Personalized Psychiatry and Neurology

Article

Study of Associations of the *GRM8* Gene with Antipsychotic-induced Hyperprolactinemia

Evgeniya G. Poltavskaya, Vladimir V. Tiguntsev, Anastasiia S. Boiko, Elena G. Kornetova, Svetlana A. Ivanova, and Olga Yu. Fedorenko*

Mental Health Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, Russia

* Correspondence: f_o_y@mail.ru

Citation Poltavskaya, E.G., Tiguntsev, V.V., Boiko, A.S., Kornetova, E.G., Ivanova, S.A., Fedorenko, O. Yu. Study of Associations of the GRM8 Gene with Antipsychotic-induced Hyperprolactinemia. *Personalized Psychiatry and Neurology* **2023**, *3 (1)*: 42-47. https://doi.org/10.52667/2712-9179-2023-3-1-42-47

Chief Editor: Nikolaj G. Neznanov, D Med Sci, Professor

Received: 21 March 2023
Accepted: 01 April 2023
Published: 15 May 2023

Publisher's Note: V.M. Bekhterev NMRC PN stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Hyperprolactinemia is one of the common adverse events of antipsychotic therapy. The role of genetic factors in the development of drug-induced side effects is being actively investigated. The present study examined the association of two polymorphisms rs2237748 and rs2299472 in the *GRM8* gene encoding the glutamate metabotropic receptor type 8 with antipsychotic-induced hyperprolactinemia in 536 patients with schizophrenia from several regions of Siberia (Russia). The investigated polymorphisms are not associated with drug-induced hyperprolactinemia in patients with schizophrenia. There were no associations of the *GRM8* gene polymorphisms with serum prolactin levels in patients taking antipsychotic therapy. Our results did not confirm the involvement of the *GRM8* rs2237748 and rs2299472 in the development of antipsychotic-induced hyperprolactinemia.

Keywords: *hyperprolactinemia; antipsychotic-induced; schizophrenia; gene polymorphism; GRM8*

Introduction

Hyperprolactinemia is one of the most common side effects of antipsychotic therapy. With short-term therapy, an increase in prolactin levels is recorded in 80% of patients [1] and in 30-70% of patients receiving long-term maintenance treatment [2, 3].

As it is known, a common biochemical property for all antipsychotics is their ability to block postsynaptic dopaminergic receptors. Antipsychotics that cause pronounced neuroendocrine side effects associated, in particular, with an increase in the production of the hormone prolactin, a decrease in the content of growth hormone, functional disorders in the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal systems, have a strong blocking effect on D2 receptors in the tuberoinfundibular system (hypothalamus, pituitary gland) of the brain [4-8].

Prolactin secretion is under complex neuroendocrine control, which involves agents of various natures: neurotransmitters, biologically active neuropeptides, hormones of peripheral endocrine glands. Factors involved in the regulation of prolactin secretion can be divided into two groups: inhibitory (dopamine, gamma-aminobutyric acid, gastrin, somatostatin, gonadotropin-binding protein) and stimulating (serotonin, thyrotropin-releasing hormone, gonadotropin-releasing hormone, vasointestinal peptide, opiates, neurotensin and substance P, oxytocin, angiotensin 2).

Experiments with rats have also shown that glutamate can directly influence the production of prolactin by the pituitary gland [9]. A hypothesis was tested that glutamate would stimulate prolactin release when applied directly to primary cultures of dispersed

adult female rat anterior pituitary cells. Glutamate increased the rate of prolactin release within two minutes in a self-limited manner. These experiments demonstrate that anterior pituitary lactotrophs respond to glutamate by increasing the rate of prolactin release.

Abnormal glutamatergic neurotransmission is attracting increasing attention from researchers. This is due to the fact that glutamate is the main excitatory neurotransmitter in the central nervous system (CNS), and the two main groups of glutamate receptors, namely metabotropic and ionotropic (AMPAR, NMDAR and KAR), are closely associated with the etiology of neuropsychiatric disorders [10, 11].

Metabotropic glutamate receptors (mGluR 1–8) are divided into 3 groups and belong to a heterogeneous family of G protein-coupled receptors [12]. It is assumed that they function as presynaptic regulatory mechanisms that control the release of neurotransmitters and, accordingly, modulate brain excitability through presynaptic, postsynaptic, and glial mechanisms [13].

Metabotropic glutamate receptor 8 (GRM8) has been mapped into the human genome 7q31.3-q32.1 spanning over 800 kb. [14]. Several studies have shown associations of schizophrenia with *GRM8* loci in Chinese [15], Japanese [16], and Iranian [17] populations.

