Metabolic Brain Disease (2019) 34:847–852
https://doi.org/10.1007/s11011-018-0380-6

ORIGINAL ARTICLE

GRM7 polymorphisms and risk of schizophrenia in Iranian population

Iman Azari[1] · Reza Hosseinpour Moghadam[2] · Hamid Fallah[1] · Rezvan Noroozi[1,3] · Soudeh Ghafouri-Fard[1] · Mohammad Taheri[4,5]

Received: 30 October 2018 / Accepted: 26 December 2018 / Published online: 4 January 2019

Abstract

The role of metabotropic glutamate receptors in the pathogenesis of schizophrenia or response to antipsychotic treatment has been proposed previously. The aim of the current study was to investigate the associations between two intronic variants within *GRM7* gene (rs6782011 and rs779867) and schizophrenia in Iranian population. These two single nucleotide polymorphisms (SNPs) were genotyped in 273 schizophrenic patients and 300 age and sex-matched normal controls. The frequency of A allele of the rs779867 was significantly lower in the schizophrenic patients compared with healthy subjects (OR (95% CI) = 0.71 (0.56–0.89), adjusted *P* value = 0.008). This SNP was associated with schizophrenia in co-dominant and dominant models (adjusted *P* values of 0.03 and 0.02 respectively). However, there was no difference in allele and genotype frequencies of the rs6782011 SNP between cases and controls. Consequently, the results of current study further highlight the participation of *GRM7* in the pathogenesis of schizophrenia.

Keywords GRM7 · Polymorphism · Schizophrenia

Introduction

Schizophrenia is a severe chronic psychiatric disorder described by anomalous social behavior and disturbed emotions and thought (Rahimi et al. 2017). This disorder affects 1% of the general population (Drahl 2008). As one of the most complex psychological conditions, diverse genetic, psychological and environmental factors participate in the pathogenesis of schizophrenia with heritable elements explaining 70% to 90% of the phenotype variability (Nothen et al. 2010).

The glutamate hypothesis of schizophrenia has been proposed as an adjunctive to the conventional dopaminergic theory (Lisman et al. 2008). Primary evidences for the participation of glutamate system in the pathogenesis of schizophrenia have emerged from an animal study showing the effect of a metabotropic glutamate receptor (mGluR) agonist in amelioration of the negative effects of phencyclidine on working memory, repetitive behavior, movement, and cortical glutamate outflow (Moghaddam and Adams 1998). Subsequently, a clinical trial reported the efficacy of a selective agonist for mGluR2/3 in the treatment of schizophrenic patients (Patil et al. 2007). A certain member of metabotropic glutamate receptors (GRM) namely GRM7 has been the subject of several association studies in different populations. GRM7 as one of the group III of mGluR is principally expressed in the presynaptic compartments, where it alters the production of both glutamate and gamma-aminobutyric acid (GABA) (Jalan-Sakrikar et al. 2014). This gene is located in a region on chromosome 3p which has been previously linked with schizophrenia in Indonesian population (Irmansyah et al. 2008). Several single nucleotide polymorphisms (SNPs) within this gene have been associated with schizophrenia in different populations including the rs17031835 in Indonesian population (Ganda et al. 2009),

✉ Soudeh Ghafouri-Fard
s.ghafourifard@sbmu.ac.ir

✉ Mohammad Taheri
mohammad_823@yahoo.com

1 Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Department of Pharmaceutics, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3 Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4 Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran

5 Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Table 1 Nucleotide sequences of primers used for tetra-ARMS PCR and PCR conditions

SNP	Primer sequence	Tm	Annealing temperature	PCR product size (bp)
rs6782011	Forward inner primer (T allele): GCTCTGACCAAATTACAAAATATATGTGGT	63 °C	57 °C	191 bp (T allele)
	Reverse inner primer (C allele): CACTCTGAATATTAGTACTCAAAACAGGGG	63 °C		261 bp (C allele)
	Forward outer primer: GTTAGAACATTTGGACTATAAGCATGGC	63 °C		392 bp (two outer primers)
	Reverse outer primer: ATAATAAACCAGTCTTCTGCATCAACGT	63 °C		
rs779867	Forward inner primer (A allele): AAACCAGGGTTTCCACTCTCATGTAAA	65 °C	58 °C	163 bp (A allele)
	Reverse inner primer (G allele): CATTAATCCAAGAGCATCTGTAAGCCC	65 °C		244 bp (G allele)
	Forward outer primer: GATCAAGATGATATAAGGGGGAAACAGG	65 °C		353 bp (two outer primers)
	Reverse outer primer: CTAGGTTTCATCCAGGAAGGGACTAAAG	65 °C		

rs13353402, rs1531939, rs2229902 and rs9870680 in Chinese Han population (Li et al. 2016; Niu et al. 2015) and rs3749380 in Japanese population (Ohtsuki et al. 2008). Two intronic variants within this gene (rs6782011 and rs779867) have been recently associated with autism spectrum disorder (ASD) in Iranian and Chinese populations (Noroozi et al. 2016; Yang and Pan 2013). However, associations of these variants with schizophrenia have not been explored yet.

