Association of Glutamate Receptor Metabotropic 5 Gene (Grm5) Polymorphisms With Schizophrenia Susceptibility and Symptoms in a Chinese-Han Population

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Research Article

Keywords: schizophrenia, GRM5, SNPs, association analyses, PANSS

Posted Date: March 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-253495/v1

Abstract

Objectives: Metabotropic glutamate receptor subtype 5 (mGluR5) is a potential target for the treatment of schizophrenia (SZ), with the evidence that mGluR5 modulates glutamatergic signaling through the NMDA receptor (NMDAR). Recently it was reported that the *GRM5* gene (encoding mGluR5) is associated with SZ in the Scottish population.

Methods: Here, case-control association analyses were performed in the Chinese-Han population to investigate if *GRM5* gene is implicated in SZ. Twenty-four single nucleotide polymorphisms (SNPs) were analyzed in 528 paranoid SZ and 528 control subjects.

Results: The genotypic and allelic frequencies of two SNPs, rs567990 and rs12421343 were significantly different between the case and control group (Genotype $P = 0.007$ and 0.011; Allele $P = 0.003$ and 0.021; respectively). The frequency of rs504183 allele was associated with SZ ($P = 0.030$). When subjects were stratified by gender, the rs12422021, rs567990, rs12421343, and rs7101540 remained significantly associated with SZ in female patients. Analysis of clinical features of SZ, as measured by the Positive and Negative Syndrome Scale (PANSS) inventory, displayed association of *GRM5* to features of the general phenotype of SZ, including traits representing delusions, hallucinations and negative symptoms.

Conclusion: In conclusion, our study provides further evidence that *GRM5* is associated with SZ, and implies a putative sex difference for the effect of the gene.

Introduction

Schizophrenia (SZ) is a common serious mental disorder, and its symptoms are complex and clinical diagnostic criteria are different, which is mainly characterized by mental, emotional and behavioral disorders and incoordination of mental activities (Keshavan et al. 2020; Schaefer et al. 2020). Researches based on family or twin suggest that schizophrenia has a high heritability of 64-81% and a complex genetic component (Yue et al. 2017). It is often described as a heterogeneous disorder affected by multiple genes, each with a small effect (Goldman et al. 2020). Thus far, genetics studies have suggested numerous putative chromosomal loci for this disorder.

Although the pathogenesis of schizophrenia is unclear, studies suggest that the disorder is associated with abnormal neurodevelopment (Matosin and Newell 2013), in which both genetic and environmental factors could influence the structure and function of brain (Lang et al. 2007). Glutamate has been linked to a myriad of processes surrounding cognition, memory, and perception (Thomas et al. 2017). Mounting evidence suggest that glutamatergic dysfunction may be critical in the etiology of schizophrenia (Steele et al. 2012). Glutamate is an excitatory neurotransmitter that is abundant in the brain and plays an important role in various brain functions and brain development (Uno and Coyle 2019; Wickens et al. 2018). Glutamate exerts effects through activating metabotropic glutamate receptors (mGluRs) and ionotropic receptors (iGluRs). The iGluRs include the N-methyl-D-aspartate receptors (NMDAR), a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and Kainate receptors. Metabotropic glutamate receptors are G protein-coupled receptors and modulate cellular excitability and synaptic transmission (Niswender and Conn 2010). There are different subtypes of mGluRs in presynaptic and postsynaptic membrane that could modulate glutamatergic neurotransmission. The involvement in multiple neuronal processes indicates its importance on the dysfunction of brain (Mukherjee and Manahan-Vaughan 2013).

Therefore, it is necessary to conduct in-depth systematic analyses on the relationship between candidate genes of glutamate pathway and schizophrenia. Metabolic glutamate receptor subtype 5 (mGluR5, encoded by gene *GRM5*) plays an irreplaceable role in the glutamate pathway, and convergent lines of evidence suggest that implicated in schizophrenia (Akkus et al. 2017). Studies have shown that mGluR5 critically modulate the function of NMDAR, which is strongly associated with schizophrenia, and mGluR5 abnormalities maybe contribute to pathogenesis of schizophrenia (Zurawek et al. 2018). The involvement of NMDAR in the long-term potentiation (LTP) process involved in memory and cognition, suggesting that mGluR5 may be a potential therapeutic target for the repair of cognitive deficits in schizophrenia (Kosten et al. 2016; Matosin et al. 2017). Abnormal expression or dysfunction of the mGluR5 is associated with schizophrenia (Matosin et al. 2016), and might originate at the genetic level. In a case-control study, the distribution of allele frequencies of the *GRM5* gene was found to be significantly different between schizophrenia patients and controls in the Scottish population (Devon et al. 2001).