In the presented study, the associations of the *GRM8* gene polymorphisms with hyperprolactinemia after antipsychotic treatment in patients with schizophrenia were studied.

Objective

The aim of the study was to study the association of polymorphic variants rs2237748, rs2299472 of the *GRM8* gene with the development of hyperprolactinemia in patients with schizophrenia after treatment with antipsychotic drugs.

Materials and Methods

Patients

This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, revised in Fortaleza, Brazil, 2013) for experiments involving humans. We recruited 536 patients with schizophrenia (266 men and 270 women) being treated at clinics of the Mental Health Research Institute, Siberian State Medical University, Tomsk Clinical Psychiatric Hospital (Tomsk), Kemerovo Regional Clinical Psychiatric Hospital (Kemerovo) in the Siberian region. The inclusion criteria were a verified clinical diagnosis of schizophrenia (F20) according to the World Health Organization World Mental Health Composite International Diagnostic Interview (WHO WMH-CIDI) for schizophrenia diagnostics [18], age 18–60 years, and the patient's informed consent. Exclusion criteria for all patients were non-Caucasian physical appearance (e.g., Mongoloid, Buryats or Khakassians), organic mental disorders (e.g., epilepsy, Parkinson's disease) or somatic disorders in the stage of decompensation.

Prolactin Analysis

Measurement of the hormone prolactin concentration in the blood serum was carried out by enzyme immunoassay using the PRL Test System reagent kit. The criterion for neuroleptic hyperprolactinemia was an increase in the concentration of prolactin in the blood: for men - above 20 ng/ml, for women - above 25 ng/ml [19].

Genetic Analysis

Blood samples were taken after 8 h overnight fasting in tubes containing EDTA and stored in several aliquots at −20 ◦C until DNA isolation using the standard phenol-chloroform method. Inclusion criteria for SNP selection and genotype are described elsewhere [20]. Genotyping of 2 single-nucleotide polymorphisms (SNPs) in *GRM8* (rs2237748,

rs2299472) was carried out with use a QuantStudio™ 3D Digital PCR System Life Technologies amplifier (Applied Biosystems, Waltham, MA, USA) using TaqMan Validated SNP Genotyping Assay kits (Applied Biosystems) based at The Core Facility "Medical Genomics", Tomsk National Research Medical Center of the Russian Academy of Sciences.

Statistical Analysis

Statistical analysis was performed using SPSS software for Windows, version 21. The genotype frequencies were checked for Hardy–Weinberg equilibrium using the chi-square test. Chi-square test and the Fisher's exact test, where necessary, were used for between group comparisons of genotype or allele frequencies at a significance level of $p < 0.05$. Assessment of the association of genotypes and alleles of the studied polymorphic variants of genes with a pathological phenotype was carried out using the odds ratio (OR) with a 95% confidence interval for the odds ratio (95% CI).

Results

Details about the studied patient population are presented in Table 1.

Table 1. Demographic and clinical parameters of the patients.

Patients with schizophrenia	Group 1 (with Hyperprolactinemia)	Group 2 (without Hyperprolactinemia)	χ2 p-value
n	318	218	
Gender, n (%)	Men: 136 (42.8%) Women: 182 (57.2%)	Men: 130 (59.6%) Women: 88 (40.4%)	χ2=14.717 p=0.0001*
Age, years, Me (Q2; Q3)	38 (30;48)	42 (33;53)	p=0.009*
Duration of illness, years, Me (Q2; Q3)	12 (5;20)	16 (10;24)	p=0.0001*
Daily dose of CPZeq, Me (Q2; Q3)	400 (200 – 750)	300 (200 – 750)	p=0.05

* - p<0.05

Patients with hyperprolactinemia were characterized by younger age and shorter duration of the disease. Along with this, hyperprolactinemia was observed more often in women (Table 1). Differences in the average value of chlorpromazine equivalent (Daily dose of CPZeq), which characterizes the antipsychotic therapy of patients, in the group with and without hyperprolactinemia did not reach statistical significance (p=0.05). Analysis of the frequency distribution of genotypes and alleles of the studied polymorphisms was carried out in a subgroup of men and in a subgroup of women (Tables 2 and 3).

Table 2. Distribution of allele and genotype frequencies of the *GRM8* gene polymorphisms in male schizophrenia patients with and without hyperprolactinemia.