The aim of the present study was to evaluate the associations between rs6782011 and rs779867 SNPs and schizophrenia in an Iranian population. These two SNPs were selected based on the results of a previous work in Iranian ASD patients indicating the associations between some haplotypes containing these variants and disease status (Noroozi et al. 2016). The same study was used for estimation of minor allele frequency of these SNPs in Iranian population.

Material and methods

Study participants

The current gene association study was performed on blood samples acquired from 273 schizophrenic patients who visited the psychology department of Hamadan University of Medical Sciences and 300 age and sex-matched normal controls. The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) was used for assessment of schizophrenic patients (Association, D.-A. P 2013). Inclusion criteria were: definite diagnosis of disease, clinical assessment confirming adequacy of oral medication, satisfactory decisional aptitude, and ability to provide written informed consent. Exclusion criteria were diagnosis of schizoaffective or schizophreniform disorder, mental retardation, any other psychiatric disease, pregnancy, breastfeeding or substance-induced psychosis. A structured psychiatric interview was used for assessment of control subjects. The study protocol was approved by Ethical Committee of Shahid Beheshti University of Medical Sciences. Informed written consents were obtained from all study participants or their guardians in the mentally incompetent cases.

Genotyping

The rs6782011 and rs779867 SNPs were genotyped using tetra-primer amplification-refractory mutation system (ARMS)-PCR technique as described previously (Noroozi et al. 2016). The genotyping results were confirmed by conventional Sanger sequencing of 10% of samples. Table 1 shows the nucleotide sequences of primers used for genotyping, their annealing temperatures and the predicted product sizes.

Statistical analysis

The SNP Analyzer 2.0 online tool (Yoo et al. 2008) was used for statistical analyses. Hardy-Weinberg Equilibrium test was performed for rs6782011 and rs779867 SNPs. Haplotypes were predicted from unphased diploid genotype data, and linkage disequilibrium (LD) between these SNPs was evaluated and described by D' and r values. Genetic association analyses were performed using co-dominant, dominant and

Table 2 General data of study participants

Variables	Patients	Controls
Male/Female [no. (%)]	184 (67.4)/89 (32.6)	200 (66.7)/100 (33.3)
Age (mean ± SD, Y)	35 ± 1.24	37.1 ± 0.2
Age range (Y)	19–54	18–58
Age at onset (mean ± SD, Y)	21 ± 0.8	–

Table 3 Exact test for hardy-weinberg principle

Study groups	rs6782011			P value	rs779867			P value
	TT	CT	CC		GG	AG	AA	
Patients	83	123	67	0.11	109	129	35	0.74
Controls	86	135	79	0.08	88	155	57	0.44

recessive models. False positive control was applied by multiple test correction. The associations between SNPs genotypes and age of disease onset were analyzed using t-test. *P* value, odds ratio (OR) and 95% confidence interval (CI) were reported. *P* values less than 0.05 were regarded as significant.

Results

General data of study participants

General data of study participants are summarized in Table 2.

Genotyping

The allele and genotype frequencies of both SNPs were in accordance with Hardy-Weinberg principle in both study groups (Table 3).

The frequency of A allele of the rs779867 was significantly lower in schizophrenic patients compared with healthy subjects (OR (95% CI) = 0.71 (0.56–0.89), adjusted *P* value = 0.008). So A allele is regarded as the protective allele against schizophrenia. This SNP was associated with schizophrenia in co-dominant and dominant models (adjusted *P* values of 0.03 and 0.02 respectively). Based on the observed genotype frequencies, it can be deduced that the GG genotype of rs779867 is associated with risk of schizophrenia. However, there was no difference in allele and genotype frequencies of the rs6782011 SNP between cases and controls (Table 4).

The associations between genotype frequencies of SNPs and age at disease onset were analyzed (Tables 5 and 6). No significant associations were detected between SNPs genotypes and age at disease onset.

Based on D' and r statistic, the mentioned SNPs were not in LD with each other (D' = 0.06, $r = 0.03$).

Haplotype analysis showed no significant association between estimated haplotype blocks and schizophrenia in the assessed population after correction for multiple comparisons (Table 7).

