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ORIGINAL RESEARCH

Association Between Polymorphisms in the 5′ Region of the *GALR1* Gene and Schizophrenia in the Northern Chinese Han Population: A Case–Control Study

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Background: Epidemiological studies have shown that genetic factors are among the causes of schizophrenia. Galanin receptor 1 is an inhibitory receptor of galanin that is widely distributed in the central nervous system. This study mainly explored the relationship between polymorphisms of the 5′ region of the *GALR1* gene and schizophrenia in the northern Chinese Han population.

Methods: A 1545 bp fragment of the 5′ regulatory region of the *GALR1* gene was amplified and sequenced in 289 schizophrenia patients and 347 healthy controls.

Results: Among the haplotypes composed of the 16 detected SNPs, the haplotype H3 was identified as conferring a risk of schizophrenia (p=0.011, OR=1.430, 95% CI=1.084–1.886). In addition, the haplotypes H4 and H7 were both protective against schizophrenia (p=0.024, OR=0.526, 95% CI=0.298–0.927; p=0.037, OR=0.197, 95% CI=0.044–0.885, respectively). In the subgroup analysis by sex, it was found that seven SNP alleles (rs72978691, rs11662010, rs11151014, rs11151015, rs13306374, rs5373, rs13306375) conferred a risk of schizophrenia in females (p<0.05), while allele G of rs7242919 (p=0.007) was protective against schizophrenia in females. Moreover, the rs72978691 AA+AC genotype (p=0.006, OR=1.874, 95% CI=1.196–2.937, power=0.780), rs7242919 CC+CG genotype (p=0.002, OR=2.027, 95% CI=1.292–3.180, power=0.861), rs11151014 GG+GT genotype (p=0.008, OR=1.834, 95% CI=1.168–2.879, power=0.735), rs11151015 GG+AG genotype (p=0.002, OR=2.013, 95% CI =1.291–3.137, power=0.843), rs13306374 CC+AC genotype (p=0.006, OR=1.881, 95% CI=1.198–2.953, power=0.788), and rs13306375 GG+AG genotype (p=0.006, OR=1.868, 95% CI=1.194–2.921, power=0.770) increased the risk of schizophrenia in females. The haplotype FH2 consisting of rs72978691, rs11662010, rs7242919, rs11151014, rs11151015, rs13306374, rs5373, and rs13306375 may also be associated with the risk of schizophrenia in females (p=0.024).

Conclusion: This study identified an association between polymorphisms in the 5′ region of the *GALR1* gene and schizophrenia, especially in females.

Keywords: galanin receptor 1, schizophrenia, single-nucleotide variant, genetic polymorphism, northern Chinese Han population

Introduction

Schizophrenia is a common multifactorial psychiatric disorder.[1] It is a complex disease caused by the interaction of environmental and genetic factors. Early twin studies showed that the heritability of schizophrenia can reach 80%.[2] Although the

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evidence that genetic factors play a significant role in schizophrenia is continuing to accumulate, their exact mechanistic involvement in this disease remains unclear. It has been hypothesized that many neurotransmitters are involved in the etiology of schizophrenia, including dopamine,[3] serotonin,[4] and glutamate.[5]

Galanin (GAL) is a 30-amino-acid neuropeptide widely distributed in the central nervous system and associated with many physiological activities and diseases of the nervous system, such as arousal/sleep, pain perception, learning, and memory, as well as inflammation, depression, Alzheimer's disease, epilepsy, and schizophrenia.[6,7] It works mainly through three receptor subtypes, galanin receptor 1 (GalR1), GalR2, and GalR3, which belong to the family of G-protein-coupled receptors. GalR1 works through G_i/G_o on adenylate cyclase to reduce cAMP, inhibit K^+ outflows, and make the cell membrane hyperpolarized.[8] Functionally, galanin is known to inhibit neuronal firing and the release of norepinephrine, serotonin (5-HT), dopamine, as well as glutamate and acetylcholine.[9–11] GalR1 is mainly distributed in the hypothalamus, locus coeruleus (LC), amygdala, and other cortical regions.[8] It is encoded by the *GALR1* gene, which is located at 18p23 in humans and has a length of 27,091 bp, containing three exons. A large number of reports have shown that GalR1 is closely related to many mental health conditions, such as depression, addiction, and Alzheimer's disease. In addition, an association between galanin and major depressive disorder has been reported in the Han Chinese population.[12] Studies have confirmed that galanin plays a role in promoting depression through GalR1, while GalR2 plays an anti-depressive role. Polymorphisms of the *GALR1, GALR2*, and *GALR3* genes are highly correlated with depression, suggesting that new antidepressants that act on GalR1–3 could be developed.[13] GalR1 has also been reported to be related to Alzheimer's disease, as well as heroin and opioid addiction. Rat experiments have also shown that GalR1 in LC is a target for the treatment of addiction.[14,15] Moreover, rs5371 of *GALR1* has been shown to be associated with stress and addiction in African-Americans.[16]

Although GalR1 is clearly closely related to mental health conditions, to the best of our knowledge no study has been performed on the association between the *GALR1* gene and schizophrenia. This work explores the relationship between the *GALR1* gene's 5′ regulatory sequence and schizophrenia through a case–control study.

