Turk J Pharm Sci 2020;17(6):653-658
DOI: 10.4274/tjps.galenos.2019.46793

ORIGINAL ARTICLE

A Novel Genotyping Method for Detection of the Muscarinic Receptor *M1* Gene rs2067477 Polymorphism and Its Genotype/Allele Frequencies in a Turkish Population

Muskarinik Reseptör *M1* Geni rs2067477 Polimorfizmini Belirlemek için Yeni Bir Genotipleme Yöntemi ve Türk Popülasyonunda Genotip/Alel Sıklıkları

Fezile ÖZDEMİR[1], Yağmur KIR[2], Kenan Can TOK[1], Bora BASKAK[2], Halit Sinan SÜZEN[3]*

[1]Ankara University Institute of Forensic Sciences, Department of Forensic Toxicology, Ankara, Turkey
[2]Ankara University Faculty of Medicine, Department of Psychiatry, Ankara, Turkey
[3]Ankara University Faculty of Pharmacy, Department of Pharmacology and Toxicology, Ankara, Turkey

ABSTRACT

Objectives: Gene variation in the cholinergic muscarinic receptor 1 (CHRM1) has potential to become a candidate biomarker in the development of several disorders as well as drug response. In this study, a novel polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was developed to determine the C to A single nucleotide polymorphism at position 267 in the *CHRM1* gene.

Materials and Methods: A new reverse primer and a mismatched forward primer were designed to obtain 125 bp PCR products. The PCR products were then digested with the *Hae III* restriction enzyme to detect the rs2067477 polymorphism that comprises a C to A base change. The novel assay developed was tested in 51 Turkish schizophrenia patients.

Results: The genotyping assay was successfully performed in patients with schizophrenia in order to confirm the accuracy and validity of this method. The frequency of CC, CA, and AA genotypes was 72.5%, 25.5%, and 2%, respectively. On the basis of these findings, the allele frequency of C was 0.85 and the allele frequency of A was 0.15.

Conclusion: This genotyping assay is practical for screening the *CHRM1* C267A polymorphism in pharmacogenetic studies. The present polymorphism may be used as a candidate biomarker to determine genetic susceptibility to related diseases and may contribute to the implementation of individualized drug therapy for M1-related diseases.

Key words: CHRM1, C267A, Turkish, schizophrenia, PCR-RFLP

ÖZ

Amaç: Kolinerjik muskarinik reseptör 1'deki (CHRM1) gen varyasyonu, çeşitli bozuklukların gelişimi için ve ayrıca ilaç yanıtında aday biyogöstergelerden biri olma potansiyeline sahiptir. Bu çalışmada, *CHRM1* geninde 267. pozisyondaki C'den A'ya olan tek nükleotid polimorfizmini belirlemek için yeni bir polimeraz zincir reaksiyonu-kesim parçası uzunluk polimorfizmi (PCR-RFLP) analizi geliştirilmiştir.

Gereç ve Yöntemler: Yüz yirmi beş bç PCR ürünlerini elde etmek için yeni bir geri primer ve uyumsuz bir ileri primer tasarlanmıştır. PCR ürünleri daha sonra C'den A'ya olan baz değişikliğini içeren rs2067477 polimorfizmini tespit etmek için *Hae III* restriksiyon enzimi ile kesilmiştir. Geliştirilen yeni analiz, 51 Türk şizofreni hastasında test edilmiştir.

Bulgular: Genotipleme analizi, yöntemin doğruluğunu ve geçerliliğini onaylamak için şizofreni hastalarında başarıyla uygulanmıştır. CC, CA ve AA genotiplerinin sıklığı sırasıyla %72,5; %25,5 ve %2 olarak bulunmuştur. Bu verilere dayanarak, C alel frekansı 0,85 ve A alel için frekans 0,15 olarak bulunmuştur.

