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Genetic polymorphisms of *CYP2C9* and *CYP2C19* in the Beninese and Belgian populations

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Aims To investigate the distribution of cytochrome P450 *2C9* (*CYP2C9*) and *2C19* (*CYP2C19*) genotype frequencies in the Beninese and Belgian Caucasian populations.

Methods Beninese ($n = 111$) and Belgian ($n = 121$) were genotyped for *CYP2C9*2*, **3*, **4*, **5*, and **11* as well as for *CYP2C19*2* and **3*.

Results The distribution of alleles was: *CYP2C9*1*: 95.5 *vs.* 82.2% ($P < 0.001$); *CYP2C9*2*: 0 *vs.* 10% ($P < 0.001$); *CYP2C9*3*: 0 *vs.* 7.4% ($P < 0.01$); *CYP2C9*4*: both 0%; *CYP2C9*5*: 1.8 *vs.* 0% ($P = 0.05$); and *CYP2C9*11*: 2.7 *vs.* 0.4% ($P < 0.05$). The frequencies of the *CYP2C19*2* allele were 13 *vs.* 9.1%, respectively. *CYP2C19*3* was not detected in either population. The 95% confidence intervals for the differences of frequencies of *CYP2C9*1*, *CYP2C9*2*, *CYP2C9*3*, *CYP2C9*4*, *CYP2C9*5*, *CYP2C9*11*, *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* between Belgian and Beninese were 7%, 19%; −14%, −6%; −11%, −4%; −1%, 1%; 0%, 4%; 0%, 5%; −10%, 2%; −2%, 10%; −1%; respectively. The distributions of *CYP2C9* genotypes in the Beninese and Belgian individuals were: *CYP2C9*1/*1*: 91 *vs.* 67% ($P < 0.00001$); *CYP2C9*1/*2*: 0 *vs.* 18.2% ($P < 0.0001$); *CYP2C9*1/*3*: 0 *vs.* 11.6% ($P < 0.001$); *CYP2C9*1/*5:* 3.6 *vs.* 0% ($P = 0.05$); *CYP2C9*1/*11*: 5.4 *vs.* 0.8% ($P = 0.05$); *CYP2C9*2/*3*: 0 *vs.* 1.6% (NS); CYP2C9*3/*3: 0 *vs.* 0.8% (NS). The distributions of *CYP2C19* genotypes between these ethnic groups were: *CYP2C19*1/*1*: 73.9 *vs.* 83.5% (NS); *CYP2C19*1/*2*: 26.1 *vs.* 14.9% ($P < 0.05$); *CYP2C9*2/*2*: 0 *vs.* 1.6% (NS).

Conclusions Differences of allele frequencies between Beninese and Belgian populations were statistically significant for *CYP2C9*2*, **3*, **5* and **11*, but not for *CYP2C9*4* or for *CYP2C19*2* and **3*.

Keywords: Benin, Belgium, *CYP2C19*, *CYP2C9*, genotype

Introduction

The cytochrome P450 2C (CYP2C) subfamily of enzymes metabolizes approximately 20% of drugs commonly used in clinical practice [1]. It comprises four members (CYP2C8, 2C9, 2C18 and 2C19), which exhibit a range of genetic polymorphisms. Those affecting *CYP2C9* and *CYP2C19* result in an impaired capacity to metabolize a range of drugs, the consequences of which may be clinically significant [1, 2]. The *CYP2C9* and *CYP2C19* polymorphisms have both been extensively characterized in Caucasians and Orientals, and to a lesser extent in Black populations. Whereas data are available for African-American and East-African populations, West African populations have not been studied in this context.

CYP2C9 is involved in the oxidation of a wide range of drugs, including S-warfarin, phenytoin, tolbutamide, losartan and torasemide. Several nonsteroidal anti-inflammatory drugs, including diclofenac, naproxen, piroxicam and ibuprofen, as well as the selective COX-2 inhibitor celecoxib, are also mainly metabolized by CYP2C9 [3].

