Acta Pharmacologica Sinica 2006 Aug; 27 (8): 966–970

Blackwell Publishing

Short communication

Association of *DRD2* polymorphisms and chlorpromazine-induced extrapyramidal syndrome in Chinese schizophrenic patients[1]

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Key words

dopamine receptors; basal ganglia disease; polymorphism; chlorpromazine; schizophrenia

[1] Project supported by grants from the national 973 and 863 programs, the National Natural Science Foundation of China, and the Shanghai Municipal Commission for Science and Technology.

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Received 2005-12-07
Accepted2006-03-25

doi: 10.1111/j.1745-7254.2006.00355.x

Abstract

Aim: Extrapyramidal syndrome (EPS) is most commonly affected by typical antipsychotic drugs that have a high affinity with the D2 receptor. Recently, many research groups have reported on the positive relationship between the genetic variations in the *DRD2* gene and the therapeutic response in schizophrenia patients as a result of the role of variations in the receptor in modulating receptor expression. In this study, we evaluate the role *DRD2* plays in chlorpromazine-induced EPS in schizophrenic patients. **Methods:** We identified seven SNP(single nucleotide polymorphism) (-141C*ins*>*del*, *Taq*IB, *Taq*ID, Ser311Cys, *rs*6275, *rs*6277 and *Taq*IA) in the *DRD2* gene in 146 schizophrenic inpatients (59 with EPS and 87 without EPS according to the Simpson-Angus Scale) treated with chlorpromazine after 8 weeks. The alleles of all loci were determined by PCR (polymerase chain reaction) . **Results:** Polymorphisms *Taq*ID, Ser311Cys and *rs*6277 were not polymorphic in the population recruited in the present study. No statistical significance was found in the allele distribution of -141C*ins*>*del*, *Taq*IB, *rs*6275 and *Taq*IA or in the estimated haplotypes (constituted by *Taq*IB, *rs*6275 and *Taq*IA) in linkage disequilibrium between the two groups. **Conclusion:** Our results did not lend strong support to the view that the genetic variation of the *DRD2* gene plays a major role in the individually variable adverse effect induced by chlorpromazine, at least in Chinese patients with schizophrenia. Our results confirmed a previous study on the relationship between *DRD2* and EPS in Caucasians.

Introduction

Schizophrenia is a complex and devastating brain disorder that affects 1% of the population and is ranked as one of the most costly disorders to afflict humans[1]. Chlorpromazine is a typical antipsychotic drug used for the treatment of schizophrenia since the 1950s, and became a milestone in the development of treatments for psychotic disorders. Although chlorpromazine is no longer used in some countries, it is still widely used to treat schizophrenia in China and many other developing countries. During the treatment of schizophrenics with antipsychotics, especially the typical antipsychotics, it can cause a high rate of extrapyramidal syndrome (EPS), including akathisia, acute dystonia and pseudoparkinsonism, and tardive dyskinesia, which is a serious drawback in neuroleptic treatment. However, the occurrence of EPS can be the bottleneck of chlorpromazine treatment.

In recent years, investigators have been trying to find genetic factors contributing to drug-induced EPS by paying close attention to dopamine receptor genes. Ser9Gly polymorphism in *DRD3* was studied extensively and inconsistent reports were published[2–4]. In addition, several studies have aimed to identify the relationship between the *DRD2* gene polymorphisms and the drug response or adverse effects, but the results are also controversial[5–7]. Two studies revealed that *Taq*IA and -141C*ins*>*del* were associated with drug response[6,7], but one showed a negative associa-

tion[2]. Meanwhile, an *in vitro* study demonstrated that the -141C*ins>del* polymorphism had a functional role in affecting *DRD2* expression[8]. In addition, Ser311Cys polymorphism partly affected the neuroleptics binding affinity to cause the blockade of functional activity[9]. Hence, *DRD2* is likely to be a promising candidate gene for the inducement of EPS in schizophrenic patients. In our previous study, we found that -141C*ins>del* in the *DRD2* gene may be related to the therapeutic effects of chlorpromazine in schizophrenic patients[10].