*Correspondence: E-mail: suzen@ankara.edu.tr, Phone: +90 533 345 37 99 ORCID-ID: orcid.org/0000-0003-1779-5850
Received: 20.08.2019, Accepted: 07.11.2019

Sonuç: Bu genotipleme yöntemi, farmakogenetik çalışmalarda *CHRM1* C267A polimorfizminin belirlenmesi için pratik bir yöntemdir. Bu polimorfizm, ilgili hastalıklara karşı genetik duyarlılığı göstermek için aday bir biyogösterge olarak kullanılabilir ve M1 ile ilgili hastalıklar için bireyselleştirilmiş ilaç tedavisinin uygulanmasına katkıda bulunabilir.

Anahtar kelimeler: CHRM1, C267A, Türk, şizofreni, PCR-RFLP

INTRODUCTION

Prenatal and perinatal risks, negative early life events, and genetic predisposition may cause neurodevelopmental alterations and sensitize the dopamine system in the brain, and the presence of these factors may contribute to the development of schizophrenia.[1,2] The prevalence of schizophrenia varies from 3 to 7 per 1000 worldwide and the average lifetime prevalence is 4/1000 while the lifetime risk is 7.2 per 1000.[3,4] However, studies about the prevalence of schizophrenia have shown that the disorder differs in all societies and can vary according to the characteristics of the society.[5,6] A systematic review based on a limited number of general population surveys conducted in Turkey showed that the prevalence of schizophrenia was 8.9 in 1000.[7]

The risk of schizophrenia is 10% for first-degree relatives and 40% for children if both parents have schizophrenia.[8] In addition to heredity in the development of this disease, the gene differences involved in the pharmacokinetics and pharmacodynamics of the drugs used in the treatment of schizophrenia also play a major role in treatment, response, and adverse drug reactions.

Antipsychotic drugs used in the treatment of schizophrenia such as clozapine (CLZ) and olanzapine have been found to be antagonistic to muscarinic receptors.[9] CLZ is prescribed especially in treatment-resistant schizophrenia patients and it is a weak muscarinic receptor 1 (M1) agonist, while its active metabolite, N-desmethylclozapine (NCLZ), is a potent M1 agonist receptor.[9] In addition, M1 receptor agonist DCLZ plays an important role in determining the clinical effects and pharmacotherapy in the treatment of psychotic disorders. Studies have also pointed out that a decreased density of M1 receptors particularly in the neocortical regions was associated with schizophrenia.[10] Similarly, some studies showed reduced M1 receptor mRNA levels in brain samples from schizophrenia patients.[11] Considering all of these, M1 receptor is an important target in the development and also treatment of schizophrenia.

There are five types of cholinergic muscarinic receptors, designated as M1 to M5. Among these, M1 is mostly located in the nervous system. M1 is typically found in the parasympathetic ganglia, cortical and hippocampal regions of the brain, and less in airway epithelial cells and is involved in cognitive functions such as learning and memory, as well as regulation of cardiac contractions.[12,13] M1 is encoded by the *CHRM1* gene located on chromosome 11q12.3. There are 15 single nucleotide polymorphisms (SNPs) in the *CHRM1* gene region; one of them is the C267A (rs2067477) base change. This polymorphism is a silent mutation that is a transversion of cytosine (C) to adenine (A) at position 267 in the *CHRM1* gene region. It is in the wobble site of the codon (GG**C**→GG**A**), so the protein sequence is preserved.[13,14]

In short, the determination of the SNPs in the gene regions that are potentially involved in schizophrenia are important because they could affect disease susceptibility, cognitive performance, drug response, or adverse drug reactions. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay is one of the most common, simple, effective, fast, and inexpensive methods used to determine SNPs. Thus, our aim was to develop a novel PCR-RFLP method for genotyping the *CHRM1* C267A polymorphism. Subsequently, the PCR-RFLP assay developed was performed for validation of the method and determination of genotype and allele frequencies in Turkish patients with schizophrenia.

MATERIALS AND METHODS

Study subjects and DNA isolation

Whole blood samples were obtained from 51 consecutive Turkish schizophrenia outpatients admitted to Ankara University Medical Faculty Psychiatry Department and diagnosed using the Diagnostic and Statistical Manual of Mental Disorders fourth edition[15] between October 2016 and April 2018. The inclusion criteria were being between 18 and 65 years of age and having signed the written informed consent. Patients with any additional psychiatric diagnosis or general medical comorbidity were excluded. Informed consent was obtained from all subjects and the protocol was approved by the Research Ethics Committee of the Medical Faculty, Ankara University. Genomic DNA was extracted with the high salt method from the peripheral blood of the 51 subjects.[16] The absorbance level of DNA samples for 260 and 280 nm was detected with spectrophotometric analysis and the purity of the samples was between 1.7 and 2.0.