Discussion

In the present study, two intronic SNPs within *GRM7* gene were genotyped in a population of Iranian schizophrenic patients and healthy controls and found lower frequency of A allele of the rs779867 in schizophrenic patients compared with healthy subjects. Moreover, this SNP was associated with schizophrenia in co-dominant and dominant models.

GRM7 encodes a member of mGluRs which inhibits the cyclic AMP cascade in the central nervous system (CNS) in response to L-glutamate. Schizophrenia has been associated with disturbances in the glutamatergic system and the resultant aberrations in synaptic plasticity and cortical microcircuitry (Marsman et al. 2011). The role of mGluRs in the pathogenesis of schizophrenia has been further highlighted by the observed glutamatergic dys-function (Gray et al. 2009) and

Table 4 Association between SNPs and schizophrenia

SNP	Model		Patients N (%)	Controls N (%)	OR (95% CI)	P value	Adjusted P value
rs6782011	Allele	C vs. T	257 (47) 289 (53)	293 (49) 307 (51)	0.93 (0.74–1.17)	0.55	1.00
	Co-dominant	CC vs. TT CT vs. TT	67 (24.5) 123 (45)	79 (26.3) 135 (45)	0.88 (0.56–1.37) 0.94 (0.64–1.39)	0.85	1.00
	Dominant	CC + CT vs. TT	190 (69.6) 83 (30.4)	214 (71.3) 86 (28.7)	0.92 (0.64–1.32)	0.65	1.00
	Recessive	CC vs. CT + TT	67 (24.5) 123 (45)	79 (26.3) 135 (45)	0.91 (0.62–1.33)	0.62	1.00
rs779867	Allele	A vs. G	199 (36) 347 (64)	269 (45) 331 (55)	0.71 (0.56–0.89)	0.004	0.008
	Co-dominant	AA vs. GG AG vs. GG	35 (12.8) 129 (47.2)	57 (19) 155 (51.7)	0.63 (0.41–0.96) 1.04 (0.76–1.43)	0.01	0.03
	Dominant	AG + AA vs. GG	164 (60.1) 109 (39.9)	212 (70.7) 88 (29.3)	0.62 (0.44–0.88)	0.008	0.02
	Recessive	AA vs. AG + GG	35 (12.8) 238 (87.2)	57 (19) 243 (81)	0.63 (0.40–0.99)	0.04	0.09

Table 5 Associations between allele/ genotype frequencies of rs6782011 and age at disease onset ($n = 273$, crude analysis, Patients were categorized based on mean value of age at disease onset)

Model	Genotype	Age at onset <21	Age at onset >21	OR (95% CI)	P value
Co-dominant	T/T	55 (31.1%)	28 (29.2%)	1.00	0.78
	C/T	77 (43.5%)	46 (47.9%)	1.17 (0.65–2.10)	
	C/C	45 (25.4%)	22 (22.9%)	0.96 (0.48–1.90)	
Dominant	T/T	55 (31.1%)	28 (29.2%)	1.00	0.74
	C/T-C/C	122 (68.9%)	68 (70.8%)	1.09 (0.64–1.88)	
Recessive	T/T-C/T	132 (74.6%)	74 (77.1%)	1.00	0.64
	C/C	45 (25.4%)	22 (22.9%)	0.87 (0.49–1.56)	

schizophrenia-like symptoms (Fell et al. 2008) resulted from deletion of subunits of these receptors.

Previous studies have shown associations between several SNPs within *GRM7* and schizophrenia in different populations (Li et al. 2016; Irmansyah et al. 2008). The reported dissimilarities in clinical manifestation and prognosis of schizophrenia in patients from diverse populations (Cohen et al. 2008) necessitate re-assessment of the genetic association studies in each population to find possible differences in the principal genetic factors. So the current study was conducted to assess association between *GRM7* and schizophrenia in Iranian population.

Based on the observed significant differences in alleles and genotypes frequencies of the rs779867 between schizophrenic patients and healthy subjects, the functional consequences of this observation was explored using in silico tools. Using the 3DSNP tool we found that this SNP is in strong LD with the rs712784, rs712778 and rs779760 in different populations with the first SNP having a high total score of functionality (Lu et al. 2016). So a possible explanation for the observed association between rs779867 and schizophrenia in the current study is the LD between this SNP and other functional SNPs including rs712784. HaploReg v4 software (Ward and Kellis 2011) indicated that rs779867 SNP alters the Mrg-binding domain of the encoded protein. MRG protein family includes a number of transcription factors participating in cellular senescence. Their localization in the nucleus and their predicted motifs imply their role in the chromatin remodeling processes (Bertram et al. 1999; Bertram and Pereira-Smith 2001). Previous studies have reported abnormalities in DNA cytosine methylation, histone modifications and histone variants, and chromosomal loop formations in some cases of schizophrenia (Halene et al. 2014). Future studies are needed to explore whether the rs779867 can alter epigenetic regulation of gene expression through changes in Mrg binding motif.

