CYP2C9 polymorphism in patients with epilepsy
Genotypic frequency analyzes and phenytoin adverse reactions correlation

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ABSTRACT

Objective: *CYP2C9* is a major enzyme in human drug metabolism and the polymorphism observed in the corresponding gene may affect therapeutic outcome during treatment. The distribution of variant *CYP2C9* alleles and prevalence of phenytoin adverse reactions were hereby investigated in a population of patients diagnosed with epilepsy. **Method:** Allele-specific PCR analysis was carried out in order to determine frequencies of the two most common variant alleles, *CYP2C9*2* and *CYP2C9*3* in genomic DNA isolated from 100 epileptic patients. We also analyzed the frequency of phenytoin adverse reactions among those different genotypes groups. The data was presented as mean±standard deviation. **Results:** The mean age at enrollment was 39.6±10.3 years (range, 17-72 years) and duration of epilepsy was 26.5±11.9 years (range 3-48 years). The mean age at epilepsy onset was 13.1±12.4 years (range, 1 month-62 years). Frequencies of *CYP2C9*1* (84%), *CYP2C9*2* (9%) and *CYP2C9*3* (7%) were similar to other published reports. Phenytoin adverse reactions were usually mild and occurred in 15% patients, without correlation with the *CYP2C9* polymorphism (p=0.34). **Conclusion:** Our findings indicate an overall similar distribution of the *CYP2C9* alleles in a population of patients diagnosed with epilepsy in the South of Brazil, compared to other samples. This sample of phenytoin users showed no drug related adverse reactions and *CYP2C9* allele type correlation. The role of *CYP2C9* polymorphism influence on phenytoin adverse reaction remains to be determined since some literature evidence and our data found negative results.
Key words: *CYP2C9*, polymorphism, Brazilian population, phenytoin, adverse reactions.

Polimorfismo do CYP2C9 em pacientes com epilepsia: estudo da frequência genotípica e correlação com os efeitos colaterais da fenitoína

RESUMO

Objetivo: A *CYP2C9* é uma das principais enzimas do metabolismo de drogas humano e o polimorfismo observado no respectivo gene pode afetar o resultado terapêutico durante o tratamento. Neste trabalho investigamos em uma população de pacientes portadores de epilepsia a distribuição dos alelos variantes do *CYP2C9* e a frequência de efeitos adversos da fenitoína tentando estabelecer uma correlação. **Método:** Realizamos uma análise através de uma PCR alelo específica para determinar a frequência dos alelos variantes mais comuns, *CYP2C9*2* e *CYP2C9*3*, isolados da amostra de 100 pacientes com epilepsia. Também levantamos a frequência de reações adversas da fenitoína nestes diferentes grupos genotípicos. Os dados são apresentados na forma de média e desvio-padrão. **Resultados:** A idade média na inclusão foi 39,6±10,3 anos (variando de 17-72 anos) e a duração da epilepsia era 26,5±11,9 anos (variando de 3-48 anos). A idade média dos pacientes no início da epilepsia era 13,1±12,4 anos (variando de 1 mês-62 anos). As frequências do *CYP2C9*1* (84%), *CYP2C9*2* (9%) e *CYP2C9*3* (7%) foram similares

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a outros estudos publicados. As reações adversas da fenitoína foram frequentemente leves e ocorreram em 15% dos pacientes, sem correlação com o polimorfismo do *CYP2C9* (p=0.34). **Conclusão:** Nossos achados indicam uma distribuição similar dos alelos variantes *2 e *3 nesta população de pacientes com epilepsia comparado a outros estudos. Esta amostra de usuários de fenitoína mostrou não haver correlação entre efeitos colaterais relacionados à droga e o tipo de alelo variante. O papel da influência do polimorfismo do *CYP2C9* nos efeitos colaterais da fenitoína precisam ser melhor determinados, já que algumas evidencias da literatura e este trabalho mostraram resultados negativos.

Palavras-chave: *CYP2C9*, polimorfismo, fenitoína, reações adversas.