PCR primers and conditions

The sequence data of the C267A (rs2067477) polymorphism in the human *CHRM1* gene region were obtained from the NCBI website (http://www.ncbi.nlm.nih.gov) and the new primers were designed as follows based on the published sequence: forward primer: 5'-TACTTCCTGCTGAGCCTAGCC-3'; reverse primer: 5'-GCCAGCCAGAGGTCACAAGCC-3'. The PCR reaction was carried out in a volume of 25 µL, which contained 10X PCR buffer (Amplicon, Denmark; containing 10X ammonium and 15 mM Mg), 1.1 mM $MgCl_2$, 0.1 mM dNTP, 10 pmol from each primer, 1.5 µL of DMSO, 0.45 U of *Taq* DNA polymerase (Amplicon, Denmark), approximately 100 ng of genomic DNA, and distilled water to complete the final volume to 25 µL. Moreover, 125 bp PCR product was obtained using the following PCR cycling conditions: initial denaturation at 94°C for 3 min, followed by 30 3-graded cycles, which were denaturation at 94°C for 30 s, annealing for 30 s at 59°C, and elongation at 72°C for 45 s. At the end, a final extension for 5 min at 72°C was carried out.

The PCR products (125 bp) were visualized under an ultraviolet illuminator on 1% agarose gel stained with ethidium bromide.

Restriction fragment length polymorphism conditions

The RFLP was carried out in a 20-µL volume mixture consisting of 2 µL of 10X buffer, 10 U of *Hae III* enzyme (New England Biolabs, USA), 10 µL of PCR product, and 7 µL of distilled H_2O. The reactions were incubated at 37°C overnight and the digested products were visualized under an ultraviolet transilluminator after they had been electrophoresed on 3% agarose gel containing ethidium bromide for 1 h. The digested RFLP products were obtained for a wild-type genotype, while there were undigested RFLP products for a mutant genotype on the agarose gel.

To further assess the reliability of the presented assay, the PCR product of each different genotype was verified by direct sequencing using the same set of primers.

Statistical analysis

Allele and genotype frequencies were calculated by genotype counting method. The observed genotype frequencies of *CHRM1* C267A were compared with the expected frequencies according to the Hardy-Weinberg equilibrium. The data obtained were compared with previously reported representative data in other ethnic groups. Differences in allele frequencies between schizophrenic groups were tested by Pearson's chi-square test and a p value <0.05 was considered statistically significant.

RESULTS

A novel PCR-RFLP assay was designed to detect C267A SNP in the *CHRM1* gene region in schizophrenic patients. We also evaluated the accuracy and validity of this novel method. New primers were designed and the PCR products were digested with *Hae III* restriction enzyme for determination of the variant genotypes. A schematic illustration of the assay is given in Figure 1.

The previous genotyping method for rs2067477 by Liao et al.[17] could not be perfectly applied to analyze this SNP due to the difficulties in finding primer sites. This method also did not include any information about PCR product fragments, PCR conditions, or base pairs of the restriction fragments for genotyping. In the present study, a novel genotyping assay was developed and successfully performed by utilizing a reverse primer and mismatch forward primer, which are explained above. As shown in Figure 2, the underlined A (adenine base) is the mismatched base in the forward primer, which was replaced with the ancestral base G (guanine base) to eliminate the recognition site of the *Hae III* restriction enzyme (GG▼CC) in the primer binding site. This was also confirmed by sequencing (data not shown).

The individuals with the CC genotype (wild type) yielded two bands of 83 bp and 42 bp, while those with the AA genotype (mutant type) gave an undigested band (125 bp) on 3% agarose gel. The agarose gel electrophoresis results of the RFLP products on 3% agarose gel are given in Figure 3.