In brief, the current research provided further evidences for contribution of *GRM7* in conferring the risk of schizophrenia in Iranian population. These results have potential clinical implications of in risk management or treatment. Although we did not assess the associations between genotypes and response of patients to antipsychotic treatments, based on the role of glutamate receptor in the function of antipsychotic medications (Ossowska et al. 2000), genotyping of variants

Table 6 Associations between allele/ genotype frequencies of rs779867 and age at disease onset (n = 273, crude analysis, Patients were categorized based on mean value of age at disease onset)

Model	Genotype	Age at onset <21	Age at onset >21	OR (95% CI)	P value
Co-dominant	G/G	74 (41.8%)	35 (36.5%)	1.00	0.63
	A/G	80 (45.2%)	49 (51%)	1.29 (0.76–2.22)	
	A/A	23 (13%)	12 (12.5%)	1.10 (0.49–2.47)	
Dominant	G/G	74 (41.8%)	35 (36.5%)	1.00	0.39
	A/G-A/A	103 (58.2%)	61 (63.5%)	1.25 (0.75–2.09)	
Recessive	G/G-A/G	154 (87%)	84 (87.5%)	1.00	0.91
	A/A	23 (13%)	12 (12.5%)	0.96 (0.45–2.02)	

Table 7 The results of haplotype analysis

rs6782011	rs779867	Patients	Controls	Total frequency	OR (95% CI)	P value	Adjusted *P* value
T	G	0.35	0.29	0.32	1.28 (1.01–1.63)	0.04	0.18
C	G	0.28	0.26	0.27	1.16 (0.9–1.54)	0.28	1.00
C	A	0.19	0.23	0.21	0.79 (0.61–1.03)	0.09	0.35
T	A	0.17	0.22	0.20	0.73 (0.53–1.02)	0.06	0.25

within the coding genes might be used for personalization of treatment strategies.

Our study had some limitations. Firstly, we did not access comprehensive patients' data including data on duration of untreated psychosis, duration of treatment, antipsychotic use, previous co-medication, psychopathology severity, etc. As some patients have been treated in other centers before referral to our center, the data regarding these factors were inconsistent and could not be assessed. Lack of genotyping of other functional variants within *GRM7* is stated as another limitation of the current study. A strong point of our study is the homogeneity of patients' cohort regarding clinical course and response to oral medications.

Future studies in other populations are needed to explore whether this gene might affect course of disease or patients' response to antipsychotic treatments. Moreover, screening of the entire gene for other SNPs using more advanced techniques such as whole gene/exome sequencing might be used to select patients for comprehensive screening strategies.

Acknowledgements The current study was supported by a grant from Shahid Beheshti University of Medical Sciences.

Compliance with ethical standards

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

References

Association, D.-A. P. 2013. Diagnostic and statistical manual of mental disorders. Arlington: American Psychiatric Publishing

Bertram MJ, Pereira-Smith OM (2001) Conservation of the MORF4related gene family: identification of a new chromo domain subfamily and novel protein motif. Gene 266:111–121

Bertram M, Berube N, Hang-Swanson X, Ran Q, Leung J, Bryce S, Spurgers K, Bick R, Baldini A, Ning Y (1999) Identification of a gene that reverses the immortal phenotype of a subset of cells and is a member of a novel family of transcription factor-like genes. Mol Cell Biol 19:1479–1485

Cohen A, Patel V, Thara R, Gureje O (2008) Questioning an axiom: better prognosis for schizophrenia in the developing world? Schizophr Bull 34:229–244

Drahl C (2008) Rethinking schizophrenia. Chem Eng News 86:38–40

Fell MJ, Svensson KA, Johnson BG, Schoepp DD (2008) Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist (−)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039). J Pharmacol Exp Ther 326:209–217

Ganda C, Schwab SG, Amir N, Heriani H, Irmansyah I, Kusumawardhani A, Nasrun M, Widyawati I, Maier W, Wildenauer DB (2009) A family-based association study of DNA sequence variants in GRM7 with schizophrenia in an Indonesian population. Int J Neuropsychopharmacol 12:1283–1289

