Research Article

CLINICAL INVESTIGATION

CYP2D6 genetic polymorphism and genetic ancestry on extrapyramidal side-effects of long-term treatment with classical antipsychotics in cuban patients with schizophrenia

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Abstract

Background: Different factors alter a patient's susceptibility to adverse neuroleptic reactions. The *CYP2D6* gene codes for a human cytochrome P450 2D6 enzyme, which is responsible for the metabolism of many psychiatric drugs. Ethnicity may also influence the susceptibility to adverse effects of long-term treatment with classical Antipsychotics (AP) because allele frequencies differ in correspondence with interethnic differences.

Objective: To evaluate whether the CYP2D6 allelic variants/predicted metabolic capacity status and the genetic ancestry are associated with classical antipsychotic-induced extrapyramidal side-effects (EPS) in Cuban patients with schizophrenia.

Methods and findings: A cross-sectional study was carried out. 209 patients were included: 61 of them with EPS at present and 148 patients without EPS. DNA samples were genotyped to estimate of admixture proportions and CYP2D6 genotype.

Homozygous patients with non-functional alleles have a higher risk of developing EPS (RR=3.418). These patients have a higher proportion of European genes (0.82).

Conclusion: The Poor Metabolizer (PM) genotype may be a predisposing factor for EPS. The study did not find evidence that ethnic differences played a large part in the risk of EPS.

Keywords: CYP2D6 ▪ Extrapyramidal side-effects ▪ Classical antipsychotics ▪ Genetic ancestry

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Introduction

Schizophrenia is a complex neuropsychiatric disorder characterized by delusions, hallucinations, passivity phenomena, disordered thought process, disorganized behavior and progressive cognitive deficits [1]. Pharmacological treatment focuses on the use of AP [2]. The adverse reaction most frequently associated with this therapy is the EPS, which can appear in more than 60% of patients [3]. Akathisia, Dystonia, Parkinsonism, and Dyskinesia are the main types of drug-induced EPS [4]. The occurrence of these adverse reactions in treated patients depends on many factors such as age, gender, drug availability, neuroleptic dose, the presence of other diseases and genetic factors [5,6].

Genetic polymorphisms of the drug metabolizing enzymes may contribute to inter-individual variations in plasma levels of antipsychotics. CYP2D6 is a cytochrome P450 enzyme involved in the metabolism of most neuroleptics, including Perphenazine,

Thioridazine, Haloperidol, and Zuclopenthixol [7]. Subjects can be classified into four metabolic groups based on their CYP2D6 activity: poor metabolizers (gPM), intermediate metabolizers (gIM), normal metabolizers (gNM), and ultrarapid metabolizers (gUM) [8-10].

The allelic variants that result in null activity of this enzyme should represent a risk factor for developing EPS in patients receiving AP treatment, since these individuals will show a deficient metabolization of these drugs, resulting in the accumulation and, therefore, increased blockade of dopamine receptors. There are few clinical studies of long-term treatment of schizophrenia and CYP2D6 polymorphisms. Some of them have investigated the impact of allelic variants on EPS but the results have been contradictory. A number of these studies have shown an association between the lack of a functional *CYP2D6* gene and side effects during neuroleptic treatment, in particular, tardive dyskinesia and parkinsonism, but the results were not replicated in others [5,7,11].

On the other side, the pharmacogenetic-based research must be standardized by the ethnicity of patients. Allele frequencies differ considerably between populations, in correspondence with interethnic differences [12].

Few studies have explored the relation between ancestry and EPS. A systematic review and meta-analysis about ethnic differences in the risks of adverse reactions to drugs used in the treatment of psychoses and depression concluded that there was inconsistent evidence for ethnic differences in adverse drug reactions to antipsychotic and antidepressant treatments [13].