Here, we performed a case-control analysis in the Chinese-Han population to investigate the potential associations between schizophrenia and *GRM5* SNPs, providing new evidence for schizophrenia susceptibility gene research.

Materials And Methods

The Ethical permission was obtained from the Ethical Committee of the Second Affiliated Hospital of Xinxiang Medical University. Written informed consent was obtained from all participants after an adequate explanation of the objectives and procedures about the study.

Subjects

Unrelated schizophrenic patients were recruited from Han population in northern Henan and 528 paranoid SZ were selected as cases (age from 18-35 years old). According to the diagnostic criteria the DSM-IV, at least two experienced psychiatrists make a consensus diagnosis. Individuals with a history of severe medical complications, organic brain diseases, and other psychiatric disorders were excluded. In the meantime, healthy people who had four biological grandparents of Han Chinese ancestry were recruited from surrounding communities and villages, of which 528 healthy controls matched for sex ratio (1:1 for both groups), age, and ethnicity.

The syndromes of 267 first-episode patients were assessed by the Positive and Negative Symptom Scale (PANSS).

SNP Selection

The functional analysis was performed for all SNPs in the *GRM5* gene region using the FASTSNP online tool (Yuan et al. 2006). According to the HapMap database, the SNPs with high ranking risk and minor allele frequency (MAF) ≥ 0.05 in the Chinese-Han population were screened out. Tag SNPs were selected through the same method in previous article (Carlson et al. 2004; Li et al. 2013) ($r^2 \geq 0.80$, MAF ≥ 0.05) using HapMap database by Haploview v4.1 (Barrett et al. 2005). All selected SNPs were evaluated, and the SNPs with Illumina design scores below 0.6 were excluded.

Genotyping

Genomic DNA samples of the participants were isolated from their peripheral blood using the Blood DNA System (Tiangen Biotech, Beijing, China). Genotyping was done using the Illumina GoldenGate assay with a BeadStation 500G System following the Illumina protocol. To evaluate the quality of genotyping, SNP genotyping of 100 samples was performed by DNA sequencing and 96 samples were genotyped in duplicate performed.

Statistical analysis

Power calculations were performed using the Genetic Power Calculator (Purcell et al. 2003). Genotypes and allele association test, haplotype analysis and Hardy-Weinberg equilibrium (HWE) evaluation were performed using the Haploview v4.1 software. Genotypes and allele were compared between two groups by the Pearson chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by SNPStats, a web tool (Sole et al. 2006). Bonferroni corrections for multiple tests were used to exclude type I errors.

Comparison of different genotypes in the case group with the clinical symptoms of the patients was performed using the SPSS13.0 statistical software package. The Levene variance homogeneity test was used for ANOVA, and the Krtlskal-Wailis rank sum test was used for SNP with irregular variance. The five factors scores of the PANSS were compared among different genotypes in the same SNP (Yang et al. 2015). The criterion for significance was set at $P<0.05$ for all tests.

Results

The alleles and genotype frequencies of twenty-four common SNPs at the *GRM5* gene were analyzed in all samples, with a genotyping success rate of 99.83%. The genotype concordance between GoldenGate assay and DNA sequencing was 99.25%. The total sample size (n=1056) had a power of 0.86 to detect a small effect, and a power of 1.00 to detect both medium and large effects on genotype distributions. Among the twenty-four SNPs, twenty-two yielded genotype data, with the genotype and allele frequencies shown in **Table 1**. Only rs4753778 and rs982709 were not in HWE. The genotypic and allelic frequencies of two SNPs, rs567990 and rs12421343 were significantly different between the case and control group (Genotype P = 0.007 and 0.011; Allele P = 0.003 and 0.021; respectively). Furthermore, the frequency of rs504183 allele was associated with schizophrenia (P = 0.030).