Gray L, van den Buuse M, Scarr E, Dean B, Hannan AJ (2009) Clozapine reverses schizophrenia-related behaviours in the metabotropic glutamate receptor 5 knockout mouse: association with N-methyl-D-aspartic acid receptor up-regulation. Int J Neuropsychopharmacol 12:45–60

Halene TB, Peter CJ, Akbarian S (2014) Epigenetic dysregulation in the schizophrenic brain. Curr Behav Neurosci Rep 1:86–93

Irmansyah, Schwab SG, Heriani, Handoko HY, Kusumawardhani A, Widyawati I, Amir N, Nasrun MWS, Holmans P, Knapp M, Wildenauer DB (2008) Genome-wide scan in 124 Indonesian sib-pair families with schizophrenia reveals genome-wide significant linkage to a locus on chromosome 3p26-21. Am J Med Genet B Neuropsychiatr Genet 147B:1245–1252

Jalan-Sakrikar N, Field JR, Klar R, Mattmann ME, Gregory KJ, Zamorano R, Engers DW, Bollinger SR, Weaver CD, Days EL, Lewis LM, Utley TJ, Hurtado M, Rigault D, Acher F, Walker AG, Melancon BJ, Wood MR, Lindsley CW, Conn PJ, Xiang ZX, Hopkins CR, Niswender CM (2014) Identification of positive allosteric modulators VU0155094 (ML397) and VU0422288 (ML396) reveals new insights into the biology of metabotropic glutamate receptor 7. ACS Chem Neurosci 5:1221–1237

Li W, Ju K, Li Z, He K, Chen J, Wang Q, Yang B, An L, Feng G, Sun W, Zhou J, Zhang S, Song P, Khan RAW, Ji W, Shi Y (2016) Significant association of GRM7 and GRM8 genes with schizophrenia and major depressive disorder in the Han Chinese population. Eur Neuropsychopharmacol 26:136–146

Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci 31:234–242

Lu, Y., Quan, C., Chen, H., Bo, X. & Zhang, C. 2016. 3DSNP: a database for linking human noncoding SNPs to their three-dimensional interacting genes. *Nucleic acids research*, gkw1022

Marsman A, van den Heuvel MP, Klomp DW, Kahn RS, Luijten PR, Hulshoff Pol HE (2011) Glutamate in schizophrenia: a focused review and meta-analysis of 1H-MRS studies. Schizophr Bull 39:120–129

Moghaddam B, Adams BW (1998) Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 281:1349–1352

Niu W, Huang X, Yu T, Chen S, Li X, Wu X, Cao Y, Zhang R, Bi Y, Yang F (2015) Association study of GRM7 polymorphisms and schizophrenia in the Chinese Han population. Neurosci Lett 604:109–112

Noroozi R, Taheri M, Movafagh A, Mirfakhraie R, Solgi G, Sayad A, Mazdeh M, Darvish H (2016) Glutamate receptor, metabotropic 7 (GRM7) gene variations and susceptibility to autism: a case-control study. Autism Res 9:1161–1168

Nothen MM, Nieratschker V, Cichon S, Rietschel M (2010) New findings in the genetics of major psychoses. Dialogues Clin Neurosci 12:85–93

Ohtsuki T, Koga M, Ishiguro H, Horiuchi Y, Arai M, Niizato K, Itokawa M, Inada T, Iwata N, Iritani S, Ozaki N, Kunugi H, Ujike H, Watanabe Y, Someya T, Arinami T (2008) A polymorphism of the metabotropic glutamate receptor mGluR7 (GRM7) gene is associated with schizophrenia. Schizophr Res 101:9–16

Ossowska K, Pietraszek M, Wardas J, Nowak G, Zajaczkowski W, Wolfarth S, Pilc A (2000) The role of glutamate receptors in antipsychotic drug action. Amino Acids 19:87–94

Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznanov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA, Schoepp DD (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized phase 2 clinical trial. Nat Med 13:1102–1107

Rahimi S, Sayad A, Moslemi E, Ghafouri-FARD S, Taheri M (2017) Blood assessment of the expression levels of matrix metalloproteinase 9 (MMP9) and its natural inhibitor, TIMP1 genes in Iranian schizophrenic patients. Metab Brain Dis 32:1537–1542

Ward LD, Kellis M (2011) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 40:D930–D934

Yang Y, Pan C (2013) Role of metabotropic glutamate receptor 7 in autism spectrum disorders: a pilot study. Life Sci 92:149–153

Yoo J, Lee Y, Kim Y, Rha SY, Kim Y (2008) SNPAnalyzer 2.0: a web-based integrated workbench for linkage disequilibrium analysis and association analysis. BMC Bioinformatics 9:290