CHAPTER 5

PHARMACOGENETICS OF PARKINSONISM, RIGIDITY, REST TREMOR, AND BRADYKINESIA IN AFRICAN-CARIBBEAN INPATIENTS: DIFFERENCES IN ASSOCIATION WITH DOPAMINE AND SEROTONIN RECEPTORS

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ABSTRACT

Background: To study the association between polymorphisms of genes coding for dopamine D2 (DRD2), dopamine D3 (DRD3), serotonin 2A (HTR2A), and serotonin 2C (HTR2C) receptors and Antipsychotic-Induced Parkinsonism (AIP), rigidity, bradykinesia, and rest-tremor in African-Caribbeans treated with antipsychotics.

Methods: Polymorphisms of DRD2 (-141CIns/Del, TaqIA, 957C>T), DRD3 (Ser9Gly), HTR2A (-1438A>G, 102T>C, His452Tyr), and HTR2C (-759C>T, Cys23Ser) genes were determined according to standard protocols. The Unified Parkinson Disease Rating Scale was used for the measurement of AIP, rigidity, bradykinesia, and rest-tremor. Chi-squared or Fisher's exact tests were applied for the association analyses. The t-test was applied for continuous data.

Results: 99 males and 27 females met the inclusion criteria (Schizophr.Res.1996,19:195). In males, but not in females, there were significant associations between -141CDel-allele carriership (DRD2) and rigidity (Fisher's Exact Test: p=0.021) and between 23Ser-allele carriership (HTR2C) and bradykinesia (p=0.026, χ^2=5.0) or AIP (p=0.008, χ^2=7.1). Rest-tremor was not associated with any of the polymorphisms studied. Analyses of the age, chlorpromazine equivalents, benztropine equivalents, the number of patients using anticholinergic medication, and the utilization patterns of the antipsychotic medication did not show statistically significant differences between patients with and without AIP, rigidity, bradykinesia, rest-tremor. Conducting the analysis without gender stratification did not affect our findings considerably, except for the association between bradykinesia and 23Ser-allele which failed to reach statistical significance in the total sample (p=0.0646, χ^2=3.41).

Conclusions: Since AIP's subsymptoms (rigidity, bradykinesia, and rest-tremor) may differ pharmacogenetically, our data strongly support symptom-specific analysis of AIP. However, further research is warranted to confirm our findings.

INTRODUCTION

Antipsychotic-induced parkinsonism (AIP) is an acute movement disorder that may appear in 15-40% of patients within the first few weeks following the start of an antipsychotic and may adversely affect therapeutic compliance, self-esteem, and quality of life [Fleischhacker et al., 1994; Gerlach 1999; Hirose 2006; Hofer et al., 2004].

Several risk factors have been suggested to predispose to AIP, such as old age, female gender, and high doses of antipsychotics [Caligiuri et al., 1999; Caligiuri et al., 2000; Ebadi and Srinivasan 1995; Hirose 2006; Jabs et al., 2003; Metzer et al., 1989]. However, these factors only partially explain the variance in the occurrence of AIP and hereditary predisposition is, therefore, possible [Galdi et al., 1981; Lencer et al., 2004].

Although the pharmacogenetics of movement disorders has been examined extensively, many studies tend to generalize multiple forms of drug-induced movement disorders (e.g., tardive dyskinesia, AIP, akathesia, dystonia) as being one single clinical syndrome [Armstrong et al., 1997; Gunes et al., 2007; Guzey et al., 2007; Inada et al., 1999; Nakazono et al., 2005]. It is however plausible that these movement disorders do differ in their genetic liability, since each movement disorder has a distinct clinical presentation, time to onset, prognosis, and medical management [Trosch 2004].

AIP may be caused by the antagonistic effects of antipsychotics on nigrostriatal dopamine D2 receptors (DRD2 gene) [Gerlach 1999; Lidow 2000; Reynolds 2004], which are modulated by serotonin 2A and 2C receptors (HTR2A and HTR2C genes, respectively) [Alex et al., 2005; Di et al., 2006; Haleem 2006; Lidow 2000].

DRD2, HTR2A, and HTR2C are therefore plausible candidate genes for the study of the pharmacogenetics of AIP. Studies focusing on HTR2A and HTR2C pharmacogenetics and AIP are currently scarce [Gunes et al., 2007; Hamdani et al., 2005]. Furthermore, there are currently only a few studies evaluating extensive sets of DRD2-polymorphisms in connection with AIP [Kaiser et al., 2002; Nakazono et al., 2005; Wu et al., 2006] and none were conducted in Negroid patients.

