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Clinical response to antipsychotic drug treatment: Association study of polymorphisms in six candidate genes

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Abstract

Pharmacogenetic studies have demonstrated significant associations between several candidate genes (DRD2, DRD3, 5HTR2A and 5HTR2C, COMT and MTHFR) and antipsychotic drug response. The present study investigates the effect of nine polymorphisms in these genes for an association with antipsychotic treatment response. 329 Caucasian patients with a non-affective psychotic disorder using antipsychotics were included. All patients participated in the longitudinal GROUP-study in The Netherlands. We genotyped 9 SNPs in 6 candidate genes (DRD2: TaqI_A, -141C; DRD3: Ser9Gly; HTR2A: 102-T/C, His452Tyr; HTR2C: Cys23Ser; COMT: Val158Met;

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MTHFR: 677-C/T) using standard protocols. Polymorphisms were based on previous studies showing associations with positive symptoms treatment response. The Clinical Global Impression - Improvement (CGI-I) scale was used to assess improvement in positive psychotic symptoms since the start of current antipsychotic treatment. Ordinal regression was used for association analyses. Ninety percent of the patients used second generation antipsychotics, with olanzapine (28%) and risperidone (29%) being the most prescribed drugs. Ser9Gly of the dopamine D3 receptor gene (P value 0.034) and 677-C/T of MTHFR (P value 0.019) were tested statistically significant. Gly-carriers and T-carriers, respectively, showed more clinical improvement on the CGI-I. The other polymorphisms did not show a statistically significant association (P values>0.10). In conclusion, we replicated two out of nine of the previously reported associations between polymorphisms and treatment response. The direction and magnitude of the associations presented here in DRD3 (Ser9Gly) and MTHFR (677-C/T) are in line with previous association studies in Caucasian patients. These polymorphisms may be of value for predicting clinical response.

1. Introduction

Since their introduction in the 1950s antipsychotic drugs play a key role in the treatment of psychotic disorders. However, almost half of patients with schizophrenia display insufficient response to antipsychotic treatment and many patients show low compliance as a result (Lacro et al., 2002). In turn, this may cause clinical deterioration including psychotic relapse. Factors that influence the variation in response to antipsychotic drug treatment have not been well elucidated. Inter-individual and inter-racial variability in response to drug treatment may possibly reflect genetic heterogeneity and the presence of modifier genes (Tsuang et al., 1990). Several genetic association studies have been performed showing positive associations between response and polymorphisms in genes coding for the dopaminergic, serotonergic, and several other systems. In this study we focus on polymorphisms associated with antipsychotic drug response (positive or global symptoms) in Caucasian patients only.

Dopamine D2 receptor blockade is a property of all antipsychotics. The polymorphisms Taq1A, located downstream of the dopamine receptor D2 gene (*DRD2*), and -141C Ins/Del, located in the promoter region of *DRD2*, are linked to D2 receptor density at the level of the striatum (Jonsson et al., 1999; Laakso et al., 2005; Pohjalainen et al., 1998). Several studies have associated TaqI_A and -141C with response to first (FGA) and second generation antipsychotics (SGA) (Hwang et al., 2005; Lencz et al., 2006; Schafer et al., 2001). The affinity of certain antipsychotics for the D3 receptor may reflect a part of their action, although affinity for the D3 receptor may differ among them (Schwartz et al., 2000). The Ser9Gly polymorphism of *DRD3* is an amino-acid substitution in the N-terminal extracellular part of the receptor and might influence the membrane expression of this receptor by modifying its intracellular maturation (Reynolds et al., 2005). A positive relation between response to SGA and the presence of the Gly allele was found in three studies (Staddon et al., 2002; Szekeres et al., 2004; Scharfetter et al., 1999).