When we stratified subjects by gender, the genotype and allele frequency of rs12421343 was significantly different between case and control group (P = 0.003 and 0.004 respectively) in female samples. Additional, rs12422021, rs567990, and rs7101540 allelic frequency was associated with schizophrenia (P = 0.030, 0.010, 0.040; OR = 0.600, 0.750, 0.630; 95%CI = 0.38-0.96, 0.59-0.95, 0.40-0.97; respectively) (**Supplementary Table 1**). However, there was no significant difference on genotype and allele frequency between male and control subjects (P > 0.05, **data not shown**).

The haplotype structure of our sample and associations between schizophrenia and LD blocks, then pair-wise LD of twenty-two SNPs in the schizophrenia patient and control group was analyzed by the standardized D' and r^2 values. The SNP's order and physical locations in the *GRM5* gene, the LD structure, and the D' values for all variants were presented in **Fig. 1** Haplotypes GG and AA in the LD block rs567990–rs12421343 showed significant difference between schizophrenia and controls (P = 0.003, and 0.025, respectively), and so was haplotype AA in the LD block rs7932640–rs7125164 (P = 0.041). It shows the three significantly associated haplotypes in **Table 2.**

To detect the association between the SNPs of *GRM5* gene and symptoms, the PANSS scores of 267 schizophrenia patients were analyzed. As shown in **Table 3**, there were significant difference between patients with different rs12422021 genetypes (GG, AA + AG) on total PANSS and positive, excitement, cognitive impairment factor subscores (P = 0.026, 0.019, 0.016, 0.024; respectively). Furthermore, negative factor subscores were significantly differed among the three genotypes of rs504183 (P = 0.025, and 0.040; respectively). The significantly associated with positive (rs12421343) and cognitive impairment (rs567990) factor subscores (P = 0.003, 0.020; respectively).

Discussion

The aim of this study is to investigate the *GRM5* mutations associated with schizophrenia and psychotic symptoms in the Han Chinese population. There were significant differences in genotype and allele frequencies of two SNPs (rs567990 and rs12421343) between the case group and the control group. In addition, the allele frequencies of the rs504183 SNP was significantly associated with schizophrenia in this population. In the *GRM5* gene, the haplotypes GG and AA (rs567990-rs12421343) revealed significant associations with schizophrenia, and so did haplotype AA□rs7932640-rs7125164). This indicates that variations in the *GRM5* gene may increase susceptibility to schizophrenia in the Chinese Han population.

When we divided the patients by gender, we found the association of the genotype and allele frequency of rs12421343 of *GRM5* with schizophrenia in female subjects, and the allele frequency of rs12422021, rs567990, and rs7101540, but not in male subjects. Moreover, research have shown that there was a positive correlation between the *PAWR* gene and female schizophrenia in Taiwanese Han population (Wang et al. 2008), similarly, female specificity was found in the schizophrenia Jewish population associated with rs7341475 in the *RELN* gene (Liu et al. 2010). Numerous studies have revealed the gender differences in clinical features of schizophrenia, such as age of onset, symptoms, drug response, clinical course and prognosis (Chen et al. 2018; de Boer et al. 2018). Some

researchers have demonstrated that there may be differences in etiology between male and female patients (Mendrek and Mancini-Marie 2016; Noguera et al. 2018). Gender differences in schizophrenia are thought to be associated with estrogen levels in patients. It has been demonstrated that estrogen impacts neuronal growth and death at every level, which involved in the mechanism of neurodegenerative in schizophrenia (Goncalves et al. 2019). Though the interaction between the female-specific association of the *GRM5* gene and schizophrenia is unclear, our study support differences in genetic susceptibility between the genders.