Nevertheless, the admixture proportion of the Cuban population makes this country representative of the human genetic diversity. Considering the distribution of the proportions of the ethnic admixture of the Cuban population is of great importance for the design of studies of allelic association that intend to estimate the genetic predisposition for any disease [14]. However, as far as we know, there are no previous studies exploring the association between EPS and admixture proportions.

The present study was aimed to evaluate whether the CYP2D6 allelic variants and predicted metabolic capacity status, and the genetic ancestry is associated with classical antipsychotic-induced EPS in Cuban patients with schizophrenia.

Materials and Methods

Subjects

The cross-sectional study included 209 Cuban patients with schizophrenia. The diagnosis was established using the Diagnostic and Statistical Manual of Mental Disorders criteria, 4th Edition (DSM-IV). All participants were inpatients at Havana Psychiatric Hospital (Havana, Cuba). The inclusion criteria were age over 18, patients treated with conventional antipsychotic drugs metabolized by CYP2D6: Haloperidol, Chlorpromazine, Trifluoperazine and Fluphenazine.

Patients with Parkinson's disease, essential tremor, Huntington's disease, tics and muscle dystonias diagnosed before antipsychotic treatment, and patients treated with antipsychotic drugs not metabolized primarily by CYP2D6 were excluded. All subjects underwent a neurological examination to evaluate the presence of EPS.

EPS were assessed with the Simpson Angus Scale (SAS), the Barnes Akathisia Scale and the Abnormal Involuntary Movement Scale (AIMS). The sample was divided into two groups: 61 patients were included in the group with EPS, and 148 patients without EPS.

Laboratory procedures

Blood samples from each patient (10 ml) were collected in EDTA tubes, and DNA extraction was carried out by saline precipitation method [15]. For CYP2D6 genotype determination, several allelic variants were analyzed by quantitative real-time PCR using fluorescence-based TaqMan® assays on a Fast 7300 Real-Time PCR System (Applied Biosystems, CA, USA).

To detect the presence of allelic variants harboring a CYP2D6*5 gene deletion or gene duplication, long-range (XL) PCR was performed as described in detail previously [16]. The presence of *CYP2D6*1* was estimated by the absence of other genotyped CYP2D6 polymorphisms.

Antipsychotic dose

The daily AP dose was calculated as Chlorpromazine-Equivalent Daily Dose (CEDD) [17].

Predicted CYP2D6 phenotype

To assign predicted metabolizer status from genotype, these criteria were used: Ultrarapid

Table 1. Alleles included in the study according to their perceived functionality [19]

Alleles*	Functionality
non-functional (0)	*3, *4, *4M,*5, *6,*4 × N
decreased-function (0.5)	*10, *17, *29, *41, *10 × 2
normal-function (1)	*1, *2, *35
increased-function (≥ 2)	*1 × N, *1 × 2, *1 × 3, *2 × N, *2 × 2, *2 × 3, *2 × 4, *41 × 4
*The values in parentheses indicate respective values assigned to an allele to calculate the activity score (AS) a diplotype [20]	

Table 2. Characteristics of patients

	EPS	no EPS	p.overall
n	61	148	
Sex (M/F)	24/37	64/84	
Age (years)*	65.0 (56.0;78.0)	61.0 (54.0;79.0)	0.41
Antipsychotic intake* (mg/day chlorpromazine equivalents)	400 (200;1050)	475 (169;1050)	0.88
*Age and antipsychotic intake are given as mean (minimum-maximum in parentheses)			

Metabolizer (UM, ≥ 3 normal function gene copies); Normal Metabolizer (NM, 1 or 2 normal function alleles); Intermediate Metabolizer (IM, ≥ 2 decreased function alleles or 1 decreased function and 1 no function allele); Poor Metabolizer (PM, ≥ 2 non-function alleles) [18] (Table 1).

Estimation of admixture proportions

DNA samples were genotyped using the Infinium PsychArray v1.0 and v1.1 BeadChips (Illumina Inc.) at Statens Serum Institut, Denmark. Estimates of admixture proportions were obtained with the program ADMIXMAP, using data from 128 Ancestry Informative Markers: autosomal, mtDNA and Y-chromosome markers [14].