The *CYP2C9* gene displays functional genetic polymorphisms unambiguously associated with impaired CYP2C9-mediated metabolism. The products of the

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*CYP2C9*2* (Arg144Cys) and *CYP2C9*3* (Ile359Leu) alleles (see http://www.imm.ki.se/CYPalleles) are enzymes with impaired activity towards a number of substrates, both *in vivo* and *in vitro* [1, 3–5]. The *CYP2C9*4* (Ile359Thr) polymorphism was first identified in a Japanese patient presenting with epilepsy [6], but its functional influence remains so far unclear. The *CYP2C9*5* variant is derived from a C1080G transversion in exon 7 that leads to Asp360Glu amino acid substitution. *In vitro* data suggest that *CYP2C9*5* carriers eliminate CYP2C9 substrates at a slower rate compared with those expressing the wild-type protein [7]. The *CYP2C9*11* genotype is derived from a C1003T mutation in exon 7, resulting in a Arg335Thr substitution (http://www.imm.ki.se/CYPalleles). Distribution of the *CYP2C9*11* polymorphism in Caucasian or Black populations is currently unknown, as is the distribution of the *CYP2C9*2*, **3*, **4* and **5* genotypes in the West Black African population.

Cytochrome P450 2C19 (CYP2C19) metabolizes a number of drugs, such as S-mephenytoin, omeprazole, propranolol, and imipramine. Several polymorphisms of the *CYP2C19* gene have been identified and produce an inactive enzyme. Two variant alleles account for the majority of the poor metabolizer (PM) phenotypes, namely *CYP2C19*2* which carryies a G→A nucleotide substitution in exon 5 resulting in an aberrant splice site, and *CYP2C19*3* which carries a G→A nucleotide substitution at position 636 in exon 4 and produces a premature stop codon. Whereas *CYP2C19*2* appears to be the most prevalent allele associated with the PM phenotype [8], *CYP2C19*3* is mainly found in Orientals [9].

The aim of this study was to assess the distribution of *CYP2C9* and selected *CYP2C19* genotypic variants in the Beninese population and to compare these data with the distribution observed in the Belgian Caucasian population.

Methods

Subjects

One hundred and eleven unrelated indigenous Beninese healthy adults were recruited. They all lived in the Zou department area of the country. Written informed consent was obtained from all participants. The study protocol was approved by the Ministry of Public Health of Benin and by Ethic Review Committee of Medical Faculty of National University of Benin (UNB).

Blood samples from 121 white Belgian Caucasian subjects were collected in Saint-Luc Hospital (Brussels, Belgium), in accordance with a protocol approved by the local ethics committee.

Blood collection and DNA isolation

For each subject, venous blood samples (5 ml) were collected in EDTA tubes. Genomic DNA was extracted using the Qiagen Qiamp DNA Blood kit (Qiagen, Leusden, the Netherlands), according to the manufacturer's protocol.

Genotyping

*CYP2C19*2* and *CYP2C19*3* genotyping was performed according to the single-tube tetra-primer polymerase chain reaction (PCR) assay method as described previously [10]. A new single-tube tetra-primer PCR assay was developed to detect the *CYP2C9*2* allele (Table 1). Primers 3S and 3AS complementary to exon 3 and allele-specific primers matching the wild-type (3IS) or the **2* allele (3IAS) were mixed together. The following reaction mixture was used: 5 µl of PCR buffer (10 mmol l^{-1} Tris hydrochloride, pH 8.3 and 50 mmol l^{-1} potassium hydrochloride), 2.25 mM of $MgCl_2$, 1 U of Gold Taq, 200 µM of each deoxyribonucleoside triphosphate (dNTPs), 10 µM of each primer except for primer 3S (3 µM), and 250 ng of genomic DNA in a final volume of 50 µl. Cycling conditions were as follows: 5 min at 95 °C; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s; and a final extension of 7 min at 72 °C. The PCR products were separated by 2% agarose gel electrophoresis. The expected size of 3S-3AS amplicon, used as internal control for the quality of the PCR amplification and as template for the allele-specific amplification (ASA), was 367 bp. The expected sizes of the ASA amplicon for *CYP2C9*2* and wild-type alleles were 243 and 156 bp, respectively.