In contrast to akathisia [Eichhammer et al., 2000] and tardive dyskinesia [Bakker et al., 2006; Lerer et al., 2002; Segman et al., 2000], the pharmacogenetics of dopamine D3 receptor (DRD3 gene) and AIP is still poorly understood [Chong et al., 2003; Gunes et al., 2007; Guzey et al., 2007].

The goal of the present study is to investigate the association between several polymorphisms of DRD2, DRD3, HTR2A, and HTR2C genes and AIP and three of its subsymptoms (rigidity, bradykinesia, and rest-tremor) in African-Caribbean inpatients on chronic antipsychotic treatment.

Currently, there are no studies published on the pharmacogenetics of AIP in Negroid populations. Since African-Caribbean subjects may have ethnic roots similar to those

of native Africans [Page 1997], the present study may also be of relevance for other Negroid populations.

METHODS

Subjects

In this study we utilized data obtained from predominantly African-Caribbean subjects (Negroid or Mixed), who previously had participated in an epidemiological study on antipsychotic-induced movement disorders [van Harten et al., 1996]. All of the study subjects were inpatients from the Dr. D.R. Capriles Clinic (Curaçao, Netherlands Antilles) and had received nearly all of their psychiatric care from that hospital.

The study protocol was approved by the Curaçao institutional review board. Informed consent was obtained from each patient after full explanation of the purpose of the study and subjects were requested to provide peripheral blood for DNA genotyping.

Patients were included in this pharmacogentic study regardless the presence or absence of AIP and regardless the type or severity of their mental illness. Inclusion criteria were: (i) absence of organic and neurological disorders that could cause movement disorders, (ii) a history of neuroleptic use for at least three months, and (iii) informed consent.

Clinical data

AIP and its subsymptoms were measured with the motor examination part of the Unified Parkinson Disease Rating Scale [Martinez-Martin et al., 1994]. All of the ratings were dichotomized in presence/absence of AIP, rigidity, rest-tremor. Since rest-tremor and rigidity are typical of AIP, a ‘mild’ involvement on one of these items led to case definition [van Harten et al., 1996]. If no tremor or rigidity was present, then the cut-off point for the presence of AIP was at least one ‘moderate’ or two ‘mild’ scores on the other items [van Harten et al., 1996].

To reduce the risk of false positives, we chose to categorize the presence/absence of bradykinesia by the use of a more stringent cut-off point of at least one ‘moderate’ or two ‘mild’ scores on the items speech, facial expression, hand movements, alternating hands, foot agility, arising from chair, posture, gait, postural stability, body bradykinesia and hypokinesia.

Furthermore, two junior medical doctors, who were not aware of the existence of movement disorders in the subjects, assessed patients’ medical files for information on the type, dose, and duration of the antipsychotic treatment as well as other co-medications (anticholinergics and benzodiazepines).

The dose of the antipsychotic medication was converted into chlorpromazine equivalents and the dose of anticholinergics into benztropine equivalents [Davis 1976; Moleman 1992].

DNA genotyping

Genomic DNA was extracted from EDTA whole-blood samples [Miller et al., 1988]. We genotyped the patients for several polymorphisms of DRD2 (-141CIns/Del, TaqIA, 957C>T), DRD3 (Ser9Gly), HTR2A (-1438A>G, 102T>C, His452Tyr), and HTR2C (-759C>T, Cys23Ser) genes. The genotyping was conducted blind to the clinical status of the patients.

Fluorogenic 5'-exonuclease TaqMan® assays were applied for the determination of all of the polymorphisms except three polymorphisms (102T>C, Cys23Ser, and Ser9Gly), which were determined by standard restriction fragment length polymorphism (RFLP) protocols [Ebstein et al., 1997; Lannfelt et al., 1992; Warren, Jr. et al., 1993]. All of the TaqMan–assays, except one (-141C Ins/Del), were ordered from Applied Biosystems (Nieuwerkerk aan den IJssel, the Netherlands) as Assay-On-Demand. The -141C Ins/Del polymorphism was performed by the use of user-designed TaqMan primers and probes, kindly provided by Xu et al. [2004].

In relation with the 957C>T polymorphism of DRD2 gene [Hirvonen et al., 2004], we genotyped additionally any subject with the homozygous 957TT genotype for the 1101G>A polymorphism of DRD2, since it has been reported that the effects of the 957T-allele are annulated in presence of the 1101A-allele [Duan et al., 2003]. The genotyping of 1101G>A polymorphism was conducted by a PCR-RFLP protocol, as kindly provided by Hirvonen et al. [2004].

