21.3 ± 12.8	20.8 ± 13.7	0.873
	Total			Men			Women		
rs951366 Parameters	T/T ($n = 152$)	C/T+C/C ($n = 172$)	*P* value	T/T ($n = 109$)	C/T+C/C ($n = 124$)	*P* value	T/T ($n = 43$)	C/T+C/C ($n = 48$)	*P* value
Working memory	15.5 ± 9.0	16.0 ± 9.0	0.625	16.1 ± 9.9	17.3 ± 8.5	0.31	14.0 ± 8.9	12.7 ± 9.6	0.507
Semantic fluency	30.2 ± 12.0	29.1 ± 12.6	0.421	29.3 ± 11.5	29.1 ± 12.4	0.878	32.2 ± 13.1	28.9 ± 13.3	0.237
Letter fluency	9.9 ± 5.9	9.8 ± 5.7	0.935	9.5 ± 6.2	9.9 ± 5.4	0.657	10.7 ± 5.1	9.6 ± 6.4	0.392
Verbal memory	23.1 ± 14.3	22.2 ± 14.2	0.581	22.3 ± 13.7	22.2 ± 14.0	0.888	25.0 ± 15.5	22.5 ± 14.9	0.448
Motor speed	49.7 ± 17.8	51.0 ± 17.2	0.486	52.2 ± 19.2	52.1 ± 18.2	0.978	43.3 ± 11.3	48.2 ± 14.0	0.071
Reasoning and problem solving	7.8 ± 6.8	7.2 ± 6.1	0.372	7.6 ± 6.9	7.8 ± 6.0	0.813	8.3 ± 6.5	5.5 ± 6.2	0.039
Attention and processing speed	20.9 ± 12.9	20.6 ± 15.6	0.81	20.7 ± 13.2	20.7 ± 11.9	0.994	21.5 ± 12.4	20.4 ± 14.3	0.677

Values are the mean ± SD.BACS, brief assessment of cognition in schizophrenia.

Table 8 Haplotype analysis of the three SNPs of the *nucks1* gene for the patients with schizophrenia and controls

(rs951366) - (rs4951261) - (rs823114)	Schizophrenia *n* (%)	Controls *n* (%)	*P* value	OR (95% CI)
T-A-A	879 (56.9%)	931 (57.0%)		1.000 (reference)
C-C-G	459 (29.7%)	503 (30.8%)	0.67	0.967 (0.83–1.13)
T-A-G	201 (13.0%)	194 (11.9%)	0.403	1.097 (0.88–1.36)

Haplotype frequency <0.03 in both schizophrenic patients and controls has been dropped.
OR, odds ratio; 95% CI, 95% confidence interval.

among men patients with schizophrenia and some neurocognitive functions among women patients.

The rs823114 SNP, located near the nucks1 gene region, is reported to have a close association with the transcription level of nucks1 (Zhu *et al.*, 2018; Liu *et al.*, 2011; Lv *et al.*, 2017), but the specific mechanism of action is not yet clear. Combined with our research results, we first speculated that the G allele of rs823114 may affect the expression of this gene by regulating its transcription level, thereby affecting the clinical manifestations and neurocognitive function in schizophrenia. Nucks1 is involved in regulating the levels of inflammatory cytokines (Poon *et al.*, 2017). Studies have shown that inflammatory cytokines such as IL-2, IL-8, IL-6, and TNF-α play a key role in the etiology and pathophysiology of schizophrenia (Lv *et al.*, 2015; Zhang *et al.*, 2002; Hope *et al.*, 2013). Some studies have noted that the symptoms of schizophrenia are associated with inflammatory cytokines (Zhang *et al.*, 2002), including increased proinflammatory cytokine activity, which may also play a part in the development of positive symptoms (Meyer *et al.*, 2011). In addition, neurocognitive dysfunction in schizophrenia is also considered to be related to inflammatory cytokines (Kogan *et al.*, 2018). Kogan believes that individuals with schizophrenia with increased expression of TNF-α exhibit more severe neurocognitive deficits (Kogan *et al.*, 2018). In combination with the above, we finally hypothesized that the G allele of rs823114 affects the expression of the nucks1 gene by changing its transcriptional activity. This gene then participates in the regulation of inflammatory cytokine levels, thereby affecting the clinical performance and neurocognitive function of patients. As mentioned earlier, nucks1 is a downstream target gene of miR-137 (Giunti *et al.*, 2019), and miR-137 is considered to be regulated by sex-related factors (Tamming *et al.*, 2020); therefore, this gene may also be related to sex, which may explain why men schizophrenic patients are more easily to develop positive symptoms, while the neurocognitive function of female patients is more likely to be affected. This seems to be a rational assumption, but it requires further research and verification.

Some limitations of our study should be noted. In the first place, power analysis indicated that given our research sample and assuming that the risk allele frequency is 42.9%, we would have 100.0% power to detect the relative risk of the genotype with an odds ratio of 1.5 (at the 0.05 level). Nevertheless, when the significance level was 0.05, an odds ratio of 1.1 yielded a power of only 26.6%. Therefore, we cannot exclude false-negative results. Furthermore, our sample size of this study was relatively small and may lead to false-positive or false-negative results. Second, our current resources were limited to data from Han people. It is still unclear whether this research can be reflected in other parts of China, and thus further exploration is required.

Conclusion

In summary, our study is the first to explore the possible link between nucks1 gene polymorphisms and schizophrenia. According to the data, the rs823114 polymorphism of the nucks1 gene may be related to the positive symptoms and neurocognitive function of schizophrenia. Its G allele may be related to the susceptibility of men schizophrenic positive symptoms and may be involved in affecting the neurocognitive function of women patients in patients of Southern Han nationality in China.

Acknowledgements

This study was carried out in accordance with the recommendations of the Ethics Committee of the Affiliated Hospital of Guangdong Medical University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University.

Y.W., G.M., Y.L., J.L. and Z.L. conceived and designed the experiments and revised the article. D.L., Z.D., D.Z., J.F., S.X., S.G. and F.N. did genetic analyzes. X.Z., X.N., J.Z., C.L. and J.Y. collected the cognitive and clinical data. X.W., X.X. and L.X. analyzed and interpreted the data and drafted the article. All authors were involved in the revision of the article.

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Conflicts of interest

There are no conflicts of interest.

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