The clinical characteristics of schizophrenia include positive symptoms (delusions, hallucinations and erratic behavior), negative symptoms (apathy, anhedonia and depression), and cognitive disturbances (poor learning and memory) (Albanna et al. 2014; Zurawek et al. 2018). In this study, two SNPs in *GRM5* gene were associated with schizophrenia positive symptoms and one SNP was associated with negative symptoms. This consistently demonstrated the association of *GRM5* variants with the pathophysiology of schizophrenia. Jennifer et al. found that positive allosteric modulators (PAMs) for mGluR5 may be effective in treating positive symptoms of schizophrenia (Ayala et al. 2009). Studies have also shown that negative symptoms and cognitive disorders may be the effects of glutamatergic dysfunction (McAllister et al. 2015; Volk et al. 2015). The glutaminergic theory of schizophrenia is supported by the following observations: subchronic administration of ketamine and phenylidine (PCP, the noncompetitive antagonists of the NMDAR) in healthy humans can induce similar clinical manifestations of schizophrenia, including cognitive impairment (Coyle et al. 2012). It should be noted that NMDAR blockade can regulate dopaminergic signal transduction in the marginal pathway of the mesencephalon (Yang et al. 2017). Recent evidence suggests the importance of mGluR5 in regulating the activity and function of NMDAR. Thus, *GRM5* is associated with the psychopathology of schizophrenia.

mGluR5 is an excitatory post-synaptic receptor which can regulate neuronal excitability, synaptic plasticity and synaptic selection (Hoeffer and Klann 2010). It is enriched in the brain regions involved in cognition, including hippocampus, striatum and cortex (Lopez-Bendito et al. 2002). It is believed that mGluR5 can regulate many cognitive fields and behaviors (Bird et al. 2014; Maksymetz et al. 2017; Matosin et al. 2018; Wu et al. 2020). This study showed that rs567990 and rs12422021 were significantly correlated with cognitive impairment factor subscores. This suggests that *GRM5* is associated with cognitive function in schizophrenia.

In addition, some susceptible genes of schizophrenia show a dynamic process during the early development of the prefrontal cortex. Therefore, the abnormal expression of these susceptible genes can increase the occurrence of abnormal brain function and cause schizophrenia. The expression of *GRM5* gene changes with age (Hernandez et al. 2018). Studies have shown that *GRM5* is involved in basic developmental processes such as proliferation, differentiation, and survival of cells (Purgert et al. 2014). Therefore, it reflects the important role of *GRM5* in the early development of brain function.

GRM5 is the best functional candidate gene involved in psychopathology on chromosome 11 (Devon et al. 2001). In a case-control study, Devon et al. had found a significant difference in the distribution of allele frequencies of *GRM5* gene between schizophrenic patients and controls. The difference was detected in an intron region of the *GRM5* gene using microsatellites. Based on recent studies, it has been proved that enhancing the function of *GRM5* showed therapeutic effects. This study and previous have demonstrated that *GRM5* is a candidate susceptibility gene for schizophrenia.

In many previous studies, the subjects were from vast geographic regions and genetically heterogeneous. However, we avoid the effect of group stratification, as all subjects in our study were from Xinxiang or surrounding counties, and genetic structure analysis showed that schizophrenia patients and controls belonged to the same population. Replication studies are required to be carried out in different ethnic groups, particularly in patients with definite schizophrenia phenotypes to confirm the role of *GRM5* variants in schizophrenia.

Conclusion

In summary, our study provides new evidence suggesting that *GRM5* is associated with schizophrenia. The genetic variation of *GRM5* may increase susceptibility of schizophrenia.

Declarations

Funding This work was supported in part by the National Natural Science Foundation of China (U1904130 to WQ-L; 82001407 to XS; 81971252 to LX-L; 81903688 to ML-S), Medical science and technology research project of Henan Province (LHGJ20190476 to MS; 2018020371 to XS), Key Scientific Research Project of Henan Higher Education Institutions (20B320016 to MS), The Open Fund of Henan Key lab of Biological Psychiatry (ZDSYS2018003 to MS; ZDSYS2018008 to XS), Science and technology research project of Henan Province (212102310587 to MS; 212102310589 to YF-Y), National Key Research and Development Program of China (2016YFC1307001 to LX-L).

Conflict of Interest The authors declare that they have no conflict of interest.

Availability of data and material The data is freely available to the public.

Code availability Not applicable.

