No Influence of DTNBP1 Polymorphisms on the Response to Aripiprazole

Chi-Un Pae^{a, c} Alberto Chiesa^b Laura Mandelli^b Alessandro Serretti^b^aDepartment of Psychiatry, Bucheon St. Mary's Hospital, Catholic University of Korea College of Medicine, Bucheon, Republic of Korea; ^bInstitute of Psychiatry, University of Bologna, Bologna, Italy;^cDepartment of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, N.C., USA

Key Words

Aripiprazole · Pharmacogenetics · Schizophrenia · Dysbindin gene · *DTNBP1*

Abstract

Aims: The aim of the present study was to investigate possible influences of a panel of markers in the dysbindin gene *DTNBP1* (rs3213207, rs1011313, rs2005976, rs760761 and rs2619522) on the clinical outcome and side effects associated to the treatment with aripiprazole in schizophrenic patients. **Methods:** Efficacy was assessed at baseline and weeks 1, 2, 4, 6 and 8 using the Clinical Global Impression Severity and Improvement Scales, the Brief Psychiatric Rating Scale and the Schedule for the Assessment of Negative Symptoms. Side effects were evaluated by the Simpson-Angus, Barnes Akathisia and Abnormal Involuntary Movement Scales. Multivariate analysis of covariance was used to test possible influences of single nucleotide polymorphisms on clinical and safety scores. Analysis of haplotypes was also performed. **Results:** No relevant association between *DTNBP1* variants and clinical or safety scores was observed. Additionally, haplotype analysis did not reveal any significant association with clinical and safety scores at any time as well. **Conclusion:** Our data suggest no association between the investigated alleles and genotypes in *DTNBP1* and the response to aripiprazole. However, because several limitations characterize the present study, further investigations are required.

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Introduction

Dysbindin is an evolutionarily conserved 40-kDa coiled-coil-containing protein that binds to α - and β -dystrobrevin in muscle and brain [1]. It was found to be expressed in several regions of the human brain such as the frontal cortex, temporal cortex, hippocampus, caudate, putamen, nucleus accumbens, amygdala and thalamus [2].

There is evidence of a significant role of dysbindin in schizophrenia. Levels of dysbindin mRNA as well as the expression of dysbindin in the glutamatergic terminals of hippocampal formation were found to be significantly reduced in multiple layers of the dorsolateral prefrontal cortex in patients with schizophrenia [2, 3]. These findings support a role of dysbindin in the pathophysiological mechanisms of schizophrenia via its influence on the glutamatergic signaling cascade, putatively a disrupted system in schizophrenia [4–7].

Consonant with such evidence, the gene coding for dysbindin (*DTNBP1*), located on chromosome 6 (6p24.2), has recently been recognized as one of the most eminent susceptibility genes for schizophrenia, as evidenced by a large number of independent case-control and family-based association studies in different ethnic populations [8]. Accordingly, in a former case-control association study on 908 Korean schizophrenic patients and 601 controls, we found a significant association between a haplo-

type composed by some *DTNBP1* markers and schizophrenia [9].

It has been hypothesized that genes implicated in schizophrenia, including *DTNBP1*, may also modulate the response to antischizophrenic drugs [10]. In particular, abnormal interactions among glutamatergic, dopaminergic and serotonergic circuitries may represent a pathogenic mechanism underlying schizophrenia, and they may interfere with the pharmacological action of treatments as well. Thus, an altered expression/functioning of dysbindin can also influence the response to pharmacological treatments and it poses a risk for schizophrenia.

Therefore, in the present study, we investigated the role of a panel of markers in *DTNBP1*, chosen on the basis of the previous research (rs3213207, rs1011313, rs2005976, rs760761 and rs2619522) [9], on the clinical outcome and side effects in a sample of schizophrenic patients treated with aripiprazole and followed up for 8 weeks.