One sample of each different genotype PCR product was sequenced to confirm the expected sequence of each genotype and the data obtained were consistent with our findings. The sequencing results of the three genotypes are given below in Figure 4. The PCR products of each different genotype sequencing result precisely demonstrated the reliability of our novel assay.

The allele and genotype frequencies in the 51 Caucasian Turkish schizophrenic patients are shown in Table 1 for the C267A polymorphism in the *CHRM1* gene. This is the first study to document the frequencies and genotypes of *CHRM1* C267A alleles in Turkish patients with schizophrenia. The molecular analyses revealed that, among the 51 patients tested for the C267A genotype, 37 (72.5%) were CC, 13 (25.5%) were CA, and 1 (2%) was AA. On the basis of these data, the allele frequency of C was 0.85 and the frequency of A was 0.15. The distribution of *CHRM1* genotypes in our samples is presented in Table 1. The

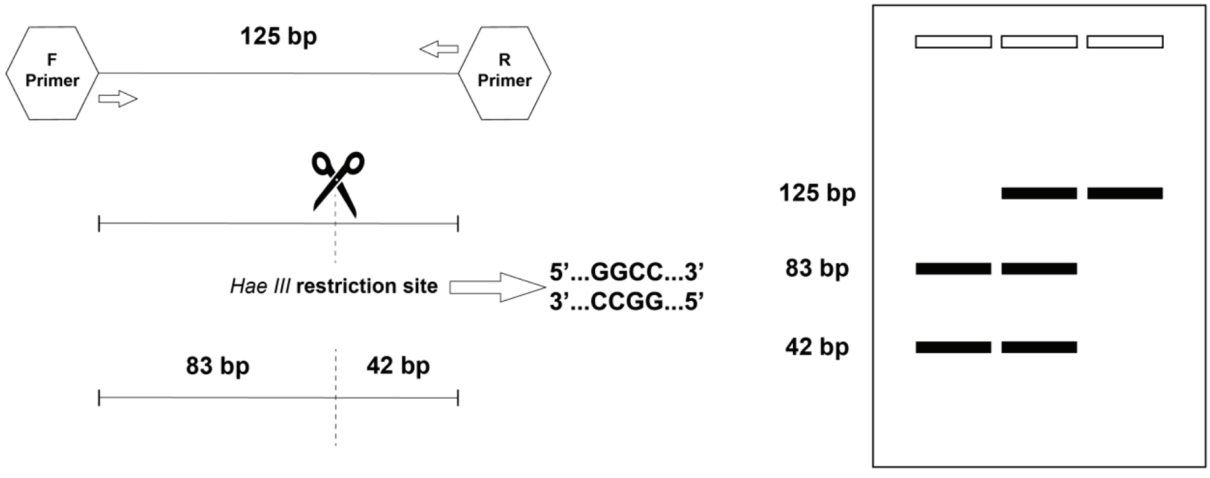

Figure 1. Diagrammatic representations of recognition sites of the *Hae III* enzyme and a schematic illustration of the restriction fragments for each genotype of *CHRM1* C267A SNP