Materials and Methods

Study Subjects

This study examined the blood samples of 636 subjects of Han ethnicity in northern China, including 289 patients in the schizophrenia group (132 males and 157 females) and 347 patients in the control group (186 males and 161 females). The mean age of the patients was 45.4 ± 8.1 (mean ± standard deviation) years, and the mean age of the healthy subjects was 44.6 ± 13.9 years. The mean age of disease onset in the case group was 34.5 ± 7.12. The disease duration was 5–23 years. All included patients were paranoid schizophrenics. All patients were assessed for age at first hospitalization, first-degree relatives with a history of mental illness, alcohol or drug abuse, reactions to antipsychotic medicine for schizophrenia, suicide attempts, and anticholinergic medication. Only patients who fully met the criteria for schizophrenia in the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM-4) were included in this study, as diagnosed by psychiatrists. The inclusion criteria for the case group were as follows: (1) Han ethnicity from northern China; (2) recruited from the Third People's Hospital of Liaoning Province; and (3) fully meeting the requirements of DSM-4 standard. The inclusion criteria for the control group were as follows: (1) Han ethnicity from northern China; (2) recruited from healthy adult blood donors; and (3) no history of mental illness in at least three generations of their family. If the participants suffered from any other mental illnesses or serious physiological diseases, or were relatives of the other participants, they were excluded. Each of the subjects provided written informed consent before participating in this study. This study was conducted in accordance with the Helsinki Declaration. Sample collection and analysis were approved by the Ethics Committee of China Medical University.

DNA Extraction

Peripheral blood samples collected from each subject were stored in EP tubes, and genomic DNA was extracted by the phenol-chloroform method.[17] The concentration and purity of the DNA were determined using a UV spectrophotometer.

Segment Selection and Primer Design

To detect polymorphisms that could potentially affect expression of the *GALR1* gene, we selected the 5′

regulatory region of the *GALR1* gene and focused on a 1545 bp (−1367 bp to +177 bp, ATG is +1) fragment containing part of it. As this part of the 5′ regulatory region is closest to the coding region, polymorphism within this fragment is more likely to affect gene expression. Primers were designed using Primer Premier 5 (www.premierbiosoft.com). The primers used in PCR were as follows: F 5′-ACGTGACTGGCCCTGCTATACC-3′, R 5′-CGCCAGCACGGTGATCACTAG-3′.

Polymerase Chain Reaction Amplification

Polymerase chain reaction (PCR) was performed on a Thermal Cycler Dice™ (TP650) (Japan). The amplification system included 0.2 μL of 5 U/μL LA enzyme (Takara, Japan), 10 μL of 2×GC Buffer I, 2 μL of dNTP, 1.5 μL of 5 pmol/μL primers, and 2 μL of 50 ng/μL DNA template; sterilized deionized water was also added to a total volume of 20 μL. PCR reaction conditions were as follows: pre-denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 62.5°C for 30 s, and extension at 72°C for 30 s; and finally extension at 72°C for 7 min.

Sequencing and Alignment

DNA was sequenced using Sanger DNA sequencing (Taihe Biotechnology Co. Ltd. Beijing China). Sequencing primers were as follows: F 5′-ACGTGACTGGCCCTGCTATACC-3′, F2 5′-CAGGAAGCCTCCC-3′, and the sequencing results were analyzed by Chromas2.23 and DNAMAN8.0 software. After successful sequencing, we aligned the sequences with the reference sequences reported in the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/gene/).

Statistical Analysis

Genotype frequency and allele frequency were calculated by direct counting, and the chi-squared test was used to assess the associations of allele, genotype, and haplotype with schizophrenia risk. The odds ratio (OR) and 95% confidence interval (CI) were calculated using SPSS 22.0 (IBM, Armonk, NY, USA). Haploview4.2 (Broad Institute, Cambridge, MA, USA) was used to analyze Hardy–Weinberg equilibrium and haplotype confirmation. Power analysis was performed with the PS program (Dupont & Plummer, 1998). In all statistical analyses, significance was set at $p<0.05$. Bonferroni correction was performed for cases with several independent trials ($p<0.05/4$ was statistically significant). Pairwise differences between genotypes [AA vs AA, AA vs AA, AA vs AA (A being the risk factor)] were used to determine an appropriate genetic model.[18]

Results

Allele and Genotype Analyses

Through the analysis of the sequencing results, we detected 16 SNPs (single nucleotide polymorphism; rs12965479, rs72978691, rs11662010, rs7242919, rs11151014, rs11151015, rs75008330, rs80131113, rs35061175, rs5372, rs13306374, rs5373, rs13306375, rs1385809035, rs142660460, and rs5374). The allele frequencies and genotype frequencies of the detected SNPs are listed in Table 1.

Among the 16 SNPs detected in the control group, the distributions of all of them with the exception of rs5372 were in Hardy–Weinberg equilibrium ($p>0.05$, when the P value is more than 0.05, it indicates the genetic balance of the population; the data are from the same Mendelian population). The genotype and allele frequency distributions of the 16 SNPs did not differ significantly between the healthy population and schizophrenia patients ($p>0.05$) (Table 1).

