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Original Article

Pharmacogenetic Analysis of Taq1A (rs1800497), T102C (rs6313) and His452Tyr (rs6314) with Clozapine Response in First Line Therapy Resistant Schizophrenia Patients in an Iranian Ethnic Group

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Pharmacogenetic studies in schizophrenia patients illustrated variable response to antipsychotic treatment. Moreover, most of patients will require long-term use of atypical antipsychotic medications which may lead to drug side effect, treatment-resistance, medication arrest, and even venture to suicide. Clozapine is the best choice in the treatment of refractory patients, although not effective in all of them and also having side effects. Therefore, any information that help to predict the outcome of each antipsychotic drug in a particular patient will be highly valuable to find the right drug for the right patient. Taq1A (rs1800497) polymorphism of dopamine receptor D2, T102C (rs6313) and His452Tyr (rs6314) polymorphisms of serotonin 2A receptor were analyzed as effective single nucleotide polymorphisms (SNPs) associated with clozapine response in schizophrenia patients in an ethnic group of Iranian population. Our data suggest that the presence of C allele for rs1800497 and rs6314 and T allele for rs6313 might be helpful for determining response to clozapine in first line therapy resistant patients. 37% of patients who had the above polymorphic alleles together, manifested improved response to clozapine versus 1.6% of clozapine responder patients who did not carry those alleles. Our data confirm that these polymorphisms are associated with clozapine response in schizophrenia patients in the studied population. Genetic screening of these three effective SNPs may be advantageous to predict clozapine response in Iranian schizophrenia refractory patients.

Keywords: Schizophrenia, clozapine, polymorphism, Iran

Schizophrenia is a chronic neuropsychiatric illness with approximately 1 percent prevalence during life time (1) which causes significant social problems, with symptoms beginning typically in late adolescence and early adulthood. It shows a multifactorial etiology with both genetic and environmental factors playing important roles (2). The risk for developing schizophrenia for monozygotic twins is more than 40% (3), while first degree relatives have 6.5% recurrence risk (3). The main treatment for schizophrenia is antipsychotic drugs which are classified into two major groups: typical and atypical. Dopamine receptors have strong affinities for typical antipsychotics while atypical ones have several targets and show greater efficacy (4).

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Schizophrenia patients show variable responses to antipsychotic treatments (5). The choice of suitable drugs for these patients depends on adverse effects of each drug and patients' genetic profile. Thus, careful treatment design is of high importance for each patient.

Often, at least two different antipsychotics are used sequentially for 6-8 weeks with great improvement occurring sometimes after 6 months treatment. Usually olanzapine and risperidone are comparably effective in decreasing symptoms as first line therapy (6). However, both are associated with adverse effects such as sedation, extrapyramidal side effect (EPS) and weight gain despite their greatest efficacy. Haloperidol and aripiprazole are administrated as second line therapy (7).

Given that most of patients will require long-term use of atypical antipsychotic medications, drug side effect, treatment resistance in nearly 20% of patients and consequently medication arrest, and even some cases of suicide may occur (8).

For treatment- resistant patients who do not respond to first-line medication, usually psychologists focus on clozapine which is prominent over other atypical drugs in the treatment of refractory patients (9). While it is effective in 30- 60% of resistant patients (10), it also has some side effects like seizures, weight gain, sedation, drowsiness , hypertension, and potentially agranulocytosis in less than 4% of cases (11). Thus, it is routinely employed only as a third-line treatment (12).

The investigation of inherited variability in response to antipsychotic drugs may lead to an optimal personalized treatment. Accordingly, neurotransmitter (dopamine and serotonin) receptors variants are in the first line of attention.

Conflicting results between different populations emphasizes the necessity of complementary studies in order to find a better correlation between genetic variants and variable response to treatment (11, 13).

The aim of this study was to examine rs1800497 at dopamine receptor D2 (DRD2) and rs6313 and rs6314 at serotonin 2A (5HT2A) receptor as most intensively examined single nucleotide polymorphisms (SNPs) (11) in order to find any possible correlation between those SNPs and clozapine response in Iranian schizophrenia patients.

Materials and methods

Patients and study protocol

Subjects included 73 schizophrenic patients (51% male and 49% female), referred to the department of psychiatry at Babol Yahyanejad hospital in north of Iran.

All patients were diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) criteria, as in antipsychotic clinical trials, DSM-IV-TR is widely used to assess symptoms of schizophrenia.

At the time of sampling, a complete clinical history and written informed consent was obtained from all participating subjects. This research was approved by the ethical committee of Babol University of Medical Sciences.

Patients initially received two or three different antipsychotic drugs, usually olanzapine and risperidone, followed by haloperidol and aripiprazole. Patients showed improvement after 6-8 weeks and maximum 6 months, forty patients with mean age 35.3±8.11 years responded to the treatment and were considered as non-clozapine responder.