CHRM1: Cholinergic muscarinic receptor 1, SNP: Single nucleotide polymorphism

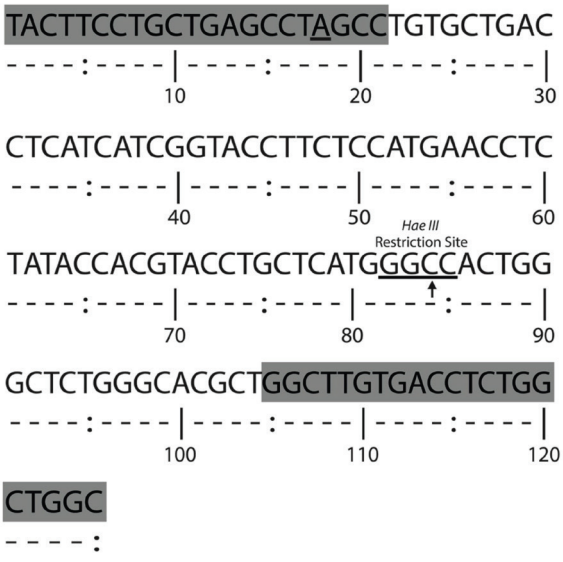

Figure 2. Restriction analysis of *CHRM1* with *Hae III* endonuclease. Forward and reverse primers are highlighted in gray. The mismatch base (A), which is used to eliminate the recognition site of *Hae III* in the forward primer, is underlined. The Hae III recognition site is depicted by underlining in the middle of the *CHRM1* sequence. This recognition site also includes rs2067477 SNP, which is depicted with capital and bold letters in the recognition site (C). In the case of the ancestral C allele at position 267 of the *CHRM1* gene 83 bp and 42 bp DNA fragments are obtained, after *Hae III* digestion. Conversely, no digestion site for *Hae III* endonuclease is found, when the C allele is replaced by an A allele at position 267, giving one fragment of 125 bp

CHRM1: Cholinergic muscarinic receptor 1, SNP: Single nucleotide polymorphism

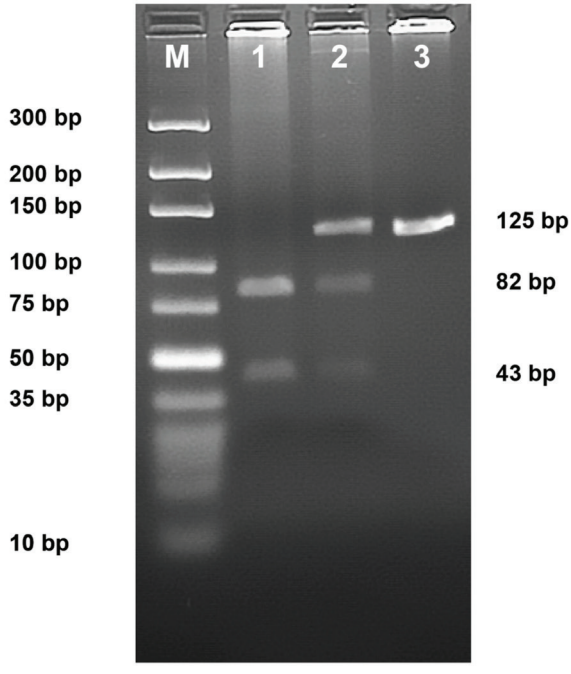

Figure 3. Agarose gel electrophoresis demonstrated the expected RFLP product sizes. The results shown in 1, 2, and 3 were in the same order as in Figure 1 (M: Thermo Fisher Scientific GeneRuler Ultra Low Range DNA Ladder Marker (10-300 bp, SM1211). 1: CC genotype, 2: CA genotype, and 3: AA genotype)

RFLP: Restriction fragment length polymorphism, PCR: Polymerase chain reaction

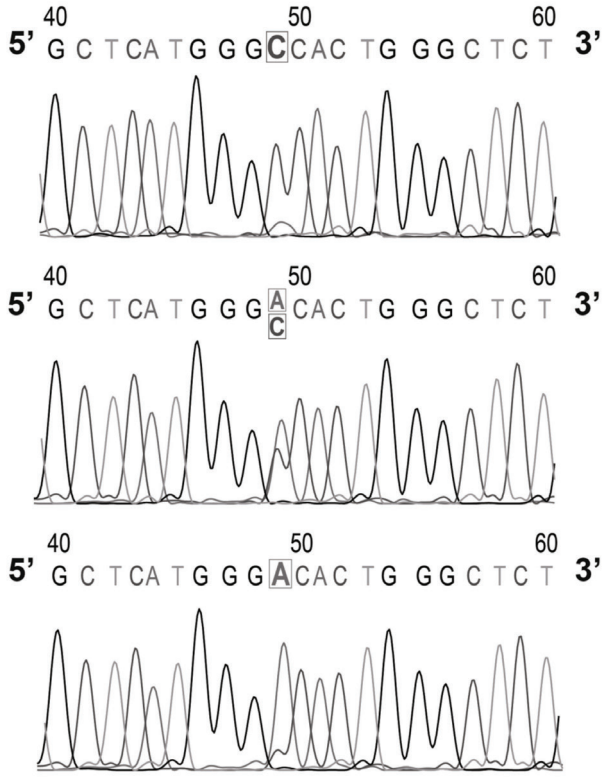

Figure 4. Examples of DNA sequencing of the polymerase chain reaction product of the *CHRM1* gene. From top the bottom the three figures represent the genotype of CC, CA, and AA, respectively, and the sequenced result of the heterozygote genotype with C and A alleles in the same position

CHRM1: Cholinergic muscarinic receptor 1

p value of the present results was p>0.05 and it was in good accordance with expected genotype distributions, calculated using the Hardy-Weinberg equilibrium (χ^2: 0.013; p=0.9).