Statistics

The associations between allele-positivity (allele carriership status) and AIP, rigidity, bradykinesia, and rest-tremor were analyzed by the use of Chi-squared or Fisher´s exact tests (2x2 contingency tables). Chi-squared or Fisher´s exact tests were also applied for the analyses of other dichotomous variables (e.g., anticholinergics and polypharmacy use).

Logistic regression was applied for the calculation of the Odds Ratio (OR) and adjusted OR (OR_adj) for polymorphisms with a significant association. For analyses of age, chlorpromazine- and benztropine equivalents the t-test was applied. The analyses were conducted twice; with and without gender-stratification. P-values < 0.050 were regarded as significant. In our study we endeavored to replicate data from previous studies (some of which not reporting an association) rather than to explore new genetic targets. Since our approach is hypothesis driven, we did not correct for multiple testing.

Departure from Hardy-Weinberg Equilibrium was calculated for all polymorphisms except those of the X–chromosomal HTR2C gene. An online tool was applied for the Chi-square goodness-of-
fit test (http://www.kursus.kvl.dk/shares/vetgen/_Popgen
/genetik/applets/kitest.htm; accessed October 28th 2007).

RESULTS

Demographic and clinical features of the subjects

One hundred and twenty six African-Caribbean subjects (99 males and 27 females) met the inclusion criteria. Table 1 presents the distribution (mean ± standard deviation) of age (years), DSM-III-R diagnosis, ethnicity, polypharmacy (number of patients using no, single or multiple antipsychotics simultaneously), lifetime exposure to antipsychotics (kilogram chlorpromazine equivalents), age on first neuroleptic use (years), daily dose of antipsychotics (mg/day chlorpromazine equivalents) and anticholinergics (mg/day benztropine equivalents) as well as the number and percentage of subjects using anticholinergic medication. Table 1 also presents the number of cases of AIP, rigidity, bradykinesia, and rest-tremor in the total sample and per gender.

Table 1. The distribution of age, diagnosis (DSM-III-R), ethnicity, polypharmacy, daily use of antipsychotics (mg/day chlorpromazine equivalents, CPZeq) on the day of assessment of the UPDRS, lifetime exposure to antipsychotics (kg CPZeq), age first neurolerptic use (years), daily use of anticholinergics (mg/day benztropine equivalents, BNZeq) on the day of assessment of the UPDRS, and the number (n) and percentage (%) of subjects using anticholinergics or benzodiazepines as well as AIP, rigidity, bradykinesia, and rest-tremor cases.

	All (126)	Male (99)	Female (27)
Age (years)	49.2±13.4	47.5±13.0	55.4±13.3
Diagnoses [a, b]**, n (%)**			
Schizophrenia [c]	88 (80.7)		
Affective disorder	5 (4.6)	-	-
Dementia	3 (2.8)		
Other	13 (11.9)		
Ethnicity, n (%) [d]			
Negroid or Mixed	108 (95.6)		
Caucasian	3 (2.7)	-	-
Other	2 (1.8)		

	All (126)	Male (99)	Female (27)
Polypharmacy, number of patients using:			
0 antipsychotics	12		
1 antipsychotic	85	-	-
2 antipsychotics	27		
3 antipsychotics	2		
Daily use of antipsychotics (mg CPZeq/day)	686.0±740.6	692.4±676.8	662.5±953.8
Lifetime exposure to antipsychotics (kg CPZeq) [e]	3.9±3.2	3.9±3.2	4.0±3.2
Age first neurolerptic use (years) [e]	29.8±10.9	28.6±10.2	33.7±12.6
Daily use of anticholinergics (mg BNZeq/day)	1.4±2.1	1.6±2.2	0.7±1.4
Patients on anticholinergics, N (%)	49 (38.9%)	42 (33.3%)	7 (5.6%)
Patients on benzodiazepines, N (%)	28 (22.2%)	23 (23.2%)	5 (18.5%)
AIP present, n (%)	47 (37.3)	35 (35.4)	12 (44.4)
RIG present, n (%)	14 (11.1)	11 (11.1)	3 (11.1)
TREM present, n (%)	21 (16.6)	18 (18.2)	3 (11.1)
BRAD present, n (%)	33 (26.2)	23 (23.2)	10 (37.0)

[a] Data from 109 patients;
[b] A patient can have several diagnosis, thus the total number exceeds 100%;
[c] Includes 295.1, 295.2, 295.3, 295.4, 295.6, 296.7, 295.9;
[d] Data from 113 patients;
[e] Data from 93 patients (72 males + 21 females).