Methods

Sample Description

A detailed sample description has been reported in a previous paper [11]. Briefly, out of 177 screened schizophrenic inpatients or outpatients according to DSM-IV criteria, 87 were included into the intent-to-treat sample. Details on inclusion/exclusion criteria can be found in our previous study [11]. The sociodemographic data of the intent-to-treat sample did not significantly differ from those of the initial sample (all $p > 0.05$ [11]). Patients were eligible for inclusion if their symptoms were not optimally controlled and/or if they did not tolerate current antipsychotic medications. None of them was at his/her first psychotic episode. The patients were randomly assigned to two different switching conditions of aripiprazole (10 mg): (1) simultaneous discontinuation of current antipsychotic, or (2) tapering off current antipsychotic over 4–6 weeks. The patients were then followed for 8 weeks with the original aim to assess the efficacy and tolerability of the different switching strategies. The last observation carried forward method was employed in case of missing evaluations at follow-up. Rescue benzodiazepine (less than 4 mg/day of diazepam or equivalent) and antiparkinsonian medication were allowed in case of acute extrapyramidal symptoms, but not for prophylactic use. Benzodiazepine users were not significantly different for any demographic and clinical features from non-users. The study protocol was approved by the institutional review board of Kangnam St. Mary's Hospital.

Efficacy and Safety Evaluations

Efficacy was assessed using the Clinical Global Impression Severity and Improvement Scales (CGI-S; CGI-I) [12]. Other measures of outcome employed were the Brief Psychiatric Rating Scale (BPRS) [13] and the Schedule for the Assessment of Negative Symptoms (SANS) [14]. Side effects were evaluated by means of

the Simpson-Angus Scale (SAS) for extrapyramidal symptoms [15], the Barnes Akathisia Scale (BAS) [16] and the Abnormal Involuntary Movement Scale (AIMS) [17]. All evaluators were blinded to the group allocation of patients. There was good reliability among the evaluators ($\kappa > 0.8$).

DNA Analysis

Genomic DNA was extracted from blood by standard methods and quantified. The high-throughput genotyping method using a pyrosequencer (Biotage AB, Uppsala, Sweden) was employed for genotyping 5 single nucleotide polymorphisms (SNPs; rs3213207, rs1011313, rs2005976, rs760761 and rs2619522) of the *DTNBP1* gene. PCR primers (Bioneer, Daejeon, Republic of Korea) and sequencing primers (Bioneer) used for the pyrosequencing assay were designed by the Pyrosequencing Assay Design Software, version 1 (Biotage AB), and 1 primer of each primer set was biotinylated. Information on SNPs, PCR primers and sequencing primers is listed in table 1.

Statistical Analysis

Statistical analyses were performed using the Statistica software (StatSoft) [18]. Multivariate analysis of covariance was used to test possible influences of SNPs on clinical and safety scores. With the aim of reducing possible sources of variance, we included sex and age as covariates into the multivariate analysis of covariance model. Haploview 3.2 was used to generate a linkage disequilibrium map and to test for Hardy-Weinberg equilibrium [19]. Tests for associations using multimarker haplotypes were performed using the statistics environment 'R' (<http://www.R-project.org>), version 2.8.1, package 'haplo.stats', to compare clinical and safety scores at every time among haplotypes. Sex and age were added as covariates. Permutations ($n = 10,000$) were performed to estimate the global significance of the results for all haplotype analyses and to validate the expectation-maximization values.

All p values were 2-tailed. According to a Bonferroni correction for multiple testing (7 tests), statistical significance was conservatively set at 0.007 in order to reduce the likelihood of false-positive results. With these parameters, we had a sufficient power (0.80) to detect a large effect size of 0.78, allowing to detect, for example, a difference of 0.87 points on the difference in week 1 CGI-S scores between males and females, corresponding to a variance explained of 13.7% [20].

Results

Sociodemographic Features of the Sample

The sociodemographic features and baseline assessment values such as gender, age, premedication, and clinical and safety scores at baseline are reported in table 2. No significant association with genetic variations could be observed. Overall, the outcome measures significantly improved during the study, whereas no significant difference from baseline was noted for the safety scores [11].