DISCUSSION

Due to several gene variations that are potentially involved in the physiopathology of mental disorders, the *CHRM1* C267A polymorphism has the probability to become a genetic biomarker. In addition, this variation might play a role in psychopharmacotherapy since the muscarinic M1 receptor is a prominent target for a considerable number of medications. There were three primary objectives in the present study. The

Table 1. The distribution of the *CHRM1* gene polymorphism in Turkish patients with schizophrenia

Gene	Genotype	Observed frequency	Expected frequency	Allele frequencies
CHRM1	CC	37	37.1	C: 0.85 A: 0.15
	CA	13	12.8	
	AA	1	1.1	
Total		51	51	1.00

CHRM1: Gene variation in the cholinergic muscarinic receptor 1

main purpose was to develop a novel genotyping assay for the *CHRM1* C267 polymorphism and to test the accuracy and validity of the developed method. The other two aims were to draw attention to the importance of the *CHRM1* gene in the pathology of schizophrenia and to determine the genotype and allele frequencies of the *CHRM1* C267 polymorphism in Turkish patients with schizophrenia.

M1 receptors could be important for neuronal disorders and cognitive function in the pathophysiology of schizophrenia due to the location in the medial prefrontal cortex and hippocampus.[18,19] Lower levels of muscarinic receptors in the central nervous system of people with schizophrenia have been found in some studies.[18,20] Scarr et al.[21] showed that decreased M1 levels in the cortical region of the brain could contribute to the pathophysiology of schizophrenia. Thus, a brain imaging test before treatment could be useful in identifying patients with low M1 levels who could be treatment resistant. Another neuroimaging study also showed that muscarinic receptors were extensively decreased in schizophrenia patients under treatment during neuroimaging.[20]

At the molecular level, Mancama et al.[22] demonstrated that the levels of *CHRM1* cDNA in schizophrenia patients were 28% lower than those in their control group. Moreover, research suggested that there could be a relationship between rs2067477 SNP and a reduction in gray matter volume in patients with schizophrenia.[23] Other studies have shown that rs2067477 might be associated with cognitive performance. In these studies the Wisconsin Card Sorting Test performance, which is a measure of prefrontal and executive functions, was better in heterozygous individuals than in homozygous wild-type carriers.[17,24] In one of these studies, 243 schizophrenic patients were assessed according to the rs2067477 genotype and the genotypes differed in responses in the Wisconsin Card Sorting Test but not in other parameters including age of onset, chlorpromazine equivalents, and Brief Psychiatric Rating Scale.[17] Contrary to these, Cropley et al.[23] indicated that the homozygous CC genotype did not have an impact on attention, visuospatial construction, verbal fluency, or working memory but they did not assess the patients using the Wisconsin Card Sorting Test. All of these studies showed the importance of the determination of *CHRM1* C267A alleles in schizophrenic patients. To the best of our knowledge, ours is the first study to document the frequencies of *CHRM1* C267A alleles and its genotype distribution in Turkish schizophrenia patients.

In the present study, the genotype distribution and allele frequencies of the *CHRM1* C267A polymorphism were obtained from 51 Turkish schizophrenia patients. The data obtained were compared with previously reported representative data in other schizophrenia patients as shown in Table 2. The present results showed that the C and A allele frequencies in Turkish patients with schizophrenia were 0.85 and 0.15, respectively. The C267A variant frequency ranged between 0.07 and 0.11 in Australian patients with schizophrenia or schizoaffective disorder, while it was 0.09 in Chinese schizophrenia patients.[17,23-26] The difference in frequency of C267A SNP between Turkish schizophrenia patients and other populations patients was not statistically significant ($p>0.05$).