Author Contributions All authors contributed to the study conception and design. The literature searches and analyses were managed by Meng Song and Wenqiang Li. Sample selection and data management were conducted by Xiaoge Guo, Qing Liu, Luwen Zhang, Yan Zhang, Minli Ding, Yanli Lu and Yongfeng Yang. The statistical analysis was performed by Wenqiang Li, Xi Su, Xiaoge Guo, Meng Song, Ming Li and Minglong Shao. The first draft of the manuscript was written by Wenqiang Li and Xi Su. All authors contributed to and have approved the final manuscript.

Ethics approval The Ethical permission was obtained from the Ethical Committee of the Second Affiliated Hospital of Xinxiang Medical University.

Consent to participate Written informed consent was obtained from all participants after an adequate explanation of the objectives and procedures about the study.

Consent for publication All participants and authors approved the publication of this manuscript.

Acknowledgments We thank the patient, their families, and the healthy volunteers for their participation, and the physicians who collect clinical data and blood samples in the Second Affiliated Hospital of Xinxiang Medical University.

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Tables

Table 1 Genotype and allele frequencies of twenty-two SNPs in the *GRM5* gene in patients with schizophrenia and controls

SNP#	dbSNP ID	Allele(D/d)[a]	Patients						Controls						*p*-value[c]	
			n[b]	HWE(*P*)	Genotype			MAF	n[b]	HWE(*P*)	Genotype			MAF		
					DD	Dd	dd				DD	Dd	dd		Genotype	A
1	rs567990	A/G	528	0.393	130	254	144	0.513	527	0.467	156	269	102	0.449	**0.007(0.165)**	**0**
2	rs12421343	A/G	528	0.275	434	87	7	0.096	528	0.093	456	72	0	0.068	**0.011(0.272)**	**0**
3	rs504183	C/A	528	0.299	224	248	56	0.341	527	0.253	255	231	41	0.297	**0.085**	**0**
4	rs7932640	A/G	528	0.791	196	249	83	0.393	526	0.965	220	240	66	0.354	0.170	0
5	rs7101540	A/T	528	0.144	428	98	2	0.097	528	0.985	451	74	3	0.076	0.130	0
6	rs7125164	C/A	527	0.844	189	255	83	0.399	527	0.991	173	258	96	0.427	0.430	0
7	rs12422021	A/G	527	0.630	453	72	2	0.072	528	0.353	469	56	3	0.059	0.290	0
8	rs2306153	G/A	528	0.763	476	51	1	0.050	528	0.963	464	62	2	0.063	0.460	0
9	rs679333	C/A	528	0.031	225	256	47	0.331	528	0.120	244	241	43	0.310	0.500	0
10	rs16915127	A/G	528	0.654	272	211	45	0.285	528	0.915	257	222	49	0.303	0.650	0
11	rs598408	A/G	528	0.258	453	74	1	0.072	528	0.811	445	79	4	0.082	0.360	0
12	rs7120151	A/G	528	0.302	241	239	48	0.317	528	0.931	259	221	48	0.300	0.510	0
13	rs11018441	C/G	527	0.643	287	201	39	0.265	527	0.095	304	183	40	0.250	0.510	0
14	rs5015672	G/A	528	0.138	464	64	0	0.061	528	0.088	455	73	0	0.069	0.410	0
15	rs5016282	G/A	528	0.155	315	178	35	0.235	528	0.269	293	207	28	0.249	0.150	0
16	rs594561	A/G	528	0.418	200	257	71	0.378	527	0.081	186	270	71	0.391	0.660	0
17	rs4753778	A/G	526	0.853	308	190	28	0.234	526	0.049	308	178	40	0.245	0.290	0
18	rs3824927	A/C	527	0.628	146	268	113	0.469	528	0.860	155	264	109	0.456	0.830	0
19	rs666229	C/A	528	0.147	253	235	40	0.298	528	0.062	246	242	40	0.305	0.900	0
20	rs982709	A/G	526	0.843	517	9	0	0.009	523	2.336E-08	516	6	1	0.008	0.450	0
21	rs591849	A/T	528	0.077	147	282	99	0.455	528	0.314	153	273	102	0.452	0.860	0
22	rs566277	C/A	527	0.804	311	189	27	0.231	527	0.998	312	187	28	0.231	0.980	1

[a] Major/minor allele, major and minor alleles are denoted by D and d, respectively.

[b] Number of samples which are well genotyped.