Genotype distribution, Hardy-Weinberg Equilibrium (HWE), and allele-carriership frequencies

None of the polymorphisms tested deviated from the HWE (Table 2). For some polymorphisms we failed to genotype 1 (DRD2 -141CIns/Del, HTR2A His452Tyr and -1438G>A) or 2 (HTR2C -759C>T) DNA samples, due to insufficient DNA quality or quantity.

Of the 5 subjects with the homozygous 957TT genotype (DRD2 957C>T polymorphism), none had the 1101AA genotype (DRD2 1101G>A).

Since we found in the total sample that for some polymorphisms the frequency of homozygote subjects could be as low as 3.2%, and becomes even lower after stratification by gender, we chose to characterize subjects either as carriers or non-

carriers of an allele, if they were heterozygote, hemizygote, or homozygote for that particular allele. However, it should be noted that this conservative approach does not reflect any specific hypothesis regarding the mode of inheritance (i.e. we do not suggest a dominant inheritance mode).

Genotype and allele-carriership frequencies are shown in Table 2.

Table 2. Genotype distribution, Hardy-Weinberg Equilibrium Chi-squared (χ^2) values, the number (n) and percentage of allele carriers.

Genotype	% of total sample (n)	χ^2 value	Genetic variation	Total sample, % (n)	Males, % (n)	Females, % (n)
DRD2 -141CIns/Del						
Ins/Ins	42.4 (53)					
Ins/Del	45.6 (57)	0.003	*carriers of the -141CDel allele*	57.6 (72)	56.6 (56)	61.5 (16)
Del/Del	12.0 (15)					
DRD2 *Taq*IA						
A2/A2	38.1 (48)					
A2/A1	46.8 (59)	0.016	*carriers of the TaqIA A1 allele*	61.9 (78)	59.6 (59)	70.4 (19)
A1/A1	15.1 (19)					
DRD2 957C>T						
957C/C	68.3 (86)					
957C/T	27.8 (35)	0.356	*carriers of 957T allele*	31.8 (40)	33.3 (33)	25.9 (7)
957T/T	4.0 (5)					
DRD3 Ser9Gly						
Gly9/Gly9	39.7 (50)					
Gly9/Ser9	46.8 (59)	0.004	*carriers of the 9Ser allele*	60.3 (76)	60.6 (60)	59.3 (16)
Ser9/ Ser9	13.5 (17)					
HTR2A -1438G>A						
-1438G/G	44.8 (56)					
-1438G/A	43.2 (54)	0.127	*carriers of the -1438A allele*	55.2 (69)	52.5 (52)	65.4 (17)
-1438A/A	12.0 (15)					

Table 2. Continued.

Genotype	% of total sample (n)	χ^2 value	Genetic variation	Total sample, % (n)	Males, % (n)	Females, % (n)
HTR2A His452Tyr						
His/His	76.0 (95)					
HisTyr	20.8 (26)	1.651	*carriers of the 452Tyr allele*	24.0 (30)	28.3 (28)	7.7 (2)
Tyr/Tyr	3.2 (4)					
HTR2A 102C>T						
102C/C	42.1 (53)					
102C/T	49.2 (62)	1.446	*carriers of the 102T allele*	57.9 (73)	54.5 (54)	70.4 (19)
102T/T	8.7 (11)					
HTR2C -759C>T						
-759C/C	96.8 (120)					
-759C/T	0.0 (0)	–	*carriers of the -759T allele*	3.2 (4)	4.0 (4)	0.0 (0)
-759T/T	3.2 (4)					
HTR2C Cys23Ser						
Cys23/Cys23	61.9 (78)					
Cys23/Ser23	11.1 (14)	–	*carriers of the 23Ser allele*	38.1 (48)	29.3 (29)	70.4 (19)
Ser23/Ser23	27.0 (34)					

Comparison of allele-carriership frequencies in cases and non-cases

AIP

As shown in Table 3, χ^2 test in the total sample indicates significant associations between AIP and HTR2C 23Ser-allele carriership (χ^2=5.35, p=0.021). All other polymorphisms were not significantly associated with AIP (data not shown).

The frequency of AIP in patients carrying the 23Ser-allele of HTR2C gene is 1.7 times higher than in non-carriers (50.0 vs. 29.5%). Furthermore, the OR for having AIP in 23Ser-allele carriers was significant (OR=2.39, p=0.022). Adjustment of the OR for age, chlorpromazine- and benztropine equivalents daily used, did not affect the results adversely (OR_adj=2.61, p=0.017).