Resistant patients, received clozapine treatment after six months typical antipsychotic treatment, and were followed up for at least six months.

The majority of treatment resistant patients which had been given clozapine, showed good improvement and were considered as clozapine responder which in our study comprised thirty three patients with mean age 34.03±9.07 years.

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Table 1. PCR primers, annealing temperatures for PCR and the restriction enzymes used for genotyping

Polymorphism	SNP alleles	Gene	Primers 5' ⟶ 3'	Annealing temperature	Restriction enzyme	Amplicon size (bp)
TaqIA (rs180049)	C/T	DRD2	F:CACGGCTGGCCAA GTTGTCTA R:CACCTTCCTGAGT GTCATCAA	60 °C	Taq1A	307 (127,180)
T102C (rs6313)	C/T	5-HT2A receptors	F:CAAGGTGAATGGT GAGCAGAAA R:TGGCAAGTGACAT CAGGAAATAGT	58 °C	Msp1	427 (170,257)
His452Tyr (6314)	C/T	5-HT2A receptors	F:GATGCCAAGACAA CAGATAATGAC R:ACTTGCTCAGTGT GCCTTCC	62 °C	Bsm1	158 (95,60)

Genotyping

Genomic DNA was extracted from venous blood (5 ml) using a commercially available kit (DNA Micro Kit, Qiagen, Germany). The quality of the extracted DNAs was analyzed by gel electrophoresis and was confirmed by optical density measurement (Thermo, USA).

DRD2 SNP was screened for TaqIA (rs1800497), and for the 5HT2A receptor T102C (rs6313) and His452Tyr (rs6314) SNPs were screened.

For genotyping, target DNA sequences, were amplified by polymerase chain reaction (PCR). The PCR reactions were performed using 0.5 µl of genomic DNA, 1.25 µl MgCl2 (20 mM), 2.5 µl 10x buffer, 0.5 µl of each forward and reverse primers (10 pM each), 0.625 µl dNTPs 10 mM, and 0.5 unit of Taq DNA polymerase, in a final reaction volume of 25 µl.

The amplification protocol comprised an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30s, annealing at 58 to 62 °C (depending on primer pairs) for 30s, and 72°C for 30s with a final extension period of 72°C for 10 min (Table 1).

Amplicons were digested overnight at 37 °C with appropriate restriction enzymes (NEB, Inc., USA) (Table 1), then separated on 3% agarose gel containing ethidium bromide and digestion patterns were visualized under UV illumination.

Statistical analyzes

Statistical analysis of the data was carried out by SPSS, version 18. Normally distributed data were expressed as mean± standard deviation (SD) or median and qualitative variables were expressed as n (%).

Statistical comparison of clinical variable between clozapine responders and non-clozapine responder patients were analyzed using chi-square ($\chi 2$) test or independent T test. In all tests, the value of $P < 0.05$ was considered as statistically significant.

The statistical difference in allelic and genotype frequencies between groups were compared using the chi-square test and odds ratio (OR) and confidence interval was also calculated with MedCalc online software (http://www.medcalc.org/).

Results

Demographic and clinical features of the patients are given in Table 2. No significant difference was observed in genders, mean age of onset, positive marital status, and positive family history between the two groups, although clozapine responders showed lower mean age at first contact (20.69± 3.49 years) and were mostly single compared to non-clozapine responder patients. Regarding occupation, the two groups showed a significant difference in having job, with most of clozapine responders being workless.

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The allele and genotype frequencies of the three examined SNPs (rs1800497, rs6314, rs6313) are shown in Table 3. None of the three SNPs showed deviation from Hardy Weinberg's equilibrium between patients groups.

Significant differences between clozapine responders and non-clozapine responders were observed in all three examined SNPs. rs1800497C, rs6314C, rs6313T alleles were more frequent in clozapine responder patients.

Table 2. Demographic and clinical characteristics of clozapine responders and non-clozapine responder patients

Characteristic	Clozapine responders n=33	Non-clozapine responders n=40	P value
Mean age (year)	34.03±9.07	35.3±8.11	0.48
Men (%)	57%	45%	0.20
Mean age at first contact (year)	20.69±3.49	24.96±7.91	0.02
Positive marital status (%)	36%	45%	0.07
Positive family history (%)	53%	40%	0.22
Having job (%)	41%	81%	0.02

Data presented as mean±SD, n (%) of cases or median.