[c] *P*-Values in the parenthesis were analyzed with Bonferroni corrections, the significance of bold values is $P<0.05$.

Table 2 Haplotype in the *GRM5* gene in patients with schizophrenia and controls

Haplotype[a]	Haplotype frequencies[b]		Chi Square	*P*-value[c]
	Patients	Controls		
rs567990-rs12421343				
GG	582.1 (0.551)	512 (0.486)	9.062	**0.003**
AG	401.9 (0.381)	442 (0.419)	3.308	0.069
AA	72 (0.068)	100 (0.095)	5.021	**0.025**
rs7932640-rs7125164				
GC	447.9 (0.424)	421.2 (0.399)	1.390	0.238
AA	369.2 (0.350)	414.6 (0.393)	4.171	**0.041**
GA	235.5 (0.223)	219.8 (0.208)	0.690	0.406
rs3824927-rs566277				
AC	482.0 (0.456)	495.2 (0.469)	0.332	0.565
CC	330.1 (0.313)	317.8 (0.301)	0.339	0.560
CG	243.9 (0.231)	243 (0.230)	0.002	0.965
rs666229-rs12422021				
AAAG	320.7 (0.304)	308.7 (0.293)	0.326	0.568
GGAG	327.5 (0.311)	298 (0.283)	1.974	0.160
GGCG	251.1 (0.238)	276.7 (0.263)	1.656	0.198
GAAG	92.4 (0.088)	89.3 (0.085)	0.059	0.808
GGCA	62 (0.059)	76 (0.072)	1.520	0.218
rs7101540-rs10830204				
TGA	568 (0.538)	557.6 (0.529)	0.165	0.685
TCG	263.7 (0.250)	277.7 (0.263)	0.523	0.469
TGG	144.3 (0.137)	116.7 (0.111)	3.276	0.070
AGG	80 (0.076)	102 (0.097)	2.956	0.086

[a] Haplotypes were omitted from analysis if the estimated haplotype probabilities were less than 5%.

[b] Frequencies are shown in parenthesis (%).

[c] *P*-Values in the parenthesis were analyzed with Bonferroni corrections, the significance of bold values is $P<0.05$.

Table 3 Association analyses between PANSS and *GRM5* SNPs in patients with SZ

dbSNPID	Genotype(n)	Total PANSS	Positive	Negative	Excitement	Depression/anxiety	Cognitive impairment
rs567990	AA (70)	85.00±26.52	15.93±5.15	25.61±10.01	12.69±5.13	14.89±5.94	15.89±7.34[a]
	AG (132)	85.48±24.72	16.11±3.82	26.78±9.35	12.02±5.28	15.36±6.08	15.21±6.73
	GG (65)	77.97±19.43	15.26±4.23	24.14±7.76	11.58±4.40	13.55±4.63	13.20±5.67
rs12421343	AA (2)	68.50±17.68	12.00±8.49	17.50±3.54	13.00±2.83	12.50±0.71	13.50±2.12
	AG (42)	80.50±20.87	14.55±4.8[a]	25.60±7.09	10.71±4.68	15.33±5.95	14.31±4.66
	GG (223)	84.23±24.78	16.14±4.12	25.95±9.57	12.37±5.09	14.71±5.74	15.02±7.06
rs12422021	GG (231)	84.75±24.40	16.15±4.25	26.18±9.30	12.36±5.12	14.91±5.78	15.15±6.84
	AA +AG (36)	75.25±18.56a	14.36±4.24a	23.33±7.46	10.19±4.13a	14.03±5.43	13.33±3.94a
rs504183	AA (113)	80.15±21.18	15.49±4.29	24.70±8.42[b]	11.58±4.585	14.44±5.03	13.81±5.95
	AC (127)	87.29±26.80	16.45±4.24	27.36±9.88[c]	12.51±5.60	14.91±6.45	16.06±7.47
	CC (27)	79.93±20.78	14.63±4.31	23.37±8.09	12.22±3.90	15.74±5.13	13.96±5.03

[a] *P* <0.05, compared with GG genotype, LSD tests.

[b] *P* < 0.05, compared with AC genotype, LSD tests.

[c] *P*< 0.05, compared with CC genotype, LSD tests.