Table 1. Primer sequences and PCR conditions for pyrosequencing of *DTNBP1* gene

SNP name	Primer sequences	Contents of PCR reaction	PCR conditions	Absolute position (chr 6)	Distance	Major/minor allele	Position
rs3213207	forward: 5'-GCCAGTTTCCTCAAAATTCCTTTA-3' reverse: 5'-Biotin-AAGCAGACCATGTATTTGAAAAGC-3' sequencing primer: 5'-TTCCTTTCGTAAGGCC-3'	DyeMix DNA polymerase (Biostream, Suwon, Korea) 10 µl, forward and reverse primers 1.0 µl each, water 7.0 µl, DNA template 1.0 µl	94°C 5 min 94°C 30 s 56°C 30 s 72°C 30 s 72°C 7 min (40 cycles)	15,736,081	5,330	A/G	intron
rs1011313	forward: 5'-Biotin-TGACTCCTTAATTCACAGGCTACA-3' reverse: 5'-GAGGCTGTATGAACAGAGTATCGA-3' sequencing primer: 5'-TGTTTTCATGCCCACTAA-3'	DyeMix 10 µl, forward and reverse primers 0.5 µl each, water 8.0 µl, DNA template 1.0 µl	94°C 5 min 94°C 30 s 58°C 30 s 72°C 30 s 72°C 7 min (40 cycles)	15,741,411	17,370	C/T	intron
rs2005976	forward: 5'-AAACGACCGATAAGCTGAAA-3' reverse: 5'-Biotin-CCAAAGTGCTGGGATTATAGGT-3' sequencing primer: 5'-AATGAGGCCGGTCGCG-3'	DyeMix 10 µl, forward and reverse primers 0.25 µl each, water 6.3 µl, DNA template 3.2 µl	94°C 5 min 94°C 30 s 65°C 30 s 72°C 30 s 72°C 7 min (40 cycles)	15,758,781	330	G/A	intron
rs760761	forward: 5'-ACCCAATAATTTTGTGTTTTTGTCT-3' reverse: 5'-Biotin-GACCAAGTCCATTGTGTCTAATTT-3' sequencing primer: 5'-AGATATAACATCAAAAGCAC-3'	same as rs1011313	94°C 5 min 94°C 30 s 56°C 30 s 72°C 30 s 72°C 7 min (40 cycles)	15,759,111	2,517	C/T	intron
rs2619522	forward: 5'-Biotin-AATTTAAAAGCAGCAAGTAGAGGG-3' reverse: 5'-TGTTTGTGTTGGGCTCTTATGTCT-3' sequencing primer: 5'-TACCATTGGTCACCT-3'	DyeMix 10 µl, forward and reverse primers 0.3 µl each, water 6.4 µl, DNA template 3.0 µl	94°C 5 min 94°C 30 s 62.5°C 30 s 72°C 30 s 72°C 7 min (40 cycles)	15,761,628		A/C	intron

Hardy-Weinberg Equilibrium and Linkage Disequilibrium for DTNBP1

All markers were in Hardy-Weinberg equilibrium: rs3213207(A/G), $p = 1.0$; rs1011313(C/T), $p = 0.41$; rs2005976(G/A), $p = 0.51$; rs760761(C/T), $p = 0.84$, and rs2619522(A/C), $p = 0.75$. A strong linkage disequilibrium was observed among rs2005976, rs760761 and rs2619522 (fig. 1). The genotypes' and alleles' prevalence rates were consistent with those retrieved in Asian populations from international databases (<http://www.snpper.chip.org>).

Allele, Genotype and Haplotype Analysis

No significant association was found between rs1011313, rs2005976, rs760761 and rs2619522 alleles or genotypes and either clinical or safety assessments at any time during the treatment (all $p > 0.007$). The only significant association was observed for rs3213207 alleles

with BPRS scores over time (BPRS scores divided according to genotypes are shown in table 3). More in detail, subjects carrying the G allele were more likely to improve over time. Nevertheless, the finding was based on only 3 subjects, and neither genotype nor haplotype analysis replicated this association, suggesting that the result was likely influenced by a stratification bias. Additionally, haplotype analysis did not reveal any significant association with clinical and safety scores at any time (all $p > 0.007$). Even when controlling for baseline scores, no significant difference was observed.

Discussion

In the present study, we investigated the potential influence of a panel of markers in *DTNBP1* on the clinical outcome and side effects associated with antipsychotic

Fig. 1. Linkage disequilibrium for *DTNBPI* rs3213207, rs1011313, rs2005976, rs760761 and rs2619522 SNPs.

treatment. No significant association between the alleles, genotypes or haplotypes under investigation and clinical or safety score was observed, suggesting that *DTNBPI* does not influence the response to aripiprazole.