Pharmacogenetics constitutes a potential tool for predicting among patients those who are likely to express the desired response to a drug, those who are likely to experience little or no benefit and those who are at risk for toxicity. Cytochrome P450 2C9 (*CYP2C9*) catalyses the metabolism of many important drugs such as phenytoin, S-warfarin, tolbutamide, losartan, torasemide, as well as, nonsteroidal antiinflamatories[1]. Genetic variation in the *CYP2C9* gene can affect metabolism, leading to altered phenotypes. Individuals with poor metaboliser alleles of *CYP2C9* gene were shown to have a reduced metabolism of phenobarbital, phenytoin and valproate compared with those with wild-type (normal) alleles[2-9]. Several studies indicate that the most common allelic variants are $Arg_{144}Cys$ (*CYP2C9*2*) and $Ile_{358}Leu$ (*CYP2C9*3*) which encode enzymes with decreased substrate turnover[10,11]. As the most commonly prescribed antiepileptic drugs (AEDs) in developed countries are metabolized by the cytochrome p450 system, the identification of patients' genotype prior to AEDs administration could potentially prevent higher serum drug concentrations leading to adverse side effects.

Phenytoin was first added to epilepsy treatment in the 60'[12] and besides the new AEDs it remains one of the most prescribed AED for partial seizures with or without secondary generalization[13]. Its main rout of elimination is through hepatic oxidation under *CYP2C9* (90%) and *CYP2C19* (10%) metabolism[8,14,15]. Phenytoin metabolism rate can be reduced by 25 to 50 % depending on the individual genetic polymorphism and drug interactions through same pathway[2,8,14,16]. Its use can be associated to several dose related or idiosyncratic adverse events. Cosmetic facial chances, in spite of generally mild, can be problematic[17]. Neuronal toxic effects including confusion, dysarthric speech, double vision, ataxic gait and neuropathy could be observed during prolonged use or in acute intoxications[13]. Moreover, some studies have tried to link the development of those side effects looking for a correlation between *CYP2C9* genetic polymorphism and phenytoin adverse reactions[18-20].

Interethnic differences in *CYP2C9* allele distribution have been described between Europeans, Asiatics and Africans[1,21]. Brazil represents one of World's most heterogeneous populations, as the result of 500 years of extensive interethnic crossover. Until now, just three studies assessed the *CYP2C9* polymorphism frequency among Brazilian population[22-24]. The genotype analyses revealed that the frequencies of *CYP2C9*2* and *CYP2C9*3* were 8-9% and 6-7%[22,23].

Thus, the purpose of the present study was to determine the frequency of the different *CYP2C9* alleles (*2 and *3) in a Brazilian group of people with epilepsy and to potentially correlate phenytoin adverse events to a specific genotypic profile.

METHOD
Subjects

Most of the individuals were characterized as having refractory drug resistant epilepsy followed in a tertiary epilepsy program at the Hospital de Clínicas, Federal University of Paraná, Brazil. All subjects come from either Curitiba or its metropolitan area, accounting for estimated 3 million people. The study protocol was approved by the Hospital de Clínicas - UFPR research ethics committee. After a detailed explanation all subjects signed a written informed consent form. All subjects in the study have had a documented use of phenytoin for over 1 year. They were selected during February 2008 until June 2009. All patients meeting those criteria were included until we have one hundred patients (52 men and 48 women). Before the blood collection hospital records were reviewed retrospectively looking for phenytoin adverse reactions (PAR) reports. Also, the patient was inquired about any adverse events during phenytoin exposure. The mean age at enrollment was 39.6±10.3 years (range, 17-72 years). The mean age at epilepsy onset was 13.1±12.4 years (range, 1 month-62 years). The epilepsy duration was 26.5±11.9 years (range 3-48 years). Seizure frequency in a given month varied from a mean of 1.6±2.1 seizures to 4.6±4.6. The most frequent epilepsy type was symptomatic localized, 22%, followed by 12% non classified, 7% crytogenic general-

Table 1. Overall studied population demographic and clinical data.