Figures

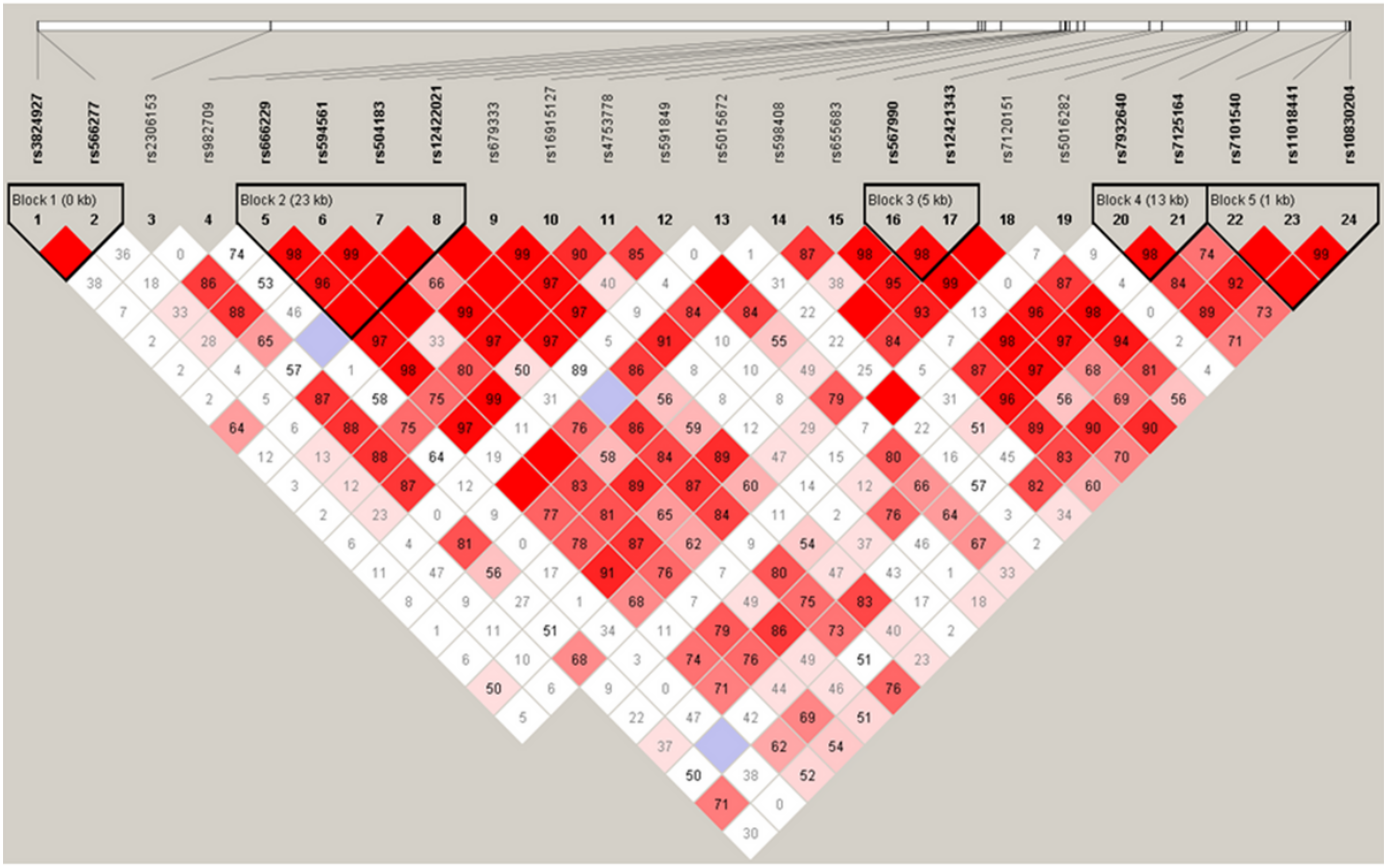

Figure 1

Haplotype block structure of the GRM5 gene in both SZ patients and HCs. The index association SNP is represented by a diamond. The colors of the remaining SNPs (circles) indicate LD with the index SNP based on pairwise r2 values from our data.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.doc