SNP	Genotypes and Alleles	Patients with schizophrenia		χ2	p-value
		Group 1 (with Hyperprolactinemia)	Group 2 (without Hyperprolactinemia)		
rs2237748	CC	64 (47.1%)	57 (43.8%)	0.310	0.857
	CT	60 (44.1%)	60 (46.2%)		
	TT	12 (8.8%)	13 (10.0%)		
	C	188 (69.1%)	174 (68.1%)	0.294	0.588
	T	84 (30.9%)	86(31.9%)		
rs2299472	AA	12 (8.8%)	13 (10.1%)	0.122	0.941
	AC	61 (44.9%)	57 (44.2%)		
	CC	63 (46.3%)	59 (45.7%)		
	A	85 (31.2%)	83 (32.2%)	0.052	0.820
	C	187 (68.8%)	175 (67.8%)		

Table 3. Distribution of allele and genotype frequencies of the *GRM8* gene polymorphisms in female schizophrenia patients with and without hyperprolactinemia.

SNP	Genotypes and Alleles	Patients with schizophrenia		$\chi 2$	p-value
		Group 1 (with Hyperprolactinemia)	Group 2 (without Hyperprolactinemia)		
rs2237748	CC	73 (40.8%)	35 (39.3%)	0.267	0.875
	CT	82 (45.8%)	40 (44.9%)		
	TT	24 (13.4%)	14 (15.7%)		
	C	228 (63.7%)	110 (61.8%)	0.182	0.670
	T	130 (36.3%)	68 (38.2%)		
rs2299472	AA	24 (13.4%)	14 (15.9%)	0.315	0.854
	AC	85 (47.5%)	40 (45.5%)		
	CC	70 (39.1%)	34 (38.6%)		
	A	133 (37.2%)	68 (38.6%)	0.111	0.740
	C	225 (62.8%)	108 (61.4%)		

We did not find any significant differences in the distribution of alleles and genotypes of rs2237748 and rs2299472 in groups of patients with schizophrenia with and without hyperprolactinemia.

In the current study, we also analyzed the level of prolactin in a group of men and a group of women with schizophrenia depending on the different genotypes of the studied polymorphic variants of the *GRM8* gene (Table 4).

Table 4. The level of prolactin in the male and female with schizophrenia.

SNP	Genotypes and Alleles	Male group			Female group		
		Level of prolactin, Me (Q2; Q3)	$\chi 2$	p-value	Level of prolactin, Me (Q2; Q3)	$\chi 2$	p-value
rs2237748	CC	20.8 (13.5 – 43.3)	1.491	0.475	46.4 (17.7 – 78.9)	0.710	0.701
	CT	20.9 (10.8 – 38.4)			39.9 (20.0 – 81.5)		
	TT	19.6 (12.5 – 29.8)			39.8 (18.7 – 65.0)		
rs2299472	AA	19.6 (12.5 – 29.8)	0.748	0.688	39.8 (18.7 – 65.0)	0.908	0.635
	AC	21.5 (11.2 – 38.8)			39.8 (20.0 – 81.5)		
	CC	20.3 (12.8 – 43.3)			47.4 (17.9 – 78.9)		

We did not find significant associations of the *GRM8* gene polymorphisms with serum prolactin levels in the patients with schizophrenia.

Discussion

Use of antipsychotic agents has been associated with hyperprolactinemia, or elevated prolactin levels. Hyperprolactinemia is more than an abnormal laboratory value; elevated prolactin levels can interfere with the functioning of reproductive, endocrine, and metabolic systems. A special role in the pathogenesis of the development of antipsychotic-induced hyperprolactinemia belongs to genetic factors, which may be the basis for the sensitivity of the development of complications in many patients [21-26].

Changes in levels of anxiety, exploratory behavior, working memory, and fear extinction identified in deficient mouse models [27] and in various neuropharmacological studies [28, 29] have provided evidence Group III metabotropic glutamate receptors (mGluR), including GRM8, should play an important role in the development of pathological processes in the brain at the molecular level.

In our study, we attempted to identify the effect of the *GRM8* gene on the level of prolactin in the blood serum and the development of hyperprolactinemia while taking antipsychotic drugs in patients with schizophrenia. We did not find any statistically significant differences for polymorphic variants of the *GRM8* gene rs2237748 and rs2299472.

Conclusions

Currently, pharmacogenetic studies remain relevant. In the study of hyperprolactinemia, more attention is paid to the role of the dopaminergic system. At the same time, the glutamatergic system is no less interesting. In this study, we did not obtain data on the association of polymorphic variants of the *GRM8* gene (rs2237748, rs2299472) with the development of antipsychotic-induced hyperprolactinemia. However, further research is needed on the role of this class of receptors in the development of endocrine side effects of antipsychotics.