Genotypes identified using the new single-tube tetra-primer assays were confirmed by sequence analysis. Identification of the *CYP2C9*3*, **4*, **5* and **11* alleles was performed by sequence analysis. The primers used for amplification of *CYP2C9*5* are shown in Table 1. For this allele, 250 ng genomic DNA was amplified in a final volume of 50 µl using 2.25 mM of $MgCl_2$, 1 U of Gold Taq, 5 µl of PCR buffer, 200 µM of each dNTPs, 10 µM of 7S, 10 µM of 7AS. PCR conditions for *CYP2C9*5* consisted of an initial denaturation of 5 min at 95 °C, followed by 40 cycles (40 s at 95 °C, 40 s at 60 °C and 1 min 20 s at 72 °C) and a final elongation of 7 min at 72 °C. The amplicons were first purified with Microcon (Amicon Millipore Corporation, Bedford, MA 01730, USA) columns. Sequence analysis was performed in both orientations with Big Dye terminators (PRISM; Applied Biosystems, Nieuwerkerk, the Netherlands), using an automated ABI 3100 capillary sequencer (Applied Biosystems).

Table 1 *CYP2C9* and *CYP2C19* genotyping: primer selection.

	Primer	*Sequence*⋆
CYP2C9		
Exon 3	3S	5′-GGAGGATGGAAAACAGAGAC-3′ (337–356)
	3AS	5′-GATATGGCCACCCCTGA-3′ (703–687)
	31S	5′-AGGAGCATTGAGGACC-3′/-3′ (548–563)
	31AS	5′-GCTTCCTCTTGAACACA-3′/-3′ (579–563)
Exon 7	7S	5′-CTCCTTTTCCATGAGTTTTTACT-3′ (34–56)
	7AS	5′-GATACTATGAATTTGGGACTTC-3′ (317–296)
CYP2C19		
Exon 5	5S†	5′-CAGAGCTTGGCATATTGTATC-3′(8–28)
	5AS†	5′-GTAAACACACAACTAGTCAATG-3′ (328–307)
	5IS[c]	5′-ATCATTGATTATTTCCCA §-3′ (100–117)
	5IAS‡	5′-AATTTGTTATGGGTTCCC §-3′ (134–117)
Exon 4	4S[c]	5′-TATGAAGTGTTTTATATCTAATGTTTACTCA-3′(21–51)
	4AS‡	5′-ACTTCAGGGCTTGGTCAATATAGA-3′(329–306)
	4IS‡	5′-GTAAGGACCCCCTGA §-3′ (220–234)
	4IAS‡	5′-GGCCTTACCTGGATC §-3′ (248–234)

⋆Nucleotide positions according to GenBank Accession no. L32982 (exon 4), L31506 (exon 5), L16878 (exon 3) and L16881 (exon 7). †From Xiao *et al*. [22]. ‡From Hersberger *et al*. [10]. §Nucleotides that correspond to the target point mutation.

Statistical analysis

Allele distributions were compared using χ^2 and Fisher's exact tests. $P \leq 0.05$ was considered significant. These analyses were performed with SPSS (SPSS Inc. Chicago, USA) for Windows (version 10.00).