The dopamine D2 receptor (DRD2) is the primary binding target of all antipsychotics. It belongs to the family of receptors coupled to heterotrimeric cyclic guanine nucleotide binding regulatory proteins (G-proteins). DRD2 activates intracellular signaling by the inhibition of cAMP synthesis through interaction with G_1-like proteins[5]. The development of EPS has been seen as a consequence of the action of typical neuroleptics on striatal DRD2. Dopamine receptor blockade in the basal ganglia is considered as the mechanism of EPS[11]. Farde *et al*[12–15], in a series of studies, have shown that: (1) typical neuroleptics from different chemical classes used at conventional doses occupy 65%–89% of the available DRD2; and (2) individuals who experience EPS have significantly higher (82%±4%) levels of DRD2 blockade as compared to those patients without EPS (74%±4%). Otherwise, clozapine, an atypical neuroleptic, has a significantly lower level of DRD2 occupancy and produces virtually no EPS at conventional doses[16]. Another study has also reported a consistent result[17]. The degree of DRD2 occupancy can be an indicate of EPS[18]. All of the above suggests that DRD2 is closely related to the onset of EPS.

In order to evaluate whether variations in the *DRD2* gene are related to drug-induced EPS, we identified more SNP (-141C*ins>del*, *Taq*IB, *Taq*ID, Ser311Cys, *rs*6275, *rs*6277 and *Taq*IA) in the *DRD2* gene of 146 Chinese schizophrenic inpatients treated with chlorpromazine.

Materials and methods

Patients and drug treatment We recruited 146 patients, who were of Han Chinese origin, from Shanghai Mental Health Center. Informed consent was obtained from all participating patients. All patients were acute inpatients with schizophrenia (mean age of onset=27.3 years, SD=9.2, 38.7% female) diagnosed according to Diagnostic and Statistical Manual of Mental Disorder, Third Edition, Revised (DSM-III-R)[19]. None of the patients had any medication for at least 1 month before this study. The dosage of chlorpromazine used in the comparison study was in a range of 300–600 mg/d. Patients treated with any other antipsychotic drugs were not included. The diagnosis for each patient with EPS was made in terms of the Simpson-Angus Scale (SAS) by at least two psychiatrists, independently, after the patients were treated with chlorpromazine for 8 weeks. As a result, 59 of 146 patients experienced EPS. The participating psychiatrists were blinded to the patients' genotypes.

SNP genotyping We chose 7 SNP (-141C*ins>del*, *Taq*IB, *Taq*ID, Ser311Cys, *rs*6275, *rs*6277 from http://www.ncbi.nlm.nih.gov/SNP/ and *Taq*IA) from *DRD2*, which spans about 270 kb where -141C*ins>del* is in the promoter region, *Taq*IB and *Taq*ID are in intron 1 and intron 2, *rs*6275 and *rs*6277 are in exon 7. *Taq*IA is in the 3'-untranslated region, which is in fact located in a novel gene, untitled X-kinase gene[20]. Genomic DNA was extracted from peripheral blood leukocytes by a standard phenol extraction procedure.

Genotyping of *Taq*IB, *Taq*ID and *Taq*IA was modified on the basis of Kaiser *et al*[5], while analysis of -141C*ins>del* was modified according to Jönsson *et al*[21]. *Rs*6275 and *rs*6277 were analyzed by direct sequencing. All amplification reactions were performed in a total volume of 25 μL, containing 10 ng DNA, 1×buffer, 200 μmol/L dNTP, 4×10^{-6} μmol/L of each primer, 1×Q solution, and 1 unit *Taq* DNA polymerase.

