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Ethnic differences in CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) genotypes in Japanese and Israeli populations

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Abstract

CYP2C9 is a major P450 2C enzyme, which hydroxylates about 16% of drugs that are in current clinical use and contributes to the metabolism of a number of clinically important substrate drugs such as warfarin. Ethnic differences in the genetic variation of CYP2C9 have been reported, and might be related to the frequencies of adverse reactions to drugs metabolized by CYP2C9 in different ethnic groups. In the present study, ethnic differences in the CYP2C9*2 and CYP2C9*3 allele distribution in Japanese and Israeli populations were evaluated using a newly developed oligonucleotide based DNA array (OligoArray^R).

The population studied consisted of 147 Japanese and 388 Israeli donors (100 Ashkenazi Jews, 99 Yemenite Jews, 100 Moroccan Jews and 89 Libyan Jews). The CYP2C9*2 [Arg144Cys (416 C>T), exon 3] and CYP2C9*3 [Ile359Leu (1061 A>C), exon 7] genotypes were determined using an OligoArray^R. The accuracy of genotyping by the OligoArray^R was verified by the fluorescent dye-terminator cycle sequencing method. A Hardy–Weinberg test indicated equilibrium (χ^2<3.84 is Hardy–Weinberg) in all populations. The CYP2C9*2 genotype (CC/CT+TT) was absent in Japanese (1/0) (OR 0.02), and its frequency was significant in Libyan Jews (0.697/0.303) (OR 2.13; 95% CI 1.07–4.24) compared with Ashkenazi Jews (0.83/0.17), Yemenite Jews (0.899/0.101), and Moroccan Jews (0.81/0.19). The frequencies of CYP2C9*3 genotype (AA/AC+CC) was significantly lower in Japanese (0.986/0.014) (OR 0.08), and was higher in Libyan Jews (0.652/0.348) (OR 3.03; 95% CI 1.5–6.1) and Moroccan Jews (0.77/0.23) (OR 1.69; 95% CI 0.62–3.48) compared with those in Ashkenazi Jews (0.85/0.15) and Yemenite Jews (0.849/0.151). Thus, the CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) variants were rare in the Japanese population, and showed different frequencies in the four Jewish ethnic groups examined.

Keywords: Single nucleotide polymorphism; CYP2C9; Genotyping; DNA array; Ethnic differences; Pharmacogenetics; Adverse drug reactions

Introduction

CYP2C9 is a major P450 2C enzyme in human liver and contributes to the metabolism of a number of clinically important substrate drugs, including some anticoagulant and antihypertensive drugs (Hermida et al., 2002). The CYP2C9*3 and CYP2C9*2 variants are associated with hypersensitivity to the anticoagulant drug warfarin. A "poor metabolizer" phenotype leading to the slow metabolism of warfarin may lead to the adverse effect of bleeding in individuals carrying the CYP2C9*3 or the CYP2C9*2 allelic variants (Higashi et al., 2002).

Ethnic differences in the genetic variation of CYP2C9 have been reported previously (Kimura et al., 1998; Gaedigk et al., 2001; Yasar et al., 2002; Schwartz, 2003; Zhao et al., 2004). In the present study, the CYP2C9*2 (Arg144Cys, exon 3) and CYP2C9*3 (Ile359Leu, exon 7) variants were determined in Japanese and Israeli populations using a newly developed oligonucleotide based DNA array (OligoArray^R).

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Study participants and methods

The populations studied consisted of 147 Japanese(96 male/51 female) and 388 Israeli donors (100 Ashkenazi Jews, 99 Yemenite Jews, 100 Moroccan Jews and 89 Libyan Jews). The DNA from all Japanese donors was obtained based on informed consent as approved by the ethics committee of Iwate Medical University (H12-25). Genomic DNA was prepared from lymphocytes using proteinase K digestion and precipitation of proteins with salt, followed by a second DNA precipitation with ethanol. The DNA from the Israeli populations was obtained from the National Laboratory for the Genetics of Israeli Populations (see: http://nlgip.tau.ac.il) based on informed consent as approved by the ethics committee of Tel Aviv University, Israel (see: http://nlgip.tau.ac.il).

Selection of candidate genes and SNPs

Two SNPs of the CYP2C9*2 [Arg144Cys (416 C>T), exon 3] and CYP2C9*3 [Ile359Leu (1061 A>C), exon 7] were selected, as shown in Table 1. We designed polymerase chain reaction (PCR) primers to amplify the regions surrounding these SNPs using a computer software (Primer Express, Ver. 1.0, Applied Biosystems, Foster City, CA). Genomic DNA sequences around the SNP sites were obtained from GenBank (http://www.ncbi.nlm.nih.gov/).