Results

CYP2C9 and *CYP2C19* allele and genotype frequencies in both populations are summarized in Table 2. Expression of the genotypes relies upon the assumption that the different mutations are not linked on the same allele [4]. Accordingly, we observed five different non homozygous wild-type *CYP2C9* genotypes in the 121 Belgian individuals: *CYP2C9⋆2/⋆3* ($n = 2$); *CYP2C9⋆3/⋆3* ($n = 1$). *CYP2C9⋆1/⋆2* ($n = 22$), *CYP2C9⋆1⋆3* ($n = 14$) or *CYP2C9⋆1⋆11* ($n = 1$). The remainder were homozygous wild-type *CYP2C9⋆1⋆1* ($n = 81$). In the 119 Beninese, four were heterozygous for the *CYP2C9⋆5* allele and six for the *CYP2C9⋆11* allele.

There was a statistically significant difference in the allelic distribution of *CYP2C9⋆2* and *CYP2C9⋆3* between Beninese Africans and Belgian Caucasians. The *CYP2C9⋆5* and *CYP2C9⋆11* alleles were statistically more common among Beninese compared with Belgians.

Eighteen of the 121 Belgians had the *CYP2C19⋆2/CYP2C19⋆1* genotype, and two were homozygous for *CYP2C19⋆*2, and would be predicted to be PMs. Allelic distributions were similar in the Beninese and Belgian subjects.

Discussion

Whereas *CYP2C* genetic polymorphisms have previously been assessed in Caucasians and Orientals, data in West African populations are lacking. As expected, the frequencies of the *CYP2C9⋆2*, *⋆3*, *⋆4* and *⋆5* alleles in the Belgian Caucasian subjects are similar to those of other Caucasian populations [5, 11, 12]. The absence of a *CYP2C9⋆2* allele in the Beninese group differs significantly from its frequency of 2.5% ($P = 0.03$) reported in 120 African-Americans by Dickmann *et al*. [7], whereas the frequency of *CYP2C9⋆3* was similar in the two groups ($P = 0.2$). The frequencies of *CYP2C9⋆2* and *⋆3* differ significantly from those in 150 Ethiopians [4% ($P = 0.001$) and 2% ($P = 0.02$), respectively] [13], and from those in 247 Egyptians [12% ($P < 0.001$) and 6% ($P < 0.001$), respectively] [14].

These discrepant data suggest genetic heterogeneity among African populations. The absence of *CYP2C9⋆2* was reported previously in Oriental and Inuit populations [11, 15, 16], and the latter did not also possess the *CYP2C9⋆3* allele [11]. In contrast, current and previous data suggest that *CYP2C9⋆2* and *⋆3* alleles are found more frequently in Caucasian (6–13%) than in Black populations [4, 5, 7, 11, 12, 17].

The frequency of *CYP2C9⋆5* allele in Beninese (1.8%) appears slightly higher than in Tanzanians (0.82%) [12], but is similar to the frequency reported in African Americans (1.7%) [8]. *CYP2C9⋆5* is not found in the Belgian or other Caucasian populations [12].

Whereas the functional importance of *CYP2C9⋆11* has not been assessed, it is thought that the activity of

Table 2 Allele frequencies (a) and genotype prevalences (b) for *CYP2C9* and *CYP2C19* in a Beninese and Belgian Caucasian population.