Alterations in the serotoninergic system have been implicated in the mechanisms of action of antipsychotics, having antagonistic properties (particularly SGA) on serotoninergic receptors, especially the serotonin 2A and 2C receptors. Two SNPs (His452Tyr and 102-T/C) of the *5HTR2A* (serotonin receptor 2A) gene were the subject of several association studies on the response to clozapine. Results were conflicting (Masellis et al., 1995, 1998; Malhotra et al., 1996; Joober et al., 1999; Jonsson et al., 1996; Arranz et al., 1995, 1996, 1998a, 1998b). The 102-T/C SNP does not entail amino acid substitution, but in Caucasian patients it is in complete linkage disequilibrium with variant -1438A/G, which is located in the gene promoter. The A allele of this polymorphism would increase the in-vitro activity of the promoter (Parsons et al., 2004). The His452Tyr polymorphism brings about functional modifications of the receptor, the Tyr variant would decrease the receptor's ability to activate C and D phospholipases (Hazelwood and Sanders-Bush, 2004). The serotonin 2C receptor is suggested to play a moderate role in the effect of antipsychotics on particularly negative symptoms (Sodhi et al., 1995; Reynolds et al., 2005; Arranz et al., 2000a; Lieberman et al., 1998). The Cys23Ser polymorphism of the *5HTR2C* (serotonin receptor 2C) gene was associated in one study with global response on clozapine (Sodhi et al., 1995). The consequences of the involved amino acid substitution are unknown.

COMT (catechol-O-methyltransferase) is involved in the degradation of dopamine in the prefrontal cortex. The *COMT* gene has a relevant Val158Met polymorphism (sometimes referred to as Val158/108Met). The Met allele was associated with a better response in two studies (Anttila et al., 2004; Bertolino et al., 2004). The MTHFR (methylenetetrahydrofolate reductase) enzyme is required for the synthesis of 5-MTHF, a cosubstrate for the conversion of homocysteine into methionine. Elevated plasma homocysteine concentration has been suggested as a risk factor for schizophrenia but the causality is still unclear. The *MTHFR* gene has a missense mutation 677-C/T. Patients possessing a copy of the T allele showed a better treatment response (Joober et al., 2000a).

In the present study we tested above mentioned nine polymorphisms for their association with response on positive symptoms in a sample of Caucasian patients with a psychotic disorder. We focused on polymorphisms associated with response on positive symptoms because antipsychotics are mainly effective for treating positive symptoms.

2. Experimental procedures

2.1. Sample

A sample of 329 in- and outpatients using antipsychotic medication was drawn from the participants in the ongoing longitudinal Genetic Risk and Outcome of Psychosis (GROUP) study. In GROUP, patients

were identified in selected representative geographical areas in the Netherlands and Belgium. Inclusion criteria for GROUP were: (i) age range 16 to 50 years, (ii) DSM-IV diagnosis of non-affective psychotic disorder and (iii) good command of Dutch language. For the present analysis the following extra inclusion criteria were applied: (iv) use of antipsychotic medication at the time of assessment for at least one month and (v) Caucasian ethnicity of Northern European ancestry. The study was approved by the Ethics Committee of the University Medical Center Utrecht and by the institutional review boards of all other participating hospitals. All subjects gave written informed consent in accordance with the committee's guidelines.

2.2. Genotyping

A total of 9 polymorphisms in 6 candidate genes were selected for the current study: *DRD2* TaqI_A and -141C; *DRD3* Ser9Gly; *HTR2A*: 102-T/C and His452Tyr; *HTR2C*: Cys23Ser; *COMT*: Val158Met; *MTHFR*: 677-C/T. These polymorphisms were chosen a priori based on findings in other association studies and determined by Sequenom (Hamburg, Germany) using the Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer. Quality check for genotyping was performed in the overall GROUP study, which encompassed exclusion of polymorphisms and individuals based on missingness and based on departure from Hardy–Weinberg equilibrium in a sample of 398 controls without a psychotic disorder.

2.3. Phenotyping

All patients were evaluated by their attending psychiatrist with the Clinical Global Impression - Improvement (CGI-I) scale at one point in time. This instrument was used to score the change in positive symptoms since the start of the current antipsychotic medication on a seven point scale (1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse). Response was dichotomized for some of the analyses: improvement was defined as a CGI-I score of very much improved or much improved. Attending psychiatrists were blinded to patient's genotype. Other clinical variables that were measured in this study were DSM-IV diagnosis, duration of illness, and antipsychotic medication and dose. Haloperidol dose equivalents were subsequently calculated using power formulas as outlined by Andreasen et al. (2010). To assess DSM-IV diagnosis one of the two structured diagnostic instruments was used: the Comprehensive Assessment of Symptoms and History (CASH (Andreasen et al., 1992)) or the SCAN Schedules for Clinical Assessment for Neuropsychiatry (SCAN 2.1 (Wing et al., 1990)). All raters were trained psychologist or psychiatrist, with extensive clinical experience. They had completed training in the CASH or SCAN. Diagnostic consensus was achieved in the presence of an independent psychiatrist. Psychiatric diagnosis was established according to the criteria of DSM-IV. Haloperidol dose equivalents were subsequently calculated using power formulas as outlined by Andreasen et al. (2010).