After gender stratification, the association between carriership of 23Ser-allele and AIP remained significant in males (χ^2=7.05, p=0.008), but not in females (Fisher's Exact Test: p=1.000). The OR and OR_adj were also significant in males (OR=3.30, p=0.009 and OR_adj=3.91, p=0.005), but not females (OR=0.73, p=0.707 and OR_adj=0.75, p=0.763).

Rigidity

As shown in Table 3, χ^2 test in the total sample indicates significant associations between rigidity and DRD2 -141CDel-allele carriership (χ^2=8.02, p=0.005). All other polymorphisms were not significantly associated with rigidity (data not shown). The frequency of rigidity in patients carrying the -141CDel allele of DRD2 gene was 9.5 times higher, compared to non-carriers (18.1 vs. 1.9%). The OR and OR_adj for having rigidity in -141CDel carriers was significant (OR=11.46, p=0.021 and OR_adj=13.21, p=0.018).

After gender stratification, Fisher's Exact Test indicated a significant association between carriership of -141CDel-allele and rigidity in males (p=0.021), but not in females (p=0.262).

The OR and OR_adj were also significant in males (OR=9.13, p=0.039 and OR_adj=8.98, p=0.049), but not females (OR, OR_adj, and p values not rateable).

Bradykinesia

As shown in Table 3, χ^2 test in the total sample indicated a non-significant trend towards an associations between bradykinesia and HTR2C 23Ser-allele carriership (χ^2=3.41, p=0.065, OR=2.13, p=0.067 and OR_adj 2.27, p=0.054). In the total sample, the frequency of bradykinesia in 23Ser-allele carriers was 1.7 times higher than in non-carriers (35.4 vs. 20.5%). All other polymorphisms were not

significantly associated with bradykinesia (data not shown). After gender stratification, the association between carriership of 23Ser-allele and bradykinesia became significant in males (χ^2=5.0, p=0.026, OR=2.95, p=0.029 and OR_adj=3.40, p=0.018), but not in females (Fisher's Exact Test: p=0.415, OR=0.46, p=0.370 and OR_adj=0.44, p=0.380).

Table 3. Cross tabulation of Antipsychotic-Induced Parkinsonism (AIP), Rigidity, and Bradykinesia in relation with 23Ser (HTR2C), -141CDel (DRD2), and 23Ser (HTR2C) allele carriership, respectively.

Genetic Variation	Symptom		Significance
	Antipsychotic-Induced Parkinsonism		
Total sample (n=126)	**AIP=no, n (%)**	**AIP=yes, n (%)**	
23Ser non-carriership	55 (70.5)	23 (29.5)	**χ^2=5.35; p=0.021**
23Ser carriership	24 (50.0)	24 (50.0)	**OR=2.39 [CI: 1.13-5.04]; p=0.022** **OR_adj=2.61 [CI: 1.19-5.74]; p=0.017**
MALES (n=99)			
23Ser non-carriership	51 (72.9)	19 (27.1)	**χ^2=7.05 ; p=0.008**
23Ser carriership	13 (44.8)	16 (55.2)	**OR=3.30 [CI: 1.34-8.14]; p=0.009** **OR_adj=3.91 [CI: 1.50-10.23]; p=0.005**
FEMALES (n=27)			
23Ser non-carriership	4 (50.0)	4 (50.0)	Fisher's Exact Test: p=1.000
23Ser carriership	11 (57.9)	8 (42.1)	OR=0.73 [CI: 0.14-3.82; p=0.707 OR_adj=0.75 [CI:0.12-4.87]; p=0.763
	Rigidity		
TOTAL SAMPLE (n=125)	**Rigidity=no, n (%)**	**Rigidity=yes, n (%)**	
-141CDel non-carriership	52 (98.1)	1 (1.9)	**χ^2=8.02; p=0.005**
-141CDel carriership	59 (81.9)	13 (18.1)	**OR=11.46 [CI: 1.45 -90.60]; p=0.021** **OR_adj=13.21 [CI: 1.57-111.07]; p=0.018**
MALES (n=99)			
-141CDel non-carriership	42 (97.7)	1 (2.3)	**Fisher's Exact Test: p=0.021**
-141CDel carriership	46 (82.14)	10 (17.9)	**OR=9.13; [CI: 1.12-74.36]; p=0.039** **OR_adj=8.98 [CI: 1.01-79.52]; p=0.049**
FEMALES (n=26)			
-141CDel non-carriership	10 (100.0)	0 (0.0)	Fisher's Exact Test: p=0.262
-141CDel carriership	13 (81.2)	3 (18.8)	Infinite OR, OR_adj, and p values due to many zero values

Table 3. Continued.