Studies have shown that sex affects the correlation between candidate genes and the risk of schizophrenia.[19] A subgroup analysis based on sex was thus performed here on the detected SNPs and their association with the risk of schizophrenia, the results of which are shown in Tables 2 and 3. Among the females, allele A of rs72978691 ($p=0.007$), allele G of rs11662010 ($p=0.032$), allele G of rs11151014 ($p=0.014$), allele G of rs11151015 ($p=0.019$), allele C of rs13306374 ($p=0.009$), allele G of rs5373 ($p=0.005$), and allele G of rs13306375 ($p=0.010$) were identified as risk alleles for schizophrenia, while allele G of rs7242919 ($p=0.007$) was identified as a protective allele for schizophrenia in this study. To further explore which genotypes are associated with this condition, a model analysis of these eight SNPs was performed (Table 4).

In the model analysis in females, these eight SNPs were all found different in the dominant model: AA+AC genotype of rs72978691 ($p=0.006$, OR=1.874, 95% CI=1.196–2.937, power=0.780), GG+AG genotype of rs11662010 ($p=0.021$, OR=1.690, 95% CI =1.079–2.646, power=0.635), CC+CG genotype of rs7242919 ($p=0.002$, OR=2.027, 95% CI =1.292–3.180, power=0.861), GG+GT genotype of rs11151014 ($p=0.008$, OR=1.834, 95% CI =1.168–2.879, power=0.735), GG+AG genotype of rs11151015 ($p=0.002$,

Table 1 Genotype and Allele Frequencies of *GALR1* SNPs in Control Subjects and Schizophrenia Patients

SNPs	Case		Control		*P* value	OR	95% CI
	N	%	N	%			
rs12965479					0.846		
A/A	329	94.81	273	94.46			
A/G	18	5.19	16	5.54			
G/G	0	0.00	0	0.00			
G Allele	18	2.59	16	2.77	0.892	0.935	0.356–2.456
rs72978691					0.261		
C/C	212	61.10	158	54.67			
A/C	114	32.85	110	38.06			
A/A	21	6.05	21	7.27			
A Allele	156	22.48	152	26.3	0.263	0.813	0.565–1.169
rs11662010					0.484		
A/A	212	61.10	163	56.4			
A/G	115	33.14	108	37.37			
G/G	20	5.76	18	6.23			
G Allele	155	22.33	144	24.91	0.459	0.871	0.603–1.256
rs7242919					0.100		
C/C	18	5.19	16	5.54			
C/G	113	32.56	117	40.48			
G/G	216	62.25	156	54			
G Allele	545	78.53	429	74.22	0.201	1.270	0.880–1.832
rs11151014					0.243		
T/T	217	62.55	162	56.06			
G/T	111	31.99	110	38.06			
G/G	19	5.48	17	5.88			
G Allele	149	21.47	144	24.91	0.316	0.828	0.572–1.198
rs11151015					0.193		
A/A	219	63.11	162	56.06			
A/G	110	31.70	110	38.06			
G/G	18	5.19	17	5.88			
G Allele	146	21.04	144	24.91	0.246	0.803	0.554–1.164
rs75008330					0.502		
G/G	290	83.57	248	85.81			
A/G	56	16.14	39	13.49			
A/A	1	0.29	2	0.69			
A Allele	58	8.36	43	7.44	0.721	1.111	0.624–1.979
rs80131113					0.115		
T/T	280	80.69	246	85.12			
C/T	66	19.02	40	13.84			
C/C	1	0.29	3	1.04			
C Allele	68	9.80	46	7.96	0.419	1.256	0.722–2.186
rs35061175					0.844		
C/C	329	94.81	275	95.16			
C/T	18	5.19	14	4.84			
T/T	0	0.00	0	0			
T Allele	18	2.59	14	2.42	0.891	1.073	0.395–2.917

(*Continued*)

Table 1 (Continued).

SNPs	Case		Control		*P* value	OR	95% CI
	N	%	N	%			
rs5372					0.837		
C/C	64	18.44	55	19.03			
C/G	213	61.38	171	59.17			
G/G	70	20.17	63	21.8			
G Allele	353	51.86	297	51.38	0.901	1.020	0.747–1.394
rs13306374					0.330		
A/A	217	62.54	164	56.75			
A/C	113	32.56	108	37.37			
C/C	17	4.90	17	5.88			
C Allele	147	21.18	142	24.57	0.322	0.829	0.572–1.202
rs5373					0.948		
C/C	158	45.53	128	44.29			
C/G	159	45.82	136	47.06			
G/G	30	8.65	25	8.65			
G Allele	219	31.56	186	32.18	0.878	0.974	0.697–1.361
rs13306375					0.368		
A/A	209	60.23	158	54.67			
A/G	120	34.58	114	39.45			
G/G	18	5.19	17	5.88			
G Allele	156	22.48	148	25.61	0.357	0.842	0.585–1.214
rs1385809035					0.107		
C/C	306	88.18	266	92.04			
C/G	41	11.82	23	7.96			
G/G	0	0.00	0	0			
G Allele	41	5.91	23	3.98	0.281	1.488	0.719–3.078
rs142660460					0.154		
C/C	320	92.22	257	88.93			
A/C	27	7.78	32	11.07			
A/A	0	0.00	0	0			
A Allele	27	3.89	32	5.54	0.369	0.715	0.343–1.491
rs5374					0.596		
T/T	159	45.83	130	44.98			
C/T	152	43.80	135	46.71			
C/C	36	10.37	24	8.3			
C Allele	224	32.28	183	31.66	0.882	1.026	0.734–1.433