Statistics

Data were analyzed using SPSS version 20.0. The differences in CYP2D6 allele frequencies were compared by using the chi-square test and/or Fisher's exact test. P-values <0.05 were regarded as statistically significant. The 95% Confidence Interval (CI) for Relative Risk (RR) was calculated.

Results

A total of 209 subjects, 88 males, and 121 females, aged 32-75 years (mean aged 62.1) were recruited in the study. The two groups were homogenous with respect to age, drug exposure and doses (Table 2). The participating patients had been continuously treated with antipsychotic drugs at least for 5 years (mean exposure time 13 years).

All alleles detected were counted and calculated for their respective frequencies. All the polymorphisms analyzed were in Hardy-Weinberg equilibrium in both groups. Twenty-two different CYP2D6 alleles were identified, including structural variants (Table 3). These included gene duplications and the CYP2D6*5 gene deletion. The most common non-functional allele was CYP2D6*4 followed by *5, and the most common decreased function alleles were CYP2D6*17 and *41.

Table 3. Percentages of CYP2D6 variant alleles in patients with EPS and in in Cuban patients with schizophrenia

CYP2D6 alleles	EPS n=61	no EPS n=148	Total N=209
*1	40.2	44	42.9
*2	9.8	11.1	10.4
*3	0.8	0.3	0.6
*4	13.1	9.2	11.3
*4M	0.7	0	0.4
*5	5.7	4.2	4.6
*6	0.8	0.3	0.5
*10	6.6	2.4	3.6
*17	7.4	8.8	8
*29	2.5	2.7	2.6
*35	2.5	2.7	2.6
*41	4.9	6.8	6.3
*1 × N	0	1.8	1
*1 × 2	0.8	0.3	0.5
*1 × 3	0	0.3	0.2
*2 × N	0	1.8	1
*2 × 2	1.6	1	1.3
*2 × 3	0	0.3	0.2
*2 × 4	0	0.3	0.2
*4 × N	2.5	1.1	1.4
*10 × 2	0	0.3	0.2
*41 × 4	0	0.3	0.2

The frequencies of non-functional alleles were slightly higher in the group with EPS than in the other group, but this difference was not statistically significant (p>0.05).

AS system was used to simplify genotype interpretation and improve phenotype prediction. In the present study, the four PM subjects have EPS, whereas IM/NM and UM were distributed between the two groups. Relative Risk (RR) comparing PM and UM predicted CYP2D6 phenotype to the IM/NM are given in Table 4. Homozygous patients with non-functional alleles have a higher risk of developing EPS than patients classified as NM or IM (RR=3.418).

Based on results from the admixture analysis it was determined that all participants in this study had a mixed proportion of genes (European, African, and Native American). There was no difference in admixture proportions in individuals with EPS compared to those without EPS (Table 5).

However, when patients are classified accordingly to the predicted metabolizer phenotype, we noticed that PM has a higher proportion of European ancestry (Table 6). The low number of patients classified as PM, only 4, is a limitation which achieves statically significant results.

Discussion

Antipsychotic drugs have been the mainstay of schizophrenia treatment since the early 1950s. Efficacious for positive symptoms, antipsychotic treatment can lead to disabling EPS. These antipsychotic-induced EPS (especially Parkinsonism) are most probably related to striatal dopaminergic blockade [21]. The Nithsdale's Schizophrenia Survey found prevalence's of Parkinson's disease, Tardive Dyskinesia and Akathisia or Pseudoakathisia of 27%, 29% and 23%, respectively, and the Yale Tardive Dyskinesia Study showed risk rates of developing this neurological adverse effect of 31.8% in the first 5 years, and 49.4 to 68.4% during the following 5 to 25 years [22].