Genetic Variation	Symptom		Significance
		Bradykinesia	
TOTAL SAMPLE (n=126)	**Bradykinesia= no, n (%)**	**Bradykinesia=yes, n (%)**	
23Ser non-carriership	62 (79.5)	16 (20.5)	χ^2=3.41; p=0.065 OR=2.13 [CI: 0.95-4.77]; p=0.067 OR_adj=2.27 [CI: 0.99-5.21]; p=0.054
23Ser carriership	31 (64.6)	17 (35.4)	
MALES (n=99)			
23Ser non-carriership	58 (82.9)	12 (17.1)	**χ^2=5.0 ; p=0.026** **OR=2.95 [CI: 1.12-7.82]; p=0.029** **OR_adj=3.40 [CI: 1.23-9.41]; p=0.018**
23Ser carriership	18 (62.1)	11 (37.9)	
FEMALES (n=27)			
23Ser non-carriership	4 (50.0)	4 (50.0)	Fisher's Exact Test: p= 0.415 OR=0.46 [CI: 0.09-2.50]; p=0.370 OR_adj=0.44 [CI: 0.07-2.79]; p=0.380
23Ser carriership	13 (68.4)	6 (31.6)	

Significant results are printed in bold. CI, 95% confidence interval; n, number of subjects; %, percentage within carriership status; OR, Odds Ratio; OR_adj, Odds Ratio adjusted for age, dose of the antipsychotic medication, and dose of anticholinergic medication.

Rest-tremor

Rest-tremor was not associated with any of the polymorphisms studied, as determined by χ^2 test, Fisher's Exact Test, and logistic regression (data not shown).

Comparison of age and the use of antipsychotics, anticholinergics and benzodiazepines

Mean age (years), mean chlorpromazine and benztropine equivalents (mg/day), and the proportion of patients using anticholinergic medication did not differ significantly neither between subjects with and without AIP, rigidity, bradykinesia, and tremor nor between carriers and non-carriers of 23Ser (HTR2C) and -141CDel (DRD2) alleles (data not shown). Furthermore, the frequency of patients using benzodiazepines did not differ significantly between cases (18.2%) and non-cases (23.7%) of bradykinesia (χ^2=0.42, p=0.516) or between carriers (18.8%) and non- carriers (24.4%) of 23Ser allele of the Cys23Ser polymorphism (χ^2=0.54, p=0.462).

Gender stratification did not alter any of the abovementioned findings (data not shown), except for the male DRD2 -141CDel allele non-carriers who had a significantly (p=0.014) higher mean chlorpromazine equivalents than male carriers of that allele (892 versus 539 mg/day, respectively).

Furthermore, we analyzed the type of the antipsychotics used. Most of the patients (n=114) used antipsychotics on the day of examination. Of whom, 7 patients were using an atypical antipsychotic as monotherapy (risperidone in all cases), and 1 patient was using risperidone in combination with a classical neuroleptic.

On the day of examination 75% of the users of antipsychotics were on monotherapy and 25% used 2 or more antipsychotics simultaneously. Of the patients using 2 or more antipsychotics, only two were using 3 antipsychotics simultaneously. To test whether polypharmacy has affected our finding, we applied 2x2 contingency tables to compare subjects without prescribed antipyschotics on the day of UPDRS-assessment versus those using at least 1 antipsychotic as well as those using 2 or 3 antipsychotics versus those with 1 or none antipsychotics in relation to AIP, rigidity, tremor, bradykinesia, and carriership of the Cys23Ser and -141C Ins/Del polymorphisms (HTR2C and DRD2 genes, respectively). In all of the comparisons made, we do not find any significant difference in the utilization patterns of the antipsychotic medication (data not shown).

DISCUSSION

The present study suggests that rigidity, bradykinesia, and rest-tremor may have different genetic vulnerability, because these neurological phenomena exhibited

differences in their association with the polymorphisms studied. We found for example in males that the Cys23Ser (HTR2C) and the -141CIns/Del (DRD2) polymorphisms are associated with bradykinesia, AIP and rigidity, respectively, but none is associated with rest-tremor. This symptom-specific relationship may probably reflect a difference in the genetic predisposition for the different parkinsonian phenomena. Of the published pharmacogenetic studies dealing with AIP as a discrete clinical entity only few [Mihara et al., 2000; Mihara et al., 2001] dissect AIP into its subsymptoms (e.g., rigidity, tremor, bradykinesia, etc.).