Notes: The SNPs with minor allele frequency <0.01 were excluded. The *p*-value was calculated by 2 × 3 and 2 × 2 chi-squared test. The false discovery rate was <0.05.
Abbreviations: SNP, single nucleotide polymorphism; 95% CI, 95% confidence interval; OR, odds ratio.

OR=2.013, 95% CI =1.291–3.137, power=0.843), CC+AC genotype of rs13306374 (p=0.006, OR=1.881, 95% CI=1.198–2.953, power=0.788), GG+GC genotype of rs5373 (p=0.042, OR=1.596, 95% CI=1.016–2.507, power=0.490), and GG+AG genotype of rs13306375 (p=0.006, OR=1.868, 95% CI=1.194–2.921, power=0.770) were risk genotypes for schizophrenia. Bonferroni correction was performed to overcome the problem of multiple comparisons. The finding of significant associations of rs11662010 and rs5373 with the risk of schizophrenia was lost after Bonferroni correction. However, the associations of rs72978691, rs7242919, rs11151014, rs11151015, rs13306374, and rs13306375 with the risk of schizophrenia were still maintained at significant levels (Table 4).

Table 2 Genotype and Allele Frequencies of *GALR1* SNPs in Control Male Subjects and Male Schizophrenia Patients

SNPs	Case		Control		*P* value	OR	95% CI
	N=132	%	N=187	%			
rs12965479					0.843		
A/A	125	94.70	178	95.19			
A/G	7	5.30	9	4.81			
G/G	0	0.00	0	0.00			
G Allele	7	2.65	9	2.41	0.845	1.105	0.406–3.004
rs72978691					0.815		
C/C	82	62.12	110	58.82			
A/C	43	32.58	65	34.76			
A/A	7	5.30	12	6.42			
A Allele	57	21.59	89	23.80	0.514	0.882	0.604–1.286
rs11662010					0.671		
A/A	84	63.64	111	59.36			
A/G	43	32.58	66	35.29			
G/G	5	3.79	10	5.35			
G Allele	53	20.08	86	22.99	0.379	0.841	0.572–1.237
rs7242919					0.891		
C/C	5	3.79	9	4.81			
C/G	45	34.09	65	34.76			
G/G	82	62.12	113	60.43			
G Allele	209	79.17	291	77.81	0.887	1.028	0.703–1.502
rs11151014					0.781		
T/T	83	62.88	113	60.43			
G/T	44	33.33	64	34.22			
G/G	5	3.79	10	5.35			
G Allele	54	20.45	84	22.46	0.775	0.945	0.641–1.393
rs11151015					0.907		
A/A	83	62.88	116	62.03			
A/G	44	33.33	62	33.16			
G/G	5	3.79	9	4.81			
G Allele	54	20.54	80	21.39	0.703	0.927	0.629–1.366
rs75008330					0.126		
G/G	120	90.91	156	83.42			
A/G	11	8.33	30	16.04			
A/A	1	0.76	1	0.53			
A Allele	13	4.92	32	8.56	0.078	0.554	0.285–1.076
rs80131113					0.057		
T/T	120	90.91	156	83.42			
C/T	10	7.58	30	16.04			
C/C	2	1.52	1	0.53			
C Allele	14	5.30	32	8.56	0.118	0.599	0.313–1.145
rs35061175					0.786		
C/C	124	93.94	177	94.65			
C/T	8	6.06	10	5.35			
T/T	0	0.00	0	0.00			
T Allele	8	3.03	10	2.67	0.789	1.138	0.443–2.922

(*Continued*)

Table 2 (Continued).

SNPs	Case		Control		*P* value	OR	95% CI
	N=132	%	N=187	%			
rs5372					0.645		
C/C	27	20.45	39	20.86			
C/G	84	63.64	111	59.36			
G/G	21	15.91	37	19.79			
G Allele	126	47.72	185	48.47	0.665	0.933	0.681–1.279
rs13306374					0.677		
A/A	86	65.15	113	60.43			
A/C	41	31.06	65	34.76			
C/C	5	3.79	9	4.81			
C Allele	51	19.32	83	22.19	0.526	0.881	0.595–1.304
rs5373					0.139		
C/C	73	55.30	84	44.92			
C/G	52	39.39	86	45.99			
G/G	7	5.30	17	9.09			
G Allele	66	25.00	120	32.09	0.052	0.706	0.496–1.004
rs13306375					0.427		
A/A	85	64.39	110	58.82			
A/G	42	31.82	67	35.83			
G/G	4	3.03	10	5.35			
G Allele	50	18.94	87	23.26	0.19	0.771	0.522–1.319
rs1385809035					0.088		
C/C	125	94.70	167	89.30			
C/G	7	5.30	20	10.70			
G/G	0	0.00	0	0.00			
G Allele	7	2.65	20	5.35	0.096	0.482	0.201–1.157
rs142660460					0.645		
C/C	121	91.67	174	93.05			
A/C	11	8.33	13	6.95			
A/A	0	0.00	0	0.00			
A Allele	11	4.17	13	3.48	0.652	1.207	0.532–2.738
rs5374					0.146		
T/T	73	55.30	88	47.06			
C/T	52	39.39	79	42.25			
C/C	7	5.30	20	10.70			
C Allele	66	25.00	119	31.82	0.062	0.714	0.502–1.017