CONCLUSION

In summary, a novel, practical, low-cost, and reproducible PCR-RFLP method was developed for genotyping the *CHRM1* C267A polymorphism. The method is based on elimination of the recognition site of *Hae III* in the forward primer binding site by utilizing a mismatch base in the forward primer. As a result of this study, the validity and accuracy of the present novel method have been proven. Thus, the genotype and allele frequencies of the *CHRM1* C267 polymorphism in Turkish patients with schizophrenia have been determined for the first time. The number of samples should be increased in further studies for more certain and reliable results. Additionally, the effect of the *CHRM1* gene in the pathology and treatment of schizophrenia is explained with the data in the literature. The developed genotyping assay and results could be useful and provide a perspective for future studies.

Financial and Conflict of interest: This work was supported in part by grant from the Research Fund of Ankara University under Project 18L0217001. The author declares no conflict of interest, financial or otherwise.

Table 2. Genotypes and allele frequencies of C267A SNP in *CHRM1* in this study and other populations

Study population	n	Genotype frequency n (%)			Allele frequency		Reference
		CC	CA	AA	C	A	
Turkish patients with schizophrenia	51	37 (72.5)	13 (25.5)	1 (2)	0.85	0.15	Present study
Chinese patients with schizophrenia	243	202 (83.1)	40 (16.5)	1 (0.4)	0.91	0.09	7
Australian patients with schizophrenia and schizoaffective disorder	97	83 (86)	14 (14)	-	0.93	0.07	24
Australian patients with schizophrenia or schizoaffective disorder	267	191 (84.1)	35 (15.4)	1 (0.4)	0.92	0.08	23
Australian patients with schizophrenia and schizoaffective disorder	176	147 (83.5)	29 (16.5)	-	0.92	0.08	25
Australian patients with schizophrenia and schizoaffective disorder	147	114 (77.6)	33 (22.4)	-	0.89	0.11	26