Table 3. The allele and genotype frequencies of rs1800497, rs6314, and rs6313 in clozapine responder and non-clozapine responder

SNP	Clozapine responders (%)	Non-Clozapine Responders (%)	OR	P value
rs1800497(Taq1A)	N=33	N=40		
CC	23(69.6%)	18(45%)	*1.00 (reference)*	
CT	9(27.2%)	20(50%)	0.35(0.12-0.95)	0.04
TT	1(3.33%)	2(5%)	0.39(0.03-4.6)	0.45
C allele frequency	55(83.3%)	56(70%)		
T allele frequency	11(16.7%)	24(30%)		
rs6314(His452Tyr)	N=33	N=40		
CC	19 (57.5%)	8 (20%)	*1.00 (reference)*	
CT	12 (36.3%)	26(65%)	0.19 (0.06-0.56)	0.001
TT	2 (6.2%)	6 (15%)	0.149 (0.02-0.84)	0.03
C allele frequency	50 (75.7%)	42 (52.5%)		
T allele frequency	16 (24.3%)	38 (47.5%)		
rs6313(T102C)	N=33	N=38		
CC	3 (9%)	10 (26.3%)	*1.00 (reference)*	
CT	21 (63.7%)	23 (60.5%)	3.04 (0.73-12.58)	0.12
TT	9 (27.3%)	5 (13.2%)	6.00 (1.10-32.55)	0.03
C allele frequency	27 (41%)	43 (53.7%)		
T allele frequency	39 (59%)	37 (46.3%)		

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Discussion

A number of studies investigated the demographic and clinical factors associated with response to clozapine. Some researchers did not observe any clinical predictors of response to clozapine (14, 15) while others reported an influence of gender and age at onset of the disorder as predictors of response to clozapine (16, 17). We did not detect differences in gender, mean age, positive marital status and positive family history between clozapine responders and non-clozapine responders, but found a significant lower mean age at first contact for clozapine responders (20.69±3.49 years vs 24.96±7.91 years; P = 0.02.)

On the other hand, a large study which combined data from 37 different countries reported that 16.2%- 22.6 % of schizophrenia patients were in paid employment, against an average employment rate of 75%-80% in the general population (18). In the present study most clozapine responders (59%) didn't have a job and were jobless (P = 0.02).

As the potency of antipsychotic drug was highly correlated with the binding affinity to dopamine receptor (25), the hypothesis that genetic polymorphisms in dopamine and serotonin genes may affect clinical response to clozapine has been tested by several studies (19). Clozapine is blocking the D2 receptor (20) and therefore, D2 receptor gene (DRD2) variants may be important to the variability in response to clozapine and many performed studies showed contradictory results in this regard (21-23).

One of the most intensively examined DRD2 SNPs is TaqIA (rs1800497), for which the A1 allele (allele C), has been associated with reduced gene expression levels in *in vitro* (24) as well as *in vivo* studies (25). Studies with Taq1A polymorphisms produced contradictory results (26, 27). However, correlation studies of Taq1A polymorphism with antipsychotic drug response, revealed that carriers of C allele were more responsive to antipsychotic drugs and homozygotes for T allele showed low response after treatment (28-30).

5HT2A receptor plays an important role in response to treatment of schizophrenia (31). T102C (rs6313) polymorphism of this gene was shown to be associated with schizophrenia, and has a major role in determining response to clozapine response (32-34) with the T allele being more prevalent among clozapine responders (33), although this finding was not replicated in some populations (30, 35).

With respect to HTR2A, positive association was also reported between His452Tyr variant (rs6314) as non-synonymous SNP and clozapine response (35) but some other studies failed to detect this association (11, 36).

In the present study, all 3 studied polymerphisms, rs1800497, rs6313 and rs6314 were associated with clozapine response in schizophrenia patients (Table 3). Our data showed that first line therapy resistant patients, who carried rs1800497C allele and especially rs6314C allele had good response to clozapine treatment (P = 0.04 and 0.001, respectively).

Also, refractory patients who carried rs6313T allele were more responsive to clozapine in comparison with other antipsychotic atypical drugs (P = 0.03).

Our data suggest that the presence of C allele for rs1800497 and rs6314 and T allele for rs6313 might be helpful for determining the response to clozapine in first line therapy resistant patients. Our data showed that 37% of patients, who had the above polymorphic alleles together, manifested improved response to clozapine versus 1.6% of clozapine responder patients who did not carry those alleles.

In conclusion, this research showed that three polymorphisms, Taq1A (rs1800497), T102C (rs6313) and His452Tyr (rs6314) were associated with clozapine response in schizophrenia patients in our population.

Furthermore, regarding long-term use of an atypical drug and treatment-resistance to first line therapy, genetic screening of these three effective SNPs may be advantageous to predict clozapine

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response in Iranian schizophrenia refractory patients. Further pharmacogenetic comparative studies with larger sample size are needed to confirm the efficacy screening of these three SNPs with response to clozapine in resistant schizophrenia patients.

Conflict of interest

The authors declared no conflict of interest.

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