The PCR program of all these reactions consisted of 36 cycles, including an initial denaturation at 94 ºC for 5 min, and a terminal extension period at 72 ºC using a Gene Amp 9700 thermcycler (Applied Biosystems, Foster City, CA). The condition of the cyclic PCR was as follows. For the -141C*ins>del* polymorphism: 94 ºC for 45 s, 55 ºC for 30 s, and 72 ºC for 1 min. For *Taq*IB and *Taq*ID: 94 ºC for 45 s, 53 ºC for 30 s, and 72 ºC for 1.5 min. For *rs*6275 and *rs*6277: 94 ºC for 45 s, 55 ºC for 30 s, and 72 ºC for 45 s. For *Taq*IA: 94 ºC for 45 s, 56 ºC for 30 s, 72 ºC for 1 min; and for Ser311Cys, 94 ºC for 45 s, 60 ºC for 1 min, 72 ºC for 1.5 min.

All but *rs*6275 and *rs*6277 PCR products were digested with restriction enzymes according to the manufacturer's protocol, separated by 2.0% agarose gel electrophoresis and stained with ethidium bromide for UV visualization. For *rs*6275 and *rs*6277, we first amplified a fragment including the two SNP, and then purified the PCR product with shrimp alkaline phosphatase. The purified PCR product was used to carry out sequencing reaction by using sense primer and a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) as a total volume of 5 μL. The sequence analysis was performed in an ABI PRISM model 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Statistical methods The difference of allele distribution

between patients with EPS and without EPS was investigated using CLUMP version 1.6[22] based on 1000 stimulations. The *P*-values reported were two tailored and the limit of significance was set to 0.05. The pair-wise linkage disequilibrium (LD), as measured by D'[23], was estimated with 2LD software from haplotype frequencies based on alleles at all possible pairs of SNP loci[24]. EHPLUS was used to estimate the haplotype frequency by performing model-free analysis and permutation tests of allelic association based on EH[25]. It uses marker genotypes from a group of unrelated individuals or a group of cases and a group of controls and employs gene-counting algorithm to estimate haplotype frequencies and output asymptotic and permutation test statistics. We used an online calculator to test the departure from Hardy-Weinberg equilibrium in both groups (Online Hardy-Weinberg equilibrium calculator http://www.kursus.kvl.dk/shares/vetgen/ Popgen/genetik/applets/kitest.htm).

Results

Extensive genetic variations, including restriction sites *Taq*IB, *Taq*IA, and *rs*6275, exist in the *DRD2* gene of schizophrenics. However, *Taq*ID,Ser311Cys with an amino acid substitution and *rs*6277 showed low frequency of variation in the subjects. Frequencies of all SNP genotypes revealed no significant deviation from Hardy–Weinberg equilibrium. The result of analysis of the SNP by CLUMP is presented in Table 1. No statistical significance between 59 patients with EPS and 87 patients without EPS was observed in both genotype and allele distribution on each single marker.

The results showed that *Taq*IB, *rs*6275, and *Taq*IA were in relative strong linkage disequilibrium, or in a LD block. The frequency of any two haplotypes consisting of the three SNP in linkage disequilibrium had no statistical difference (data not shown).

Discussion

It is generally recognized that genetic variants in *DRD2* are promising as predictors for adverse affects of antipsychotic medication in schizophrenia patients, including EPS. Many studies have reported on the relationship between the *DRD2* gene and the occurrence of schizophrenia and drug response in schizophrenia, but few have reported on the correlation between SNP in *DRD2* and response to chlorpromazine, which is widely and routinely used in China and developing countries. In this work, however, we genotyped 7 SNPs, including -141C*ins>Del*, *Taq*IA, *Taq*IB, Ser311Cys, *rs*6275, *rs*6277, and *Taq*ID, but only four of them were informative enough to carry out statistical analysis. The results showed no statistical differences in allele and genotype frequency of -141C*ins>del*, *Taq*IB, *rs*6275, and *Taq*IA ($P>0.05$). A strong level of LD was detected in *Taq*IB, *rs*6275, and *Taq*IA ($D'>0.5$). No significant difference was

Table 1. Statistical analysis of polymorphisms in *DRD2*.