Characteristics	Mean	SD	95% CI
Age	39.6 years	10.3	37.6-41.7
Maximum seizure frequency/month	4.6	4.6	3.7-5.5
Minimum seizure frequency/month	1.6	2.1	1.2-2.0
Epilepsy onset	13.1 years	12.4	10.7-15.6
Epilepsy duration	26.5 years	11.9	24.1-28.8

SD: standard desviation; CI: confidence interval.

Table 2. Correlation between genotype, epilepsy and drug treatment.

	Overall	Variants *2 and *3	Wild-type	p valor
Number of cases	100	28	72	
Epilepsy duration - mean years (SD)	26.58 (±11.91)	22.85 (±13.53)	27.92 (±10.99)	0.66*
Duration of phenytoin usage - mean years (SD)	8.33 (±6.91)	5.04 (±7.14)	8.88 (±7.56)	0.18*
Maximum phenytoin dosage - mean mg (SD)	312.6 (±77.6)	326.9 (±91.9)	308.2 (±77.72)	0.31*
Reported side effects - number of individuals (%)	15 (15%)	6 (21%)	9 (12%)	0.34**

*Mann-Whitney U-test; **Fisher's exact test.

ized , 5% localized cryptogenic and 3% idiopathic generalized. Demographic features of the studied population are summarized in Table 1. The mean use of phenytoin was 8.3 years (±6.9 years) for the entire group, with an average maximum daily dosage of 301.5 mg (±78 mg) (Table 2). All patients had their phenytoin regimen titrated according to clinical efficacy versus adverse side effects, over a period of approximately 3 to 6 months.

CYP2C9 genotyping

The crude DNA samples were analyzed using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method for *Arg144Cys* (*CYP2C9*2*) and *Ile359Leu* (*CYP2C9*3*) variant alleles using the primers mentioned by Sullivan-Klose et al. 1996[10].

Data analysis

Allelic frequencies were derived by gene counting. The Chi-square test was used to compare allele frequencies. Mann-Whitney U-test was used to compare time of phenytoin usage, phenytoin maximum dosage and epilepsy duration since data do not assume a Gaussian distribution. Frequency of phenytoin adverse events were compared with Fisher's exact test. The observed genotype frequencies of *CYP2C9* were also analyzed by Hardy Weinberg Equilibrium (HWE) for the predicted frequencies. The value P<0.05 was considered statistically significant.

RESULTS

The genotype frequencies for the study population

Table 3. Frequency (%) of *CYP2C9* genotypes in an epileptic South Brazilian population.

Genotype	Number of subjects	Observed frequency (%)	Predicted frequency(%)*
*1/*1	72	72	72.2
*1/*2	13	13	14.4
*1/*3	11	11	11
*2/*2	1	1	0.7
*3/*3	0	0	0.4
*2/*3	3	3	1.1

*Predicted frequency calculated according to the Hardy-Weinberg equation. There are no significant diference between the observed and predicted groups.

are listed in Table 3. The variant alleles *CYP2C9*2* and *CYP2C9*3* were detected in 9 % (n=18 alleles) and 7 % (n=14 alleles), respectively, of the overall study population. Among the study population, one subject were homozygous for *CYP2C9*2* and three were heterozygous for both *2 and *3 variants. No patient was found carrying the *CYP2C9*3* homozygous. The observed frequencies for the overall study population was concordant with HWE (Table 2).

There were no significant differences regarding the maximum phenytoin daily dose (Table 3), between the variant group (326.9 mg±91.9) and the wild-type group (308.2 mg±77.72, p=0.31). Other variables such as duration of phenytoin usage (p=0.18) and duration of epilepsy (p=0.66) were not significant, as well (Table 3).