DNA genotyping by oligonucleotide based DNA array (OligoArray^R)

PCR amplification

All SNPs were amplified simultaneously in a 50 μl reaction containing 25–100 ng of genomic DNA or a sample of DNA extracted from human blood, 25 μl of Multiplex PCR Master Mix (Qiagen Inc., Valencia, CA), and 200 nM of each primer. PCR was performed using a DNA engine (MJ Research Inc., Waltham, MA) as follows: preheating 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, with a post-cycling extension at 72 °C for 3 min.

Oligonucleotide based DNA array (OligoArray^R) preparation

Oligonucleotides for the OligoArray^R were commercially synthesized (Nisshinbo Industries, Inc., Chiba, Japan). Each SNP was located at the center of the oligomers. DNA oligomers were immobilized on CarboStation^R slides (Nisshinbo Industries, Inc., Chiba, Japan) according to the manufacturer's instructions as follows. Each 20 pmol/μl aliquot of oligomer was mixed with the same volume of Micro Spotting Solution (Telechem, Tokyo, Japan) and spotted on CarboStation^R slides. The slides were irradiated with UV light at 600 mJ/cm^2 using a UV crosslinker (Stratagene, LaJolla, California) and then placed in blocking solution (per liter, 6.1 g 2-amino-2-hydroxymethyl-1, 3-propanediol, 5.9 g NaCl, 0.3 g Triton-X-100, 15.0 g BSA [Sigma, A2153]) for 30 min. The slides were then washed with TE buffer (10 mM Tris–HCl, 1 mM EDTA, 0.1 M NaCl, pH 8.0), and air-dried at room temperature.

Hybridization and signal detection

The schematic steps used in OligoArray^R hybridization are shown in Fig. 1. Four microliters of denatured PCR product and 16 μl of hybridization buffer (Nisshinbo Industries, Inc., Chiba, Japan) were mounted on an OligoArray^R with a cover glass, and hybridized at 37 °C for 2 h. After hybridization, the slide was washed in a washing buffer (Nisshinbo Industries, Inc.) at 37 °C for 5 min. Hybridization signals were visualized using the ABC method for coloring. A conjugate solution 1.4 ml was prepared from a kit (Nisshinbo Industries, Inc., Chiba, Japan) according to the manufacturer's instructions, and was mounted on the OligoArray^R which was then incubated for 30 min at room temperature. Each slide was washed twice with coloring buffer (Nisshinbo Industries, Inc., Chiba, Japan) at room temperature for 5 min. The coloring solution, prepared from the above kit according to the manufacturer's instructions, was mounted on the OligoArray^R and the assembly was then incubated for 30 min at room temperature. The OligoArray^R was rinsed with distilled water, air-dried, and then scanned using a GT-8700F scanner (Epson, Tokyo, Japan).

Statistical analysis

Data were compiled according to genotype. Statistical analyses were calculated by Fisher's exact test, and odds ratio and 95% confidence interval (CI) were calculated with the frequency of the genotype in Ashkenazi Jews. The Hardy–Weinberg test was applied to all populations. The Hardy–Weinberg test indicated equilibrium if $\chi^2<3.84$. InStat (GraphPad software, CA, USA) was used for statistical calculations.

Table 1
Selection of candidate genes and SNPs for the CYP2C9*2 and CYP2C9*3 variants

Gene	Allele	Nucleotide change	Primer
CYP2C9	CYP2C9*2	Arg144Cys	F: aagaaatggaaggagatccgg
		(416 C>T)	R: tgatatggagtagggtcacccac
CYP2C9	CYP2C9*3	Ile359Leu	F: ctccttttccatcagtttttact
		(1061A>C)	R: gatactatgaatttgggacttc

Results

The CYP2C9*2 (416 C>T, exon 3) and CYP2C9*3 (1061 A>C, exon 7) variants were determined by the OligoArray^R method. The accuracy of this method was verified by a fluorescent dye-terminator cycle sequencing method. Three representative cases in each genotype are presented in Fig. 2. The results of the Hardy–Weinberg tests are presented in Table 2. The Hardy–Weinberg test demonstrated equilibrium ($\chi^2<3.84$) in all populations. The CYP2C9*2 genotype (CC/CT+TT) was absent in Japanese (1/0) (OR 0.02), and its frequencies were higher in Libyan Jews (0.697/0.303) (OR 2.13; 95% CI 1.07–4.24) compared with Ashkenazi Jews (0.83/0.17), Yemenite Jews (0.899/0.101), or Moroccan Jews (0.81/0.19). The frequency of the CYP2C9*3 genotype (AA/