Notes: The SNPs with minor allele frequency <0.01 were excluded. The *p*-value was calculated by 2 × 3 and 2 × 2 chi-squared test. The false discovery rate was <0.05.
Abbreviations: SNP, single nucleotide polymorphism; 95% CI, 95% confidence interval; OR, odds ratio.

Linkage Disequilibrium and Haplotypes

In linkage disequilibrium analysis, rs72978691, rs11662010, rs7242919, rs11151014, and rs11151015 were shown to be strongly linked together (any two SNPs, D′> 0.96, $r^2 > 0.89$) (Figure 1). In the control group, among the 16 SNPs, there were a total of 63 different haplotypes. Eight of these haplotypes (frequency > 1%; Table 5) were analyzed. It was found that H3 was associated with the risk of schizophrenia (p=0.011, OR=1.430, 95% CI=1.084–1.886), while H4 and H7 were protective against it (p=0.024, OR=0.526, 95% CI=0.298–0.927; p=0.037, OR=0.197, 95% CI=0.044–0.885, respectively) (Table 6).

In the female group, the eight SNPs rs72978691, rs11662010, rs7242919, rs11151014, rs11151015, rs13306374, rs5373, and rs13306375 were observed to be associated with the risk of schizophrenia, so they

Table 3 Genotype and Allele Frequencies of *GALR1* SNPs in Control Female Subjects and Female Schizophrenia Patients

SNPs	Case		Control		*P* value	OR	95% CI
	N=157	%	N=160	%			
rs12965479					0.967		
A/A	148	94.27	151	94.38			
A/G	9	5.73	9	5.63			
G/G	0	0.00	0	0.00			
G Allele	9	2.87	9	2.81	0.968	1.02	0.399–2.603
rs72978691					**0.022** *		
C/C	76	48.41	102	63.75			
A/C	67	42.68	49	30.63			
A/A	14	8.92	9	5.63			
A Allele	95	30.25	67	20.94	**0.007****	1.638	1.141–2.351
rs11662010					0.071		
A/A	79	50.32	101	63.13			
A/G	65	41.40	49	30.63			
G/G	13	8.28	10	6.25			
G Allele	91	29.00	69	21.56	**0.032***	1.484	1.034–2.130
rs7242919					**0.008****		
C/C	11	7.01	9	5.63			
C/G	72	45.86	48	30.00			
G/G	74	47.13	103	64.38			
G Allele	220	70.06	254	79.38	**0.007****	0.608	0.423–0.874
rs11151014					**0.030***		
T/T	79	50.32	104	65.00			
G/T	66	42.04	47	29.38			
G/G	12	7.64	9	5.63			
G Allele	90	28.66	65	20.31	**0.014***	1.576	1.093–2.273
rs11151015					**0.041***		
A/A	79	50.32	103	64.38			
A/G	66	42.04	48	30.00			
G/G	12	7.64	9	5.63			
G Allele	90	28.66	66	20.63	**0.019***	1.546	1.074–2.227
rs75008330					0.553		
G/G	128	81.53	134	83.75			
A/G	28	17.83	26	16.25			
A/A	1	0.64	0	0.00			
A Allele	30	9.55	26	8.13	0.526	1.194	0.689–2.070
rs80131113					0.465		
T/T	126	80.25	124	77.50			
C/T	30	19.11	36	22.50			
C/C	1	0.64	0	0.00			
C Allele	32	10.19	36	11.25	0.667	0.895	0.541–1.482
rs35061175					0.610		
C/C	151	96.18	152	95.00			
C/T	6	3.82	8	5.00			
T/T	0	0.00	0	0.00			
T Allele	6	1.91	8	2.50	0.614	0.76	0.261–2.215

(*Continued*)

Table 3 (Continued).