In fact, it is plausible that some of these symptoms have distinct neurological circuits, etiology, pathophysiology, and/or genetic liability. For example it has been shown that stimulation of certain brain regions in patients with Parkinson Disease (which has symptoms similar to those of AIP) may lead to differential effects on rigidity and tremor [Bejjani et al., 1997; Gross et al., 1999; Krack et al., 1998]. Furthermore, the symptomatic treatment of the Parkinson Disease is symptom-dependent [Koller 1992; Siemers 1992]. Anticholinergic preparations for instance are generally considered effective for tremor and rigidity but not for bradykinesia, which is better treated with Levodopa.

Recent work [Lerer et al., 2005] on another movement disorder (tardive dyskinesia) supports this approach too, because two subclasses of tardive dyskinesia (orofacial- and limbotruncal tardive dyskinesias) do differ in their association with particular genetic markers.

Moreover, examination of the published papers in this field reveals that the majority of the pharmacogenetic papers do not even assess AIP as a discrete clinical syndrome, but rather pool this syndrome with other antipsychotic-induced movement disorders. Although there is some overlap between the different types of antipsychotic-induced movement disorders (tardive dyskinesia, AIP, akathisia, and dystonia) [van Harten et al., 1997], these different types of movement disorders do differ considerably from each other in many points [Trosch 2004]. Pooling these different types of movement disorders may, therefore, be detrimental for the analyses.

The significant associations observed in this study were exhibited in males, but not in females. Although gender-related effects can not be excluded, the lack of associations in the female sample is probably due to the small number of female subjects included in our study (i.e., insufficient power).

In the current study we find a significant association between bradykinesia or AIP and HTR2C Cys23Ser polymorphism, which -although debated [Fentress et al., 2005; Jonsson et al., 2004]- has been suggested to be functional [Okada et al., 2004]. This finding is completely in line with the findings of the only other published study [Gunes et al., 2007]. In our sample, the minor-allele frequency of the Cys23Ser polymorphism (Ser allele) was 0.26, which is higher than that of Asians (0.03) and Europeans (0.16) (www.genecards.org; accessed October 28th 2007).

In relation with HTR2A gene, we find no evidence for any association with its polymorphisms (-1438A>G, 102T>C, and His452Tyr). This lack of association

between AIP and -1438A>G polymorphism has also been reported by Hamdani et al. [2005]. Gunes et al. [2007] reported a higher 102C allele frequency in 25 Estonian patients with either parkinsonism (n=23) or akathisia (n=2). However, the utilization of allele frequencies, instead of allele-positivity (as in our study), may has led to biased conclusions as discussed by Ohashi et al. [Ohashi et al., 2001; Ohashi and Tokunaga 1999]. Indeed, when Gunes et al. [2007] compared the median SAS scores between the three genotype classes of 102T>C polymorphism, there was no significant difference observed. Furthermore, Gunes et al. [2007] reported that the frequency of the 452Tyr allele (His452Tyr polymorphism) was not significantly different in cases as compared to non-cases. Taken all together, currently, there is no strong evidence for an association between these polymorphisms and AIP.

Notably, our data do not support the view that genetic variations of the DRD2 gene significantly predispose to AIP -when measured as a total syndrome- and hence do replicate findings of three other studies in German [Kaiser et al., 2002] and Asian [Chong et al., 2003; Wu et al., 2006] patients, respectively. However, we do find a significant association between rigidity and the DRD2 promoter variant -141CIns/Del, which -although debated [Pohjalainen et al., 1999; Ritchie and Noble 2003]- has been suggested to be functional [Arinami et al., 1997; Jonsson et al., 1999] or in linkage disequilibrium with another functional polymorphism [Duan et al., 2003]. This relationship with rigidity was not reported by the only co-existing study on 52 Japanese subjects [Mihara et al., 2001], who are ethnically different from African-Caribbeans. The frequency of the minor allele of this polymorphism (the Del allele) was 0.35 in our sample, which is 2-3 times higher than that of Asians (0.16) and Europeans (0.11) (SZGene, www.schizophreniaforum. org; accessed October 28th 2007).

Two other studies did report an association between the -141CIns/Del polymorphism and extrapyramidal symptoms [Inada et al., 1999; Nakazono et al., 2005]. However, these studies are probably biased due to pooling of parkinsonism with other types of movement disorders (akathisia, dyskinesia, and dystonia).