2.4. Statistical analysis

Age, duration of illness and haloperidol dose equivalents were compared between patients with and without response and were tested for statistical significance using a Mann–Whitney test or *t*-test when appropriate. We compared response between the most frequently prescribed antipsychotics and between diagnoses and tested differences for statistical significance using the χ^2 test. Ordinal regression (Scott et al., 1997), with the logit link function, was used to quantify the association between the highly skewed CGI-I score on positive symptoms and polymorphisms, while adjusting for age and gender. Sequential low prevalent outcome groups were pooled to fulfill the assumptions of ordinal regression, i.e. high enough cell counts in each genotype/outcome group (minimum of 1). The null hypothesis of parallel lines was tested for each polymorphism using χ^2 tests. We considered initially an additive model with the number of minor alleles as independent variable for all polymorphisms. When genotype/outcome group cell counts were too low, a dominant model for the polymorphism was considered. Polymorphisms positioned on the X-chromosome were tested in a dominant model only. All statistical analyses were performed using SPSS 16.0 for Windows. Since all nine polymorphisms were based on earlier positive association studies in Caucasian patients, we refrained from adjusting for the multiple testing. The significance level of this study was set at 0.05, two-sided. Replication was established when the effect was in the same direction as in the original study and statistically significant.

3. Results

Descriptive statistics of our study sample are presented in Table 1 according to response on positive symptoms. Patients with response had a two years higher age (Mann Whitney P value 0.031) and a two months longer treatment period of the current antipsychotic (Mann Whitney P value 0.039) than patients without response. Haloperidol equivalents, duration of illness, DSM-IV diagnoses and type of antipsychotic were not significantly different between response groups.

All polymorphisms were validated and had a missing genotype rate below 10% in the total sample. No polymorphism deviated from Hardy–Weinberg equilibrium. The allele and genotype frequencies are shown in Table 2. Due to the low prevalence of scores 4, 5, 6 and 7 on the CGI-I, we used three ordinal groups of response: a CGI-I score of 1, 2, and 3 or higher. Rs6314 and rs1799732 showed too low cell counts when tested additive, and were subsequently tested dominantly. The null hypothesis of parallel lines was not rejected for any of the polymorphisms tested.

In Table 3 the associations with response are depicted. Two of the nine polymorphisms showed a significant association with response on positive symptoms. The Gly (C) allele of Ser9Gly of the *DRD3* gene was associated with more improvement on positive symptoms (P value 0.034). The T-allele of 677-C/T of the *MTHFR* gene also showed more improvement (P value 0.019). Both results were in the same direction as the previous association studies reporting on these polymorphisms (Staddon et al., 2002; Szekeres et al., 2004; Scharfetter et al., 1999; Joober et al., 2000a). The Gly-allele and T-allele showed odds ratios of 1.39 (95% CI 1.03–1.90) and 1.45 (95% CI 1.06–1.98) for being in a better response category, respectively.

4. Discussion

In this study we aimed to replicate previously reported significant findings from candidate gene studies on positive symptoms improvement in patients with psychotic disorders treated with antipsychotics. Of the nine tested polymorphisms two were tested significantly: Ser9Gly of the *DRD3* gene and 677-C/T of the *MTHFR* gene.

Previous positive association studies with Ser9Gly of *DRD3* in Caucasian patients were performed in prospective studies with patients using clozapine (Scharfetter et al., 1999), olanzapine (Staddon et al., 2002), and several SGA (Szekeres et al., 2004). In all three studies the Gly allele was associated with better response. All three studies used different symptom scales and treatment duration varied between 12 weeks and 6 months. Three other studies (two clozapine and one FGA) with Caucasian patients showed no significant association

Table 1 Descriptive statistics of 329 Caucasian patients using antipsychotics, stratified by improvement on positive symptoms.