SNPs	Case		Control		*P* value	OR	95% CI
	N=157	%	N=160	%			
rs5372					0.299		
C/C	28	17.83	25	15.63			
C/G	87	55.41	102	63.75			
G/G	42	26.75	33	20.63			
G Allele	171	54.46	168	52.50	0.621	1.082	0.792–1.478
rs13306374					**0.022***		
A/A	78	49.68	104	65.00			
A/C	67	42.68	48	30.00			
C/C	12	7.64	8	5.00			
C Allele	91	28.98	64	20.00	**0.009****	1.632	1.131–2.355
rs5373					0.114		
C/C	55	35.03	74	46.25			
C/G	84	53.50	73	45.63			
G/G	18	11.46	13	8.13			
G Allele	120	38.22	89	27.81	**0.005****	1.605	1.150–2.242
rs13306375					**0.022***		
A/A	73	46.50	99	61.88			
A/G	72	45.86	53	33.13			
G/G	12	7.64	8	5.00			
G Allele	96	30.57	69	21.56	**0.010****	1.602	1.119–2.293
rs1385809035					0.416		
C/C	141	89.81	139	86.88			
C/G	16	10.19	21	13.13			
G/G	0	0.00	0	0.00			
G Allele	16	5.10	21	6.56	0.431	0.764	0.391–1.494
rs142660460					0.189		
C/C	136	86.62	146	91.25			
A/C	21	13.38	14	8.75			
A/A	0	0.00	0	0.00			
A Allele	21	6.69	14	4.38	0.202	1.567	0.782–3.139
rs5374					0.289		
T/T	57	36.31	72	45.00			
C/T	83	52.87	73	45.63			
C/C	17	10.83	15	9.38			
C Allele	117	37.26	103	32.19	0.180	1.251	0.902–1.736

Notes: The SNPs with minor allele frequency <0.01 were excluded. The *p*-value was calculated by 2 × 3 and 2 × 2 chi-squared test. The false discovery rate was <0.05. The bold text indicates p<0.05. *p<0.05, **p<0.01.

were then subjected to haplotype analysis in females. In the control group, there were a total of 17 different haplotypes consisting of the eight above-mentioned SNPs. We chose four haplotypes (frequency > 1%; Table 7) to analyze associations with the risk of schizophrenia, the results of which are shown in Table 8. Among them, our findings suggested that FH2 may be correlated with the occurrence of schizophrenia (p=0.024, OR=1.858, 95% CI=1.080–3.196).

Discussion

In this study, the distribution of rs5374 in the control group did not conform to Hardy–Weinberg equilibrium, which may be related to the insufficiently random

Table 4 The Model Analysis of Eight SNPs in Females

SNPs	Model	*P* value	OR	95% CI	Power
rs72978691 C>A	AAvsCC	0.099	2.088	0.859–5.076	
	AAVSAC	0.782	1.138	0.456–2.840	
	AA+ACvsCC	**0.006****	1.874	1.196–2.937	0.780
	AAvsAC+CC	0.259	1.643	0.690–3.913	
rs11662010 A>G	GGvsAA	0.252	1.662	0.693–3.989	
	GGvsAG	0.965	0.980	0.397–2.420	
	GG+AGvsAA	**0.021***	1.690	1.079–2.646	0.635
	GGvsAG+AA	0.486	1.354	0.576–3.186	
rs7242919 C>G	CCvsGG	0.259	1.701	0.671–4.313	
	CCvsCG	0.673	0.815	0.314–2.115	
	CC+CGvsGG	**0.002****	2.027	1.292–3.180	0.861
	CCvsCG+GG	0.613	1.264	0.509–3.140	
rs11151014 T>G	GGvsTT	0.222	1.755	0.705–4.371	
	GGvsGT	0.914	0.949	0.370–2.435	
	GG+GTvsTT	**0.008 ****	1.834	1.168–2.879	0.735
	GGvsGT+TT	0.470	1.389	0.568–3.394	
rs11151015 A>G	GGvsAA	0.231	1.738	0.698–4.330	
	GGvsAG	0.914	0.949	0.370–2.435	
	GG+AGvsAA	**0.002****	2.013	1.291–3.137	0.843
	GGvsAG+AA	0.470	1.389	0.568–3.394	
rs13306374 A>C	CCvsAA	0.143	2.000	0.780–5.128	
	CCvsAC	0.884	1.075	0.408–2.830	
	CC+ACvsAA	**0.006****	1.881	1.198–2.953	0.788
	CCvsAC+AA	0.333	1.572	0.625–3.958	
rs5373 C>G	GGvsCC	0.121	1.863	0.842–4.122	
	GGvsGC	0.641	1.596	0.553–2.623	
	GG+GCvsCC	**0.042***	1.596	1.016–2.507	0.490
	GGvsGC+CC	0.317	1.464	0.692–3.100	
rs13306375 A>G	GGvsAA	0.135	2.034	0.791–5.230	
	GGVSAG	0.840	1.104	0.422–2.891	
	GG+AGvsAA	**0.006****	1.868	1.194–2.921	0.770
	GGvsAG+AA	0.333	1.572	0.625–3.958	

Notes: The *p*-value was calculated by 2 × 3 and 2 × 2 chi-squared test, in which the codominant model, the recessive model, and the allele model were corrected by Bonferroni correction and $p < 0.05/4$ was considered statistically significant. The statistical power was considered to be sufficient to detect any significant difference at power >0.8. The false discovery rate was <0.05. The bold text indicates p<0.05. *p<0.05, **p<0.01.