A possible explanation may rely on the mechanism of action of aripiprazole, which operates predominantly at the level of dopamine and serotonin receptors [21]. Apart from the relevance of the interaction between glutamatergic and other aminergic systems, altered dysbindin function may play a more substantial role in the efficacy of agents directly influencing glutamate neurotransmission [10].

Nevertheless, our findings have to be taken with some caution because of the preliminary nature of the study and several methodological limitations. Furthermore, the results cannot be generalized to other antipsychotic agents as well as to other psychotropic treatments. For instance, there is some evidence of an influence of *DTNBPI* on the response to antidepressants [22, 23].

A major limitation of the present study is represented by the small size of our sample, which could have limited the possibility to detect smaller effects of genetic variations on outcome scores. Indeed, we did not have sufficient power to identify small effects like those that are usually exerted by single genes. A more than twofold larger sample ($n = \text{approx. } 200$) would have been required to have sufficient power to detect smaller effect sizes of $f = 0.25$. Ethnicity should also be considered since susceptible and protective haplotypes could vary according to

Table 2. Baseline scores of the sample

Sample size, n	87
Sex, n	
Females	50 (57%)
Males	37 (43%)
Age, years	36.50 ± 9.7
Previous antipsychotic, n	
Risperidone	38 (44%)
Olanzapine	38 (44%)
Quetiapine	8 (9%)
Amisulpride	2 (2%)
Not reported	1 (1%)
CGI-S score	3.43 ± 1.2
BPRS score	22.37 ± 14.69
SANS score	21.41 ± 19.3
SAS score	10.40 ± 1.9
BAS score	0.39 ± 1.4
AIMS score	1.05 ± 3.5

Values denote means $\pm$ SD unless specified otherwise.

Table 3. Clinical baseline scores of the sample displayed according to the genotype

SNPs	BPRS scores	f	p
rs3213207	A/A: 22.19 ± 14.23 A/G: 27.66 ± 28.28	0.39	0.53
rs1011313	C/C: 23.42 ± 14.06 C/T: 21.68 ± 16.13 T/T: 17.25 ± 14.35	0.27	0.60
rs2005976	G/G: 22.56 ± 13.74 G/A: 21.75 ± 17.89 A/A: 22.56 ± 13.74	0.04	0.82
rs760761	C/C: 22.48 ± 13.78 C/T: 21.94 ± 18.47	0.02	0.89
rs2619522	A/A: 22.21 ± 13.69 A/C: 23.00 ± 18.47	0.04	0.84

Values denote means $\pm$ SD unless specified otherwise.

ethnic differences [7, 24]. For example, the minor allele frequency for rs760761 is 0.20–0.30 in Caucasians and 0.02–0.09 in Asians. Thus, we cannot exclude a possible association between *DTNBPI* and the response to aripiprazole in non-Korean samples. Furthermore, we did not investigate a number of factors potentially influencing the course of symptoms over time independently of treatment, such as prior duration of illness, number of epi-

sodes, and environmental stressors. In addition, we did not control whether systematic differences in preswitch antipsychotic dosages could result in pharmacodynamic differences at the time of the switch. This could have influenced the results, given that a higher level of preswitch dosing of D₂ antagonists could lead to a more abrupt functional hyperdopaminergia after switching to a partial agonist. Even though we did not record the dosage of prior antipsychotics at study entry, to be eligible for the present study, patients had to be treated with an antipsychotic at its standard therapeutic dose. Additionally, we cannot rule out a possible lack of treatment compliance because a formal control of drug consumption or plasma levels of aripiprazole was not implemented. Finally, the choice of SNPs was based on previous research; neverthe-

less, other variants may have a more significant effect on the expression of the gene and/or functionality of the protein.

Taking into account such restrictions, there is a preliminary indication of no influence of *DTNBP1* on the response to aripiprazole treatment in the short term. However, further research is required to establish the role of dysbindin in the efficacy and tolerability of antipsychotic treatments.

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