	Improvement (n=247)	No improvement (n=82)	P-value
Age (years), median (range)	27 (16–47)	25 (16–42)	0.031
Gender (male)	197 (80%)	63 (77%)	0.52
Duration of illness (years), median (range)	3.4 (0.2–21.4)	3.2 (0.2–12.0)	0.99
Duration of current treatment (months), median (range)	4 (1–60)	6 (1–110)	0.039
Current dose (haloperidol equivalents [a]), median (range)	4.8 (0.6–18.2)	6.6 (0.6–21.0)	0.33
DSM-IV diagnosis			0.45
Schizophrenia	163 (66%)	57 (70%)	
Schizo-affective disorder	37 (15%)	9 (11%)	
Schizophreniform disorder	6 (2%)	3 (4%)	
Psychotic disorder NOS	28 (11%)	9 (11%)	
Delusional disorder	6 (2%)	4 (5%)	
Other	7 (3%)	0 (0%)	
Antipsychotic			0.24
Risperidone	55 (22%)	20 (24%)	
Olanzapine	68 (28%)	12 (15%)	
Quetiapine	14 (6%)	4 (5%)	
Clozapine	22 (9%)	8 (10%)	
Haloperidol	19 (8%)	5 (6%)	
Aripiprazole	2 (1%)	3 (4%)	
Other	16 (7%)	6 (7%)	
Multiple antipsychotics	25 (10%)	12 (15%)	
Missing	26 (11%)	12 (15%)	

[a] Calculated as outlined by Andreasen et al. (2010).

(Joober et al., 2000b; Malhotra et al., 1998; Shaikh et al., 1996). In vitro studies indicate that the Gly variant is associated with higher dopamine binding affinity (Lundstrom and Turpin, 1996). However, the biological function is hard to interpret since the Gly variant was mostly associated with poor response in Chinese populations (Reynolds et al., 2005; Lane et al., 2005). This different direction of association with response may be explained by a linkage disequilibrium status with another functional polymorphism.

Only one study has been performed regarding 677-C/T of *MTHFR* and antipsychotic response (Joober et al., 2000a). The T allele was found more often in responders compared to non-responders, all having FGA. The 677-C/T polymorphism is functional (Frosst et al., 1995) and is possibly associated with risk of schizophrenia itself (Shi et al., 2008; Allen et al., 2008). MTHFR is required for the synthesis of 5-methyltetrahydrofolate, the primary circulatory form of folate and the carbon donor for homocysteine remethylation to methionine. Homozygous individuals (TT) have around one third of the expected MTHFR enzyme activity, and heterozygotes (CT) have around two third activity, compared to the most common genotype CC (Frosst et al., 1995). Some patients with homocysteinuria, an inborn error of metabolism that can be caused by rare and severe mutations in the MTHFR gene, have exhibited schizophrenia-like symptoms (Freeman et al., 1975). Furthermore, high levels of homocysteine have been

Table 2 Frequencies of alleles and genotypes of polymorphisms in 329 Caucasian patients with a non-affective psychotic disorder.

Gene	rs-id	Chromosome position	Variant name	Alleles [a]	MAF [b]	Genotypes		
						11	12	22
DRD2	rs1800497	Chr11:113270827	TaqI_A	C/T	0.17	228 (69%)	89 (27%)	12 (4%)
	rs1799732	Chri11:113346252	-141C	C/Del	0.11	262 (80%)	60 (18%)	5 (2%)
DRD3	rs6280	Chr3:113890814	Ser9Gly	T/C	0.32	150 (46%)	139 (43%)	36 (11%)
HTR2A	rs6314	Chr13:47409033	His452Tyr	C/T	0.1	255 (80%)	59 (19%)	3 (1%)
	rs6311	Chrl3:47471477	102-T/C	C/T	0.45	92 (28%)	177(54%)	59 (18%)
HTR2C	rs3813929	X:113818519	-759C_T	C/T	0.18	244 (83%)	–	49 (17%) [c]
	rs6318	X:113965734	Cys23Ser	G/C	0.16	249 (84%)	–	48 (16%) [c]
COMT	rs4680	Chr22:19951270	Val158Met	A/G	0.46	91 (28%)	169 (52%)	68 (21%)
MTHFR	rs1801133	Chrl:11856378	677-C/T	C/T	0.32	153 (47%)	141 (43%)	35 (11%)

[a] Major allele given first.
[b] MAF = minor allele frequency.
[c] Carriers of variant allele, polymorphism positioned on X-chromosome.

Table 3 Results of association analyses between polymorphisms and clinical improvement on positive symptoms in 329 Caucasian patients with a non-affective psychotic disorder.