The pharmacogenetics exerts an evermore

Table 4. Predicted CYP2D6 phenotype and analysis of the dependent risk for e × trapyramidal symptoms

Predicted CYP2D6 phenotype	AS	N=209 (%)	EPS		no EPS		RR
			n=61	%	n=148	%	
gPM	0	4 (1.9%)	4	6.5	0	0	3.418 (2.737-4.269)
gIM /NM	0.5	188 (90.0%)	6	90.2	12	89.9	Reference category
	1		17		32		
	1.5		13		37		
	2		19		52		
gUM	>2	17 (8.1%)	2	3.3	15	10.1	0.3 (0.07-1.36)

Table 5. Distribution of average ancestral contributions in patients with EPS and in patients without EPS

	EPS	no EPS	p.overall
AFR	0.25 (0.04;0.60)	0.34 (0.05;0.67)	0.241
EUR	0.70 (0.34;0.91)	0.62 (0.27;0.90)	0.390
AMR	0.03 (0.01;0.06)	0.02 (0.01;0.05)	0.209
EAS	0.01 (0.00;0.02)	0.01 (0.00;0.01)	0.979
Abbreviations: AFR: African; EUR: European; AMR: Native American; EAS: East Asian			

Table 6. Distribution of average ancestral contributions in patients according to predicted CYP2D6 phenotype

	gPM	gIM /NM	gUM	p.overall
AFR	0.14 (0.03;0.33)	0.29 (0.05;0.64)	0.44 (0.07;0.67)	0.512
EUR	0.82 (0.62;0.94)	0.68 (0.28;0.91)	0.51 (0.30;0.85)	0.344
AMR	0.04 (0.03;0.06)	0.02 (0.01;0.05)	0.04 (0.02;0.12)	0.191
EAS	0.00 (0.00;0.01)	0.01 (0.00;0.02)	0.01 (0.00;0.01)	0.575
Abbreviations: AFR: African; EUR: European; AMR: Native American; EAS: East Asian				

important influence on daily use of these drugs. CYP2D6 genotype or phenotype can undoubtedly be used to identify the patients with side effects caused by the changes in the metabolism of drugs that are substrates for this enzyme [23].

The CYP2D6 is a highly polymorphic gene localized on chromosome 22q13.1. More than 110 allelic variants have been described for this gene [24]. Some of them are present in a cross-section of populations at similar frequencies, whereas others are observed at largely different frequencies or have only been detected in a certain ethnic group. In this sample of Cuban schizophrenic patients, the frequencies for the non-functional alleles *3 and *4 were similar to the Americas Allele Frequencies (0.60% and 10.76%; respectively); lower than Caucasian (European and North American) alleles frequencies (1.3% and 18.17%) and higher than African American Alleles Frequencies (0.27% and 6.38%). In the case of allele *5, the frequency was higher than caucasian alleles frequency and Americas Allele Frequency (2.8% and 2.1%), and lower than African American Allele Frequency (6.4%). In this group of the non-functional alleles, CYP2D6*6 had the lowest frequency in all populations: 0.2% in African American sample; 0.96% in caucásicos [25].

In relation with decreased-function alleles, the frequency of the allele *10 is similar to the rest of the populations, except in East Asians, where presented the highest frequency (42.4%) followed by South/Central Asian Allele Frequency (17.4%) [22]. CYP2D6*17 is more frequent in Black Africans (22.36%) followed by African Americans (18.13%) [25,26]. In this study, the frequency for this allele was even lower than the reported for African Admixed (12.63%) [26]. The average of African ancestry in the patients in whom this allele was identified was 61%.

In the same way, the frequency of the allele *29 is more frequent in African Americans (6.44%) and African Admixed (4.55%) than in other ethnic groups. Although in this case, the frequency was lower than these populations, it exceeds that reported by Llerena et al. for the American Admixed population, in which Cuba was included, and it was estimated at 0.72% [26].