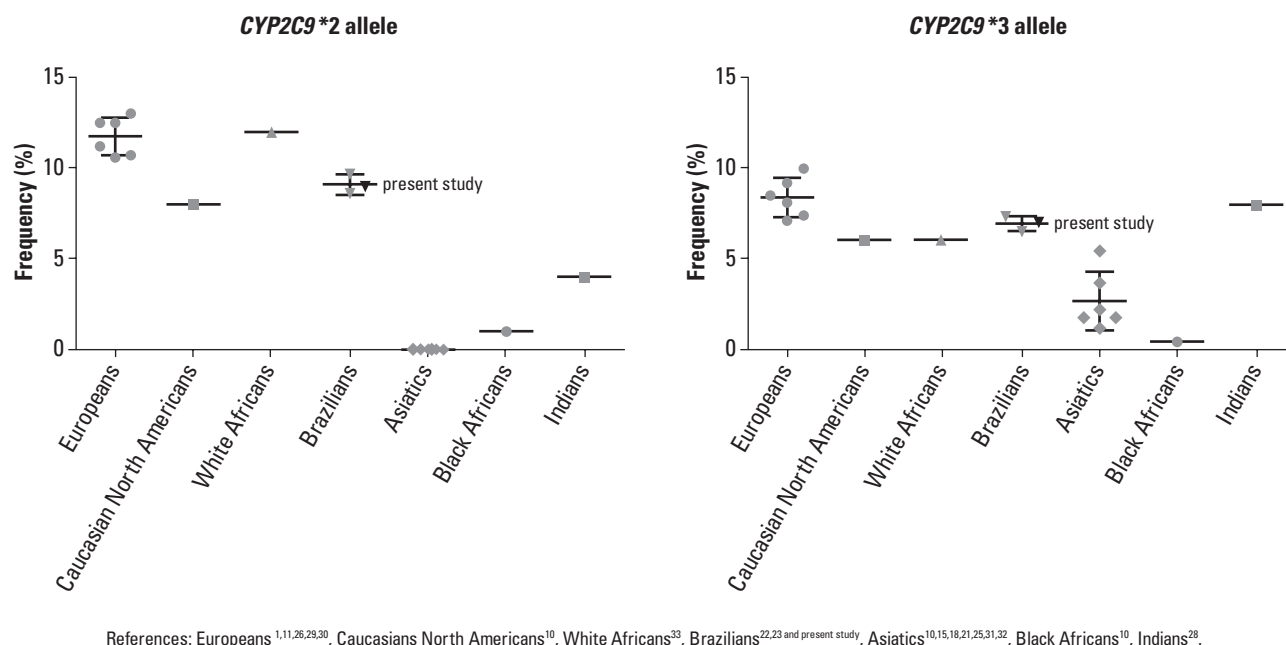The frequency of PAR in the overall study population was 15% (n=15) and for the group with at last one

CYP2C9 *2 allele

CYP2C9 *3 allele

Figure. Frequency (%) of *CYP2C9* alleles around the world.

References: Europeans [1,11,26,29,30], Caucasians North Americans [10], White Africans [33], Brazilians [22,23 and present study], Asiatics [10,15,18,21,25,31,32], Black Africans [10], Indians [28].

variant allele it was 21% (n=6), a non-statistically significant difference (p=0.34), comparing variants with mutants (Table 3). Subgroups analysis regarding heterozygous, homozygous or variant type were not possible due to the limited size of our population. The most common PAR observed were gingival overgrowth, in 8% (n=8), and cerebellar intoxication signs, in 7% (n=7). Mild nystagmus on lateral forced gaze, without any other cerebellar signs were not considerate as PAR, since it is frequently found during phenytoin titration and has no clinical significance.

As the majority of patients were characterized as refractory epilepsy a high number of individuals used more than one drug. At some point during the phenytoin treatment period 76% used another concomitant drug therapy. The most common associated used drugs were phenobarbital, 27%, carbamazepine, 26% and lamotrigine, 20%. Also, topiramate, clobazam, primidone and valproic acid were used less frequently.

DISCUSSION

The characterization of *CYP2C9* genetic polymorphism might contribute to the optimization of therapy in a range of clinically important drugs like antiepileptics. Many studies have shown considerable interethnic differences in *CYP2C9* polymorphism around the World[1,10,11,16,18,23,25-30].