Author Contributions: Conceptualization, O.Yu.F. and S.A.I.; methodology, O.Yu.F. and S.A.I.; software, V.V.T.; validation, O.Yu.F. and E.G.K.; formal analysis, E.G.P.; investigation, O.Yu.F., E.G.P., V.V.T. and A.S.B.; resources, O.Yu.F. and S.A.I.; data curation, O.Yu.F. and V.V.T.; writing—original draft preparation, E.G.P. and O.Yu.F.; writing—review and editing, O.Yu.F.; visualization, O.Yu.F.; supervision, S.A.I.; project administration, O.Yu.F.; funding acquisition, O.Yu.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation (project no. 21-15-00212).

Institutional Review Board Statement: This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, revised in Fortaleza, Brazil, 2013) for experiments involving humans and according to the protocol approved by the Bioethical Committee of the Mental Health Research Institute of the Tomsk National Research Medical Center of the Russian Academy of Sciences (protocol N142 of 14.05.2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated for this work will not be made publicly accessible, although they are available on reasonable request to Olga Yu. Fedorenko (f_o_y@mail.ru), following approval of the Board of Directors of theMHRI, in line with local guidelines and regulations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Laita, P., Cifuentes, A. et al. Antipsychotic relatedabnormal involuntary movements and metabolic and endocrine side effects in children and adolescents. *J Child Adolesc Psychopharmacol* **2007;** 17: 487–502. doi: 10.1089/cap.2006.0039
2. Bushe, C., Shaw, M., Peveler, R. A review of the association between antipsychotic use and hyperprolactinaemia. *J Psychopharmacol* **2008;** 22*:* 46–55. doi: 10.1177/0269881107088435.
3. Cookson, J., Hodgson, R., Wildgust, H.J. Prolactin, hyperprolactinaemia and antipsychotic treatment: a review and lessons for treatment of early psychosis. *J Psychopharmacol* **2012;** 26: 42–51. doi: 10.1177/0269881112442016
4. Beasley, C.M. Jr., Magnusson, M., Garver, D.L. TSH response to TRH and haloperidol response latency in psychoses. *Biol. Psychiatry* **1988,** 24(4): 423-431. doi: 10.1016/0006-3223(88)90179-5.
5. Farde, L., Nordstrem, A.L. et al. Positron emission tomographic analysis of central Dl and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: relation to extrapyramidal side-effects. *Arch. Gen. Psychiatry* **1992**; 49: 538–544. doi: 10.1001/archpsyc.1992.01820070032005.
6. Kufferle, B., Tauscher, J., Asenbaum, S. IBZM SPECT imaging of striatal dopamin-2 receptors in psychotic patients treated with the novel antipsychotic substance quetiapine in comparison to clozapine and haloperidol. *Psychopharmacology* **1997;** 133: 323-328. doi: 10.1007/s002130050409.
7. Kapur, S., Remington, G. Dopamine (D2) receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. *Biol. Psychiatry* **2001;** 50(11): 873-883. doi: 10.1016/s0006-3223(01)01251-3.
8. Perkins, D.O. *Prolactin- and Endocrine-Related Disorders in Schizophrenia / Medical Illnes and Schizofrenia*, ed. by Meyer J.M. and Nasrallah H.A.; Amer. Psychiatr. Publ. Washington, London, **2003**; 215-232.
9. Login, I.S. Direct stimulation of pituitary prolactin release by glutamate. *Life Sci.* **1990,** 47(24): 2269-2275. doi: 10.1016/0024-3205(90)90158-n.
10. Ohi, K., Hashimoto, R. et al. Glutamate networks implicate cognitive impairments in schizophrenia: genome-wide association studies of 52 cognitive phenotypes *Schizophr Bull* **2014;** 41(4): 909-918. doi: 10.1093/schbul/sbu171.
11. Sanacora, G., Zarate, C.A. et al. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders *Nat Rev Drug Discov* **2008,** 7: 426–437. doi: 10.1038/nrd2462.