Locus	Genotype (%)			*df*	*P*	Hardy–Weinberg equilibrium *P* value	Allele (%)		*df*	*P* value
-141C*ins>del*	I/I	I/D	D/D				Ins	Del		
With EPS	43(72.9)	15(25.4)	1(1.7)				101(85.6)	17(14.4)		
Without EPS	73(83.9)	13(14.9)	1(1.2)	2	0.27	0.98	159(91.4)	15(8.6)	1	0.12
*Taq*IB	G/G	G/A	A/A				G	A		
With EPS	19(32.2)	32(54.2)	8(13.6)				7 0(59.3)	48(40.7)		
Without EPS	25(28.7)	46(52.9)	16(18.4)	2	0.69	0.56	96(55.2)	78(44.8)	1	0.48
rs6275	T/T	T/C	C/C				T	C		
With EPS	20(33.9)	26(44.1)	13(22.0)				66(55.9)	52(44.1)		
Without EPS	27(31.0)	44(50.6)	16(18.4)	2	0.24	0.95	98(56.3)	76(43.7)	1	0.94
*Taq*IA	G/G	G/A	A/A				G	A		
With EPS	16(27.1)	28(47.4)	15(25.4)				60(50.8)	58(49.2)		
Without EPS	27(31.0)	35(40.3)	25(28.7)	2	0.69	0.25	89(51.1)	85(48.9)	1	0.95

detected in distribution of haplotype constituted by the three SNP. This indicates that the four variations of *DRD2* do not play an important role in the development of EPS. Compared with other similar studies, our results are inconsistent with those of Suzuki *et al*[8] and Mihara *et al*[26], who found a positive association between the polymorphisms in *DRD2* and EPS, but more agreeable with other more comprehensive studies that show no association between the polymorphisms of *DRD2* and EPS in Caucasian people[5,27,28]. In our study, as only one drug was used and patients had no other medication at least one month before this study, the detecting power was much higher than using different neuroleptics. In addition, relatively large sample sizes and more polymorphisms were analyzed in our study and that of Kaiser *et al*[5], although both showed negative results. The mechanism of EPS is more complex than its phenotype. Although the D2 receptor is shown to have a direct effect on the inducement of EPS, the polymorphisms themselves in *DRD2* gene do not play a major role. Instead, they may cause EPS during medication with antipsychotics by interaction with other genes, which code drug metabolizing enzymes and other receptors, such as *CYP2D6* and *DRD3*. Moreover, the impact of polymorphisms in *DRD2* is not large enough for detection using the current method, but the effect may become obvious in specific gene-gene and gene-environment interactions. So, we cannot exclude a role of *DRD2* in further pharmacogenetic and pharmacogenomic studies.

One negative factor of the present study is that the diagnosis of the patients with EPS recruited was made after 8 weeks of treatment with chlorpromazine, which was relatively short. The variations in *DRD2* analyzed here affected late-onset EPS after long-term treatment but had little effect on early-onset EPS. Chen *et al*[28] showed that *Taq*IA polymorphism was associated with the occurrence of tardive dyskinesia after long-term treatment.

In conclusion, *DRD2* is the rational candidate gene as a predictor of the neurological adverse effects from treatment with antipsychotic drugs. However, the 4 genetic variants in the *DRD2* gene analyzed here have not been shown to play a major role in the inducement of EPS in Chinese schizophrenic patients. In our further study, more relative genes, such as *DRD3*, will be studied to clarify this and the interaction of the genes involved will also be investigated.

Acknowledgements

We are deeply grateful to all members of the families participating in this study, as well as the psychiatrists and mental health workers who helped us in identifying the families.

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