				Ordinal regression [a]		
Gene	rs-id	Variant name	Alleles [b]	Beta	Odds ratio	P-value
DRD2	rs1800497	TaqI_A	C/T	−0.24	0.79	0.21
	rs1799732	-141C	C/Del	−0.36	0.70	0.17
DRD3	rs6280	Ser9Gly	T/C	**−0.33**	**0.72**	**0.034**
HTR2A	rs6314	His452Tyr	C/T	−0.17	0.84	0.53
	rs6311	T102C	C/T	0.10	1.11	0.53
HTR2C [c]	rs3813929	-759C_T	C/T	0.08	1.08	0.79
	rs6318	Cys23Ser	G/C	−0.47	0.63	0.13
COMT	rs4680	Val158Met	A/G	0.08	1.08	0.62
MTHFR	rs1801133	677-C/T	C/T	**−0.37**	**0.69**	**0.019**

[a] Ordinal regression analysis with CGI-I improvement as dependent variable, and the number of minor alleles (0, 1 or 2) of the polymorphism as independent variable (additive model), corrected for age and gender. A negative beta or odds ratio<1 means more improvement (lower CGI score) per extra minor allele. Significant results ($p<0.05$) are shown in bold.
[b] Major allele given first.
[c] Polymorphisms tested in a dominant model.

observed in schizophrenia patients (Regland et al., 1995). Several reports link a high plasma level of homocysteine to various neurological conditions also, such as pregnancies complicated by neural tube defects (Bakker and Brandjes, 1997) and migraine (Oterino et al., 2010). Together with the association we replicated, this points out that MTHFR may be involved in the interaction with antipsychotic medication.

In this study we did not replicate seven of the previously associated polymorphisms with response on positive symptoms. When a more conservative correction for multiple testing was applied, such as Bonferroni, even the two replications would not be significant. An important question regards the explanation of our findings. A likely explanation for the variation in results from pharmacogenetic studies is the heterogeneity in the definition and characterization of the phenotype as well as in the characterization of the genetic variability. This concerns the present study as well. An important difference of our study from other studies is the time between start of the medication and assessment of improvement. The time of assessment is variable in our study, because we measured CGI-I cross-sectionally in our cohort. However, since most patients show response in the first month after the start of an antipsychotic (Derks et al., 2010) we consider our method valid for pharmacogenetic purposes. We have a relatively big group of patients showing improvement, what can be expected when response is cross-sectionally measured. Patients who do not respond well are less likely to continue an antipsychotic and will eventually switch to another antipsychotic. This might have underestimated the results, because we have a smaller range of response outcome. In addition, due to the cross-sectional nature of our study we had to use a relatively generic measure, in this case the CGI-I, to assess response to antipsychotics. This approach might be responsible for a relatively imprecise measurement of the true response. To perform a true replication study, all methodologies and patient characteristics should be identical. This is however not possible when testing different polymorphisms at once, all tested before in different studies with different approaches. Another difference between pharmacogenetic studies on antipsychotics (including ours) is the inclusion of all antipsychotics as opposed to focusing on a single antipsychotic. Different drug types, treatment duration and response assessment methods may significantly alter study results (Arranz et al., 2000b). We believe that this is a fairly homogeneous group of patients, all with a non-affective psychotic disorder. However, it might very well be possible that pharmacogenetic associations still differ between the diagnoses we included. Of course publication bias might be an important contributing factor as well, i.e. positive results have a better chance of being published than negative results. A combination of above mentioned aspects is probably the reason why most pharmacogenetic association results regarding response on antipsychotics are conflicting: positive associations are often not replicated. Apart from the limitations of the cross-sectional design we used, our study has also several strengths. Compared to most other candidate studies we have a relatively large sample size of more than 300 patients using antipsychotics. Our study does not suffer from heterogeneity with regard to ethnicity as we have a homogeneous group of Caucasian patients of Northern European ancestry.

In conclusion, two of the previously reported associations between polymorphisms and treatment response were replicated in the present study. Heterogeneity in patient samples and outcome variables as well as publication bias may all play a role in lack of replication, found in our study, as in others. The direction of the associations presented here in *DRD3* (Ser9Gly) and *MTHFR* (677-C/T) is in line with previous association studies in Caucasian patients. These polymorphisms may be of clinical value if their added value to other clinical predictors of response can be demonstrated in future research.

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ZonMw – or any of the Mental Health Care organizations – had no further role in the study design; in the analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

All authors designed the study. The GROUP investigators wrote the study protocol. Author Vehof managed the literature searches (together with GROUP investigators and Al Hadithy) and statistical analyses (together with Burger, Snieder and Alizadeh). All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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