12. Pin, J.P., Duvoisin, R. The metabotropic glutamate receptors: Structure and functions. *Neuropharmacology* **1995**; 34: 1–26. doi: 10.1016/0028-3908(94)00129-g.
13. Plitman, E., Nakajima, S. et al. Glutamatemediated excitotoxicity in schizophrenia: a review. *Eur Neuropsychopharmacol: J Eur Coll Neuropsychopharmacol* **2014**; 24(10): 591-1605. doi: 10.1016/j.euroneuro.2014.07.015.
14. Scherer, S.W., Duvoisin, R.M. et al. Localization of two metabotropic glutamate receptor genes,GRM3andGRM8, to human chromosome 7q. *Genomics* **1996;** 31: 230–233. doi: 10.1006/geno.1996.0036.
15. Zhang, L., Zhong, X. et al. Association analysis of the GRM8 gene with schizophrenia in the Uygur Chinese population. *Hereditas* **2014**; 151(6): 140-144. doi: 10.1111/hrd2.00045.
16. Takaki, H., Kikuta, R. et al. Positive associations of polymorphisms in the metabotropic glutamate receptor type 8 gene (GRM8) with schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* **2004**; 128B(1): 6-14. doi: 10.1002/ajmg.b.20108.
17. Tavakkoly-Bazzaz, J., Azarnezhad, A. et al. TCF4 and GRM8 gene polymorphisms and risk of schizophrenia in an Iranian population: a case-control study. *Mol Biol Rep* **2018**; 45(6): 2403-2409. doi: 10.1007/s11033-018-4406-2.
18. World Health Organization. International Statistical Classification of Diseases and Health Related Problems ICD-10; World Health Organization: Geneva, Switzerland, **2004.**
19. Peuskens, J., Pani, L. et al. The effects of novel and newly approved antipsychotics on serum prolactin levels: a comprehensive review. *CNS Drugs* **2014;** 28(5): 421-453. doi: 10.1007/s40263-014-0157-3.
20. De Hert, M., Detraux, J., Peuskens, J. Second-generation and newly approved antipsychotics, serum prolactin levels and sexual dysfunctions: a critical literature review. *Expert Opinion on Drug Safety* **2014;** 13(5): 605–624. doi: 10.1517/14740338.2014.906579.
21. Tandon, R., Belmaker, R.H. et al. World Psychiatric Association Pharmacopsychiatry Section statement on comparative effectiveness of antipsychotics in the treatment of schizophrenia; Section of Pharmacopsychiatry, World Psychiatric Association. *Schizophrenia Research* **2008;** 100(1–3): 20–38. doi:10.1016/j.schres.2007.11.033.
22. Reynolds, G.P., McGowan, O.O., Dalton, C.F. Pharmacogenomics in psychiatry: the relevance of receptor and transporter polymorphisms. *British Journal of Clinical Pharmacology* **2014;** 77(4): 654–672. doi:10.1111/bcp.12312.
23. Ivanova, S.A., Osmanova, D.Z. et al. Prolac in gene polymorphism (-1149 G/T) is associated with hyperprolactinemia in patients with schizophrenia treated with antipsychotics. *Schizophrenia Research* **2017;** 182: 110–114. doi: 10.1016/j.schres.2016.10.029.
24. Ivanova, S.A., Osmanova, D.Z. et al. Identification of 5- hydroxytryptamine receptor gene polymorphisms modulating hyperprolactinaemia in antipsychotic drug-treated patients with schizophrenia. *The World Journal of Biological Psychiatry* **2017;** 18(3): 239–246. doi: 10.1080/15622975.2016.1224926.
25. Alladi, C.G., Mohan, A. et al. Risperidone-Induced Adverse Drug Reactions and Role of DRD2 (-141 C Ins/Del) and 5HTR2C (-759 C/T) Genetic Polymorphisms in Patients with Schizophrenia. *J Pharmacol Pharmacother* **2017;** 8(1): 28–32. doi: 10.4103/jpp.JPP_197_16.
26. Fedorenko, O.Y., Loonen, A.J. et al. Hyperprolactinemia and EYP2D8, DTD2, JVT2E genes polymorphism in patients with schizophrenia. *Physiology & Pharmacology* **2017;** 21(1): 25–33.
27. Vadasz, C., Saito, M. et al. Glutamate receptor metabotropic 7 is cis-regulated in the mouse brain and modulates alcohol drinking. *Genomics* **2007;** 90: 690–702. doi: 10.1016/j.ygeno.2007.08.006.
28. Fabbri, C., Drago, A., Serretti, A. Early antidepressant efficacy modulation by glutamatergic gene variants in the STAR*D. *Eur Neuropsychopharmacol: J Eur Coll Neuropsychopharmacol* **2013**; 23: 612–621. doi: 10.1016/j.euroneuro.2012.07.006.
29. Fonseca, F., Gratacos, M. et al. Response to methadone maintenance treatment is associated with the MYOCD and GRM6 genes. *Mol Diagn Ther* **2010;** 14*:* 171–178. doi: 10.1007/BF03256370.