	Frequency in Belgian population % (95% CI)	*Frequency in Beninese population % (95 CI)*	*95% CI on the difference*	P-*value*
a Variant allele				
CYP2C9 *allele*				
*CYP2C9*1*	82.2 (76.7, 86.7)	95.5 (91, 97.3)	0.07, 0.19	<0.001
*CYP2C9*2*	10.0 (6.6, 14.5)	0	−0.14, −0.06	<0.001
*CYP2C9*3*	7.4 (4.6, 11.7)	0	−0.11, −0.04	<0.01
*CYP2C9*4*	0	0	−0.01, 0.01	NS
*CYP2C9*5*	0	1.8 (0.5, 4.8)	0.00, 0.04	0.05
*CYP2C9*11*	0.4 (0.0, 2.6)	2.7 (1.1, 6)	0.00, 0.05	<0.05
CYP2C19 *allele*				
*CYP2C19*1*	90.9 (86.4, 94.1)	87 (81.6, 91)	−0.10, 0.02	NS
*CYP2C19*2*	9.1 (6, 13.6)	13 (9, 18.4)	−0.02, 0.10	NS
*CYP2C19*3*	0	0	−0.01, 0.01	NS
b Genotype				
CYP2C9				
*CYP2C9*1/*1*	67 (57.7, 75.0)	91 (83.6, 95.3)	0.13, 0.34	<0.00001
*CYP2C9*1/*2*	18.2 (12, 26.4)	0	−0.26, −0.12	0.0001
*CYP2C9*1/*3*	11.6 (6.7, 19)	0	−0.18, −0.06	0.001
*CYP2C9*1/*5*	0 (0, 3.8)	3.6 (1.1, 9.5)	0.00, 0.09	0.05
*CYP2C9*1/*11*	0.8 (0.04, 5.2)	5.4 (2.2, 11.8)	0.00, 0.10	0.05
*CYP2C9*2/*3*	1.6 (0.3, 6.4)	0	−0.06, 0.02	NS
*CYP2C9*3/*3*	0.8 (0.04, 5.2)	0	−0.04, 0.02	NS
CYP2C19				
*CYP2C19*1/*1*	83.5 (75.4, 89.4)	73.9 (64.5, 81.5)	−0.20, 0.01	NS
*CYP2C19*1/*2*	14.9 (9.3, 22.7)	26.1 (18.4, 35.4)	0.00, 0.22	<0.05
*CYP2C19*1/*3*	0	0	−0.03, 0.03	NS
*CYP2C19*2/*2*	1.6 (0.3, 6.4)	0	−0.06, 0.02	NS
*CYP2C19*2/*3*	0	0	−0.03, 0.03	NS
*CYP2C19*3/*3*	0	0	−0.03, 0.03	NS

CI, Confidence interval; NS, no significant differences ($P > 0.05$). *CYP2C9*1* and *CYP2C19*1* wild-type alleles frequencies have been inferred from the observed frequencies of mutated alleles, as assessed in the study. Calculation of allele frequencies was made on the assumption of compound heterozygosity when two polymorphisms were observed.

CYP2C9 in heterozygous genotypes is less than in homozygous wild-type genotypes [17]. Individuals homozygous for the *CYP2C9*3* allele show markedly decreased metabolic capacities for most CYP2C9 substrates [1, 3, 5]. The frequency of these PM subsets (0.8%) in the Belgian is similar to that in other Caucasian populations [4, 5, 13, 17].

Our data on *CYP2C19*2* and **3* allele frequencies are also in agreement with previous data obtained from Zimbabweans and Tanzanians [18], as well as in Dutch [19], French [20], German [21] or Egyptian populations [14]. In the Venda (South Africa), *CYP2C19*2* was found at a high frequency (21.7%) [18], but *CYP2C19*3* was also absent. The frequency of *CYP2C19*3* was reported to be higher (between 5 and 11%) in Oriental populations [2, 14, 22]. Considering that *CYP2C19*2* and **3* account for approximately 83% of all PM alleles in Caucasians [8], we estimate a PM phenotype frequency of approximately 2% in the Belgian population. In contrast, we found no homozygous or compound heterozygous *CYP2C19* mutations in the Beninese population. These findings suggest that the prevalence of PM phenotype with respect to CYP2C19 in the Beninese could be lower than in Belgians.

In conclusion, frequencies of *CYP2C9*2* and **3* alleles appear higher in Caucasian than in Black populations. Conversely, the frequency of *CYP2C9*5* allele is higher in the Beninese population than in Belgians or other Caucasian populations. Likewise, the frequency of the *CYP2C9*11* allele is significantly higher in Beninese than in Belgian Caucasians. The allelic distributions of *CYP2C19*2* and **3* are comparable in both ethnic groups.

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