We chose DRD3 gene because many studies have shown that it may be associated with tardive dyskinesia [Bakker et al., 2006; Lerer et al., 2002]. Our study however provides no evidence for a relationship between this polymorphism and symptoms of AIP, which is in agreement with the literature [Chong et al., 2003; Gunes et al., 2007; Guzey et al., 2007]. This finding suggests that the genetic vulnerability for tardive dyskinesia may differ from that for antipsychotic-induced parkinsonism.

A limitation of our study is that we did not correct for variation in the psychopathology of the patients. However, our study sample can be considered as homogenous, since the majority of the patients was of African-Caribbean origin and had schizophrenia. Furthermore, the presence of organic and neurological disorders that may cause movement disorders was a stringent exclusion criterion. Since we followed our patients for ten years [van Harten et al., 2006], neurological diseases such as Morbus Parkinson would have been detected [Hausner 1983].

Factors other than genetic polymophisms (i.e., confounders) may also cause the parkinsonian symptoms. Tremor for example may be an essential tremor or may be induced by other drugs (such as antidepressants or antiepileptics). However, these tremors are predominantly postural, whereas we examined our patients for rest tremor, which is more specifically related to antipsychotics and AIP.

Furthermore, we compared the means of age, chlorpromazine and benztropine equivalents as well as the number of patients using anticholinergic medication and the number of antipsychotics used per patient (polypharmacy) in cases and non-cases of AIP, rigidity, bradykinesia, and rest-tremor and in carriers and non-carriers of 23Ser (HTR2C) and -141CDel (DRD2) alleles. Overall there were no statistically significant differences between the compared groups, except for male carriers of the -141CDel allele (DRD2) who had significantly lower mean chlorpromazine equivalents than non-carrier male patients (a fact which may only reinforce our findings). Of note, the majority of the patients were using typical (first generation) antipsychotics. There is, therefore, no indication for biased findings due to difference in the utilization patterns of antipsychotics.

Bradykinesia might be caused by psychiatric symptoms or sedation. We therefore chose a more stringent cut-off point (see Methods). Additionally, we evaluated the utilization of benzodiazepines in our sample and found that the number of patients using benzodiazepines does not differ significantly between bradykinesia cases and non-cases or between carriers and non- carriers of 23Ser allele (HTR2C).

Another limitation of the present study is that the UPDRS is not developed specifically to measure AIP. However, the UPDRS is a reliable and a valid instrument that has been extensively tested and used for Parkinson's disease. Since the phenomenology of Morbus Parkinson is not essentially different from the phenomenology of drug-induced parkinsonism, we assume that the UPDRS is able to measure AIP. In fact, several studies have utilized the UPDRS for the measurement of drug-induced parkinsonism [Hassin-Baer et al., 2001; Jamora et al., 2007; Lera and Zirulnik 1999; van Harten et al., 1996; van Harten et al., 2006]. Additionally, the UPDRS is far more comprehensive and balanced than the often used Simpson-Angus Scale (SAS), which has been criticized for overemphasizing rigidity items (6 out 10 items measure rigidity, while only 1 item measures tremor) as well as other shortcomings recently highlighted by Loonen and van Praag [Loonen and van Praag 2007].

In our study we have endeavored to replicate data from previous studies rather than to explore new genetic targets. Since our approach is hypothesis driven, we did not correct for multiple testing. In fact, the issue of correction for multiple testing is a subject of ongoing debate. The European Society of Human Genetics states that "How to correct for this [multiple testing for genetic association] is still under debate. The Bonferroni correction could overcorrect for the inflated false-positive rate, and as a consequence, valid information would be discarded …What is even more concerning than the incidence of false-positives is the potential lack of detecting genuine effects" (http://www.eshg.org; accessed November 11th 2007). To give an example, since we tried to replicate a number of studies simultaneously

in the same sample, correction for multiple testing would obviously lower the chance of statistically significant confirmation of studies investigating only 1 SNP.

In conclusion, the present study suggests an association between the -141CIns/Del (DRD2) polymorphism and rigidity and between the Cys23Ser (HTR2C) polymorphism and AIP or bradykinesia in African-Caribbeans. However, this study does not provide evidence for a genetic association between rest-tremor and any of the polymorphisms studied.

Since our data suggest that AIP and its more specific subsymptoms rigidity, rest-tremor, and bradykinesia may differ pharmacogenetically, our data strongly support symptom-specific approach in contrast to recent pharmacogenetic studies that pool even the different extrapyramidal symptoms in their analyses. Further research is however warranted to confirm our findings.

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