Global distribution analyses of *CYP2C9* polymorphism suggests that *CYP2C9*2 and *CYP2C9*3 variants are more frequently found in European populations. Some studies demonstrated that *CYP2C9*2 frequency varied from 10.7%[26] to 16.5%[30] and the *CYP2C9*2 from 7.1%[30] to 9.2%[1] in such population. Asiatics[10,18,21,25,31,32] present no *CYP2C9*2 mutation and very low incidence of *CYP2C9*3, ranging from 1.1[32] to 5.4[18]. These findings may imply that the *CYP2C9* allele evolved quite recently. In accordance, Asiatics and Africans[1] also had shown a low incidence of both *CYP2C9*2 (4%) and *CYP2C9*3 (2%) alleles. Similarly, black North [10] and South Americans[22,23] showed low mutation rates of *CYP2C9*2 and *CYP2C9*3 alleles, 1.0-4.5 and 0.5-3.2 respectively. The frequencies of the *CYP2C9*2 (9%) and *CYP2C9*3 (7%) found in our epileptic Brazilian population were very close to those found in European populations (Figure).

Almost all genetic studies reveal the predominance of *CYP2C9*2 mutation over the *CYP2C9*3. By contrast we find higher rates of *CYP2C9*3 allele in comparison with *CYP2C9*2 in populations such Asiatics (Japanese[3], Chinese[25], Korean[32]), Canadian Native Indians[28] and Indians[14].

The South Brazilian epileptic patients in this study showed similar genotyping results consistent with evidence that subjects from many parts of the World have a significantly higher frequency of both *CYP2C9*2 and *CYP2C9*3 alleles than do black, Asiatic or Indian subjects. The frequency analysis of *CYP2C9* polymorphism in Brazilian healthy volunteers[23,24] showed overall results very similar to ours. In this study, when a comparison

with the genotypic distributions reported for other populations was performed no significant differences were observed between white Brazilians and other European or between black Brazilians and either Africans from Ethiopia or African Americans[23].

A few studies have tried to established a relationship between the development of phenytoin side effects and the presence of *CYP2C9**2 and *CYP2C9**3 variants[18,20]. Incidence of phenytoin induced gingival overgrowth in epileptic patients may be as high as 57%[19], but whether this or other phenytoin side effects may be influenced by cytochrome P450 polymorphism remains to be yet determined. In our study only 8% showed this adverse reaction. Subjects with more severe gingival overgrowth exhibited significantly higher serum phenytoin concentration, but the degree of gingival overgrowth did not directly correlate with *CYP2C9* polymorphisms[18]. However, *CYP2C9**3 variant could play a role in the proportion of patients with diphenylhydantoin induced cutaneous adverse reaction[20].

We could expect that variant group would have reached a lower phenytoin maximum dosage, because the lower metabolization rate, but it was not founded. The mean maximum dosage used was similar in both groups. Although PAR were almost twice more frequent in variant group (21%×12%), these findings were no statistical significant. Maybe if we were able to do subgroup analyses splitting in heterozygote and homozygote some PAR genotype correlation could exist.

We were not able to find an association between the report of PAR and *CYP2C9* genetic polymorphism. However, it seems to be clear that several factors may contribute to the lack of correlation between *CYP2C9* genetic polymorphism and PAR. Not only *CYP2C9* but also *CYP2C19* has been reported to catalyze phenytoin[8,14]. Some studies also indicate the role of P-glycoprotein in the disposition of phenytoin. Unfortunately, we are unable to do such genotyping. Moreover, concomitant drug therapies maybe could significant interfere with the phenytoin metabolism. In our study 76% of patients used concomitant medications at some point during the treatment which could possible modify the phenytoin metabolism. In this sample we do not had the actual or past phenytoin plasma concentrations.

Of course, this work has some limitations. A main problem could be the reduced number of subjects with PAR. Perhaps a larger sample may overcome this issue. Moreover, this study seems underpowered in this respect. But, besides the small sample, our findings indicate an overall similar distribution of the *CYP2C9* alleles in the South Brazilian epileptic population compared to European or Brazilian samples. Up to this point, development of side adverse effects related to phenytoin were

no different among the *CYP2C9* genotypes, but larger studies with control of the interfering factors are needed to clarify some of the pending issues described above. The characterization of *CYP2C9* genetic polymorphism influence on phenytoin adverse events might contribute to a better therapy optimization.

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