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

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Received 1 December 2004; accepted 12 April 2005

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®).

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®. The accuracy of genotyping by the OligoArray® was verified by the fluorescent dye-terminator cycle sequencing method. A Hardy–Weinberg test indicated equilibrium ($\chi^2 < 3.84$ 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.

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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®).
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 (OligoArrayR)

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 (OligoArrayR) preparation

Oligonucleotides for the OligoArrayR were commercially synthesized (Nissinbo Industries, Inc., Chiba, Japan). Each SNP was located at the center of the oligomers. DNA oligomers were immobilized on CarboStationR 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 CarboStationR slides. The slides were irradiated with UV light at 600 mJ/cm² 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 OligoArrayR hybridization are shown in Fig. 1. Four microliters of denatured PCR product and 16 μl of hybridization buffer (Nissinbo Industries, Inc., Chiba, Japan) were mounted on an OligoArrayR with a cover glass, and hybridized at 37 °C for 2 h. After hybridization, the slide was washed in a washing buffer (Nissinbo 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 (Nissinbo Industries, Inc., Chiba, Japan) according