The duplication/multiplication of active alleles is higher in Middle Easterners (6.72%), Black Africans (5.80%) and Mediterranean-South Europeans (3.85%) than in the rest. In our sample the total frequency of these alleles (*1 × N, *1 × 2, *1 × 3, *2 × N, *2 × 2, *2 × 3, *2 × 4, *41 × 4) is equal to 4.6% (Table 3); also higher than that reported by Llerena et al. for the American Admixed population with 2.55% [25].

The combination of these alleles in the genotype allowed predicting the CYP2D6 phenotype of the patients. The frequency of PM in the entire sample was lower than the values previously reported for the Cuban population by Llerena et al. In this paper the frequencies of PM were 3.9% in Cuban Mestizos and 5.3% in White Cubans. The sample was divided into two groups: 'Whites' that were those individuals with four Caucasian grandparents and 'Mestizos' that represented the rest [27]. It is important to clarify that these are the frequencies reported for healthy Cuban volunteers.

Possible involvement of CYP2D6 in susceptibility to schizophrenia arises from the evidence of CYP2D6 expression in certain regions of the Central Nervous System, which appear to be impaired in schizophrenia patients, and may indicate the role of CYP2D6 in a number of endogenous interactions that are of potential clinical importance for neurological diseases. Based on this hypothesis, some association studies between schizophrenia and CYP2D6 observed an underrepresentation of PMs in schizophrenics whereas other studies did not find this association [28].

Our results are in general agreement with the investigations which found that there is a possible association between the CYP2D6 PM genotype with the appearance of antipsychotic-induced EPS in Cuban schizophrenic patients. Similar results have been reported for Korean schizophrenic [5], Italian schizophrenic patients [29], and in Spanish patients receiving AP therapy [30].

Moreover, as in our sample, the frequency of UM in the group without EPS was higher than the group with EPS; this status seems to play an important role in the occurrence of EPS, at least theoretically, as a protective condition.

There have been few studies reporting the relationship between extrapyramidal symptoms due to the use of neuroleptics and ethnic origin. Some of them suggest that East Asian patients have a significantly higher risk of extrapyramidal symptoms compared with non-East Asian patients [13]. In our sample, the East Asian ancestry component was very low and didn't show the difference between the groups. The European ancestral contribution was greater in absolute values in patients with EPS, and the African

contribution in the group without EPS was higher, but differences were not statically significant.

The analysis of ancestral contributions with predicted CYP2D6 phenotype indicates the frequency of CYP2D6 alleles can vary across ethnic groups. Gaedigk et al. propose that diplotype frequencies predicting poor metabolism are highest in Europeans (average, 5.4%) and in the Ashkenazi Jewish population (6%) [25]. According to that, in this study, PM group had the highest proportion of genes of European origin; over even the average contribution of genes of European origin reported for the Cuban population of 72% by Marcheco-Teruel et al. [14].

Conclusion

In this study, the CYP2D6 allelic variants and predicted metabolic capacity status is associated with classical antipsychotic-induced EPS, because the PM genotype may be a predisposing factor for this adverse effect, although results cannot be considered conclusive due to the small number of PM patients. In these patients, there is an average of the ancestry of European origin higher than that reported for the Cuban population. However, the ethnic differences did not increase the risk of EPS. It represents the first report of the frequencies of CYP2D6 polymorphisms in Cuban patients with schizophrenia.

Ethics Approval and Consent to Participate

The protocol was approved by the Research Ethics Committee of the National Centre of Medical Genetics of Cuba, and written informed consent to participate in the study was obtained from the patients or their relatives.

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Competing and Conflicting Interests

There is no known conflict of interest.

Executive summary

Background: Different factors alter a patient's susceptibility to adverse neuroleptic reactions. The CYP2D6 gene codes for a human cytochrome P450 2D6 enzyme, which is responsible for the metabolism of many psychiatric drugs. Ethnicity may also influence the susceptibility to adverse effects of long-term treatment with classical antipsychotics (AP) because allele frequencies differ in correspondence with interethnic differences.

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