Abbreviation: SNP, single nucleotide polymorphism; 95% CI, 95% confidence interval; OR, odds ratio.

sampling, small population size, and presence of more than 16 SNPs in the analyzed fragment. The purpose of this study was to explore the correlation between the *GALR1* gene and the risk of schizophrenia in the Han Chinese.

In this study, H3 was identified as a risk haplotype of schizophrenia, while H4 and H7 were shown to be haplotypes protective against schizophrenia. The findings indicated that polymorphisms of the 5′ regulatory region of the *GALR1* gene are correlated with schizophrenia, and that the roles of haplotypes in this condition warrant further study. Although no clear mechanism has been found to date, reasonable biological evidence for this is available: GalR1 is mainly expressed in cortical areas associated with emotion and emotion control, and experiments have proven that GalR1 in the cerebral cortex is closely related to the occurrence of depression.[20]

Moreover, numerous animal studies have shown that GalR1 is associated with depressive behavior.[21–27] In rodents, activation of GalR1 was shown to lead to

Table 5 Haplotypes of 16 SNPs in the *GALR1* Gene in Control Subjects

Haplotype	rs12965479	rs72978691	rs11662010	rs7242919	rs11151014	rs11151015	rs75008330	rs80131113	rs35061175	rs5372	rs13306374	rs5373	rs13306375	rs1385809035	rs142660460	rs5374
H1	A	C	A	G	T	A	G	T	C	C	A	C	A	C	C	T
H2	A	C	A	G	T	A	G	T	C	G	A	C	A	C	C	T
H3	A	A	G	C	G	G	G	T	C	G	C	G	G	C	C	C
H4	A	C	A	G	T	A	A	C	C	G	A	G	A	C	C	C
H5	A	C	A	G	T	A	G	T	C	C	A	C	A	C	A	T
H6	G	C	A	G	T	A	G	T	T	C	A	C	A	C	C	T
H7	A	C	A	G	T	A	G	T	C	C	A	C	A	G	C	T
H8	A	C	A	G	T	A	G	C	C	G	A	G	A	C	C	C

Note: The eight haplotypes are those at a frequency >1% among the total of 63 haplotypes. Haplotypes with frequency <1% were excluded.
Abbreviation: SNP, single nucleotide polymorphism.

Table 6 Haplotype Analysis of *GALR1* SNPs in Control Subjects and Schizophrenia Patients

Haplotype	Case (n=578)		Control (n=694)		*P* value	OR	95% CI
	N	%	N	%			
H1	222	38.4	265	38.2	0.935	1.010	0.804–1.267
H2	103	17.8	126	18.2	0.877	0.978	0.733–1.303
H3	132	22.8	119	17.1	**0.011***	1.430	1.084–1.886
H4	18	3.1	40	5.8	**0.024***	0.526	0.298–0.927
H5	31	5.4	22	3.2	0.051	1.731	0.991–3.024
H6	11	1.9	13	1.9	0.969	1.016	0.452–2.286
H7	2	0.4	12	1.7	**0.037***	0.197	0.044–0.885
H8	2	0.4	9	1.3	0.129	0.264	0.057–1.228

Notes: The bold text indicates p<0.05 *p<0.05.
Abbreviations: SNP, single nucleotide polymorphism; 95% CI, 95% confidence interval; OR, odds ratio.

Table 7 Haplotypes of Eight SNPs in the *GALR1* Gene in the Female Control Subjects

Haplotype	rs72978691	rs11662010	rs7242919	rs11151014	rs11151015	rs13306374	rs5373	rs13306375
FH1	C	A	G	T	A	A	C	A
FH2	A	G	C	G	G	C	G	G
FH3	C	A	G	T	A	A	G	A
FH4	C	A	G	T	A	C	G	G

Note: The four haplotypes are those at a frequency of >1% among the total of 17 haplotypes. Haplotypes with frequency <1% were excluded.
Abbreviations: SNP, single nucleotide polymorphism.

Table 8 Haplotype Analysis of Eight *GALR1* SNPs in Female Control Subjects and Schizophrenia Patients

Haplotype	Case (n=314)		Control (n=320)		*P* value	OR	95% CI
	N	%	N	%			
FH1	190	60.5	213	66.7	0.113	0.77	0.557–1.065
FH2	86	27.4	54	16.8	**0.001****	1.858	1.266–2.727
FH3	21	6.7	27	8.3	0.495	0.778	0.430–1.407
FH4	1	0.3	7	2.2	0.069	0.143	0.017–1.168

Notes: The bold text indicates p<0.05. **p<0.01.
Abbreviations: SNP, single nucleotide polymorphism; 95% CI, 95% confidence interval; OR, odds ratio.