CHRM1: Cholinergic muscarinic receptor 1, SNP: Single nucleotide polymorphism

Ethical conduct of research: All authors state that the appropriate institutional review board approval had obtained and the informed consent has been obtained from the participants involved study. The authors state that all experiments had followed the principles outlined in the Declaration of Helsinki.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Howes OD, Murray RM. Schizophrenia: an integrated sociodevelopmental cognitive model. Lancet. 2014;383:1677-1687.
2. Owen MJ, Sawa A, Mortensen PB. Schizophrenia. Lancet. 2016; 388:86-97.
3. Mc Garth J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 2008;30:67-76.
4. DSM, American Psychiatric Association. Section 2: Diagnotic Criteria and Codes, Schizophrenia Spectrum and Other Psychotic Disorders. In: Diagnostic and Statistical Manual of Mental Disorders, 5th edition. Ed: First MB, Ward MN. American Psychiatric Publishing, Washington, DC. 2013;99-105.
5. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med. 2005;2:413-433.
6. Esan OB, Ojagbemi A, Gureje O. Epidemiology of schizophrenia-An update with a focus on developing countries. Int Rev Psychiatry. 2012;24:387-392.
7. Binbay T, Ulaş H, Elbi H, Alptekin K. The psychosis epidemiology in Turkey: a systematic review on prevalence estimates and admission rates. Turk Psikiyatri Derg. 2011;22:40-52.
8. Ayano G. Schizophrenia: a concise overview of etiology, epidemiology diagnosis and management: review of literatures. J Schizophr Res. 2016;3:1-7.
9. Weiner DM, Meltzer HY, Veinbergs I, Donohue EM, Spalding TA, Smith TT, Mohell N, Harvey SC, Lameh J, Nash N, Vanover KE, Olsson R, Jayathilake K, Lee M, Levey AL, Hacksell U, Burstein ES, Davis RE, Brann MR. The role of M1 muscarinic receptor agonism of N-desmethylclozapine in the unique clinical effects of clozapine. Psychopharmacology (Berl). 2004;177:207-216.
10. Scarr E, Cowie TF, Kanellakis S, Sundram S, Pantelis C, Dean B. Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. Mol Psychiatry. 2009;14:1017-1023.
11. Michel MC, Teitsma CA. Polymorphisms in human muscarinic receptor subtype genes. Handb Exp Pharmacol. 2012;208:49-59.
12. Hamilton SE, Schlador ML, McKinnon LA, Chmelar RS, Nathanson NM. Molecular mechanisms for the regulation of the expression and function of muscarinic acetylcholine receptors. J Physiol Paris. 1998;92:275-278.
13. Lucas JL, Sadee W, DeYoung JA. Single nucleotide polymorphisms of the human M1 muscarinic acetylcholine receptor gene. AAPS Pharmsci. 2001;3:57-61.
14. Brann MR, Ellis J, Jorgensen H, Hill- Eubanks D, Jones SV. Muscarinic acetylcholine-receptor subtypes - localization and structure-function. Prog Brain Res. 1993;98:121-127.
15. The American Psychiatric Association. DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders 4th Revised Edition, American Psychiatric Press Inc. 2000. Avaialable from: https://dsm.psychiatryonline.org/doi/abs/10.1176/appi.books.9780890420249.dsm-iv-tr
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. 1988;16:1215.
17. Liao DL, Hong CJ, Chen HM, Chen YE, Le SM, Chang CY, Chen H, Tsai SJ. Association of muscarinic M1 receptor genetic polymorphisms with psychiatric symptoms and cognitive function in schizophrenic patients. Neuropsychobiol. 2003;48:72-76.
18. Dean B, McLeod M, Keriakous D, McKenzie J, Scarr E. Decreased muscarinic1 receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol Psychiatry. 2002;7:1083-1091.
19. Yohn SE, Conn PJ. Positive allosteric modulation of M1 and M4 muscarinic receptors as potential therapeutic treatments for schizophrenia Neuropharmacol. 2018;136:438-448.
20. Raedler TJ, Knable MB, Jones DW, Urbina RA, Gorey JG, Lee KS, Egan MF, Coppola R, Weinberger DR. *In vivo* determination of muscarinic acetylcholine receptor availability in schizophrenia. Am J Psychiatry. 2003;160:118-127.
21. Scarr E, Hopper S, Vos V, Seo MS, Everall IP, Aumann TD, Chana G, Dean B. Low levels of muscarinic M1 receptor-positive neurons in cortical layers III and V in Brodmann areas 9 and 17 from individuals with schizophrenia. J Psychiatry Neurosci. 2018;43:338-346.
22. Mancama D, Arranz MJ, Landau S, Kerwin R. Reduced expression of the muscarinic 1 receptor cortical subtype in schizophrenia. Am J Med Genet. 2003;119B:2-6.
23. Cropley VL, Scarr E, Fornito A, Klauser P, Bousman CA, Scott R, Cairns MJ, Tooney PA, Pantelis C, Dean B. The effect of a muscarinic receptor 1 gene variant on grey matter volume in schizophrenia. Psychiatry Res. 2015;234:182-187.
24. Scarr E, Sundram S, Deljo A, Cowie TF, Gibbons AS, Juzva S, Mackinnon A, Wood SJ, Testa R, Pantelis C, Dean B. Muscarinic M1 receptor sequence: Preliminary studies on its effects on cognitionand expression. Schizophr Res. 2012;138:94-98.
25. Carruthers SP, Gurvich CT, Cropley VL, Pantelis C, Bousman C, Lenroot RK, Bruggemann JM, Weickert T; Australian Schizophrenia Research Bank, Rossell SL. The effects of a muscarinic receptor 1 gene variant on cortical thickness and surface area in schizophrenia. Psychiatry Res Neuroimaging. 2018;280:62-64.
26. Carruthers SP, Cropley V, Bousman C, Everall IP, Neill E, Pantelis C, Sumner PJ, Tan EJ; Australian Schizophrenia Research Bank, Bozaoglu K, Thomas EHX, Van Rheenen TE, Gurvich CT, Rossell SL. The effects of a muscarinic receptor *1* gene variant on executive and non-executive cognition in schizophrenia spectrum disorders. Psychiatry Res. 2019;273:178-180.