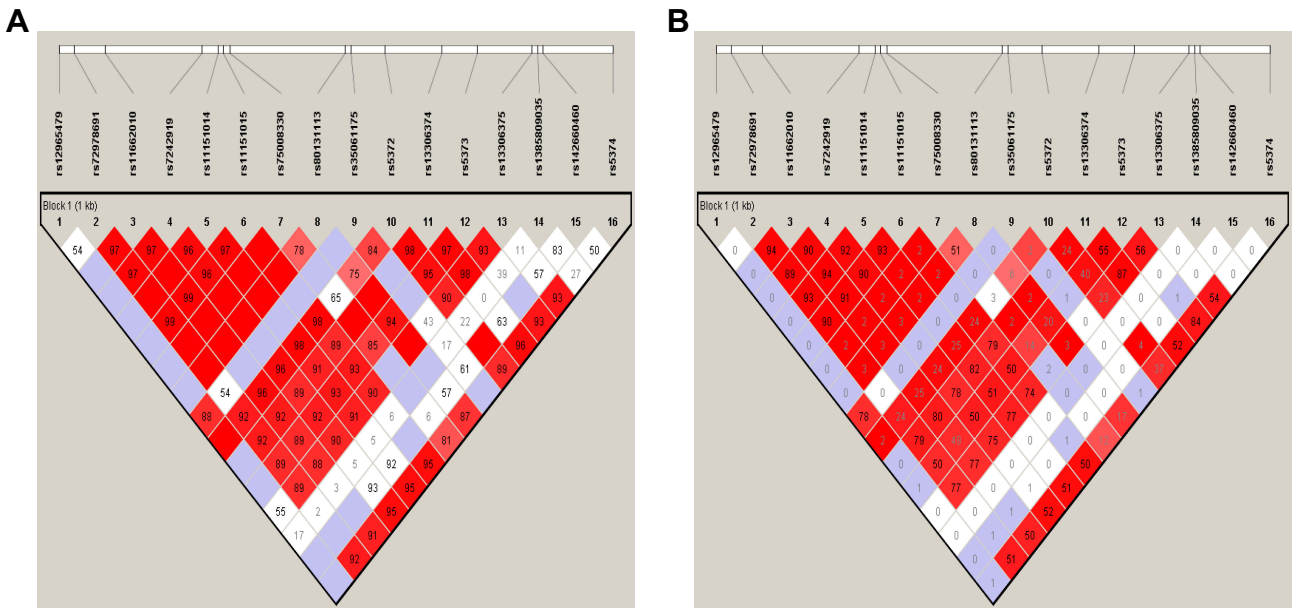

Figure 1 Linkage disequilibrium block composed of rs12965479, rs72978691, rs11662010, rs7242919, rs11151014, rs11151015, rs75008330, rs80131113, rs35061175, rs5372, rs13306374, rs5373, rs13306375, rs1385809035, rs142660460, and rs5374. The number is the value of multiallelic *D′* (**A**) and r^2 (**B**), which represents the level of recombination between the two blocks.

depression-like behavior.[27–29] Many researchers have also reported that GAL mainly functions through GalR1–5-HT1A heteroreceptor complexes.[30–32] Furthermore, polymorphisms in GALR1 were reported to be associated with a variety of psychiatric disorders, such as rs5376 associated with heroin addiction, rs5374 associated with cocaine addiction, and rs5117162 associated with both of these addictions.[16] Although to the best of our knowledge no research on the correlation between the *GALR1* gene and schizophrenia has been reported, many researchers have suggested that addiction, anxiety, depression, schizophrenia, and other psychiatric disorders might have similar genetic backgrounds.[33,34] We thus proposed that *GALR1* may be correlated with schizophrenia. In the current studies, the hypothesis of dopamine and 5-HT was widely accepted. GalR1 inhibits the release of 5-HT, thus increasing the susceptibility to schizophrenia. Thus, *GALR1* may be one of the many pathogenic factors associated with schizophrenia.

Because the incidence of schizophrenia in females is significantly higher than that in males,[35] in this study a subgroup analysis by sex was implemented. The results showed that, in the female group, rs72978691, rs7242919, rs11151014, rs11151015, rs13306374, and rs13306375 were significantly different between the schizophrenia group and the control group. No such difference was found in males. SNP is a bimodal magnetic marker with low heterozygosity. To improve heterozygosity and make more efficient use of genetic information, haplotype analysis was carried out on the female population in this study. Within the females, the haplotype FH2 was significantly different between the schizophrenia group and the control group and was identified as a risk factor for schizophrenia. It was thus indicated that there was indeed a correlation between the *GALR1* gene and schizophrenia, with this correlation being particularly strong in females. In terms of an explanation for this, it was previously reported that the GalR1 ligand GAL is closely related to estrogen production and release.[20] In addition, the correlation between rs948854 and rs694066 of the GAL gene and depression was demonstrated only in females,[12,20] although the mechanism behind this correlation needs to be confirmed by further studies. GalR1 is the main inhibitory receptor of GAL, so it is reasonable that there are also sex differences in the correlation between *GALR1* and schizophrenia.

There are some limitations of this study. First, there were not enough SNPs in the gene. Second, some deviation from HWE was identified. Third, a family-based study, which is more robust than a case–control design[36] was not included in this work. Fourth, although interactions between multiple genes might affect the risk of schizophrenia, these were not studied here.[37]

In conclusion, our study found an association between polymorphisms of the 5′ region of the *GALR1* gene and the risk of schizophrenia, especially in females. Additional

larger studies on the etiology of schizophrenia are necessary, for which our data may provide a useful reference.

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Disclosure

The authors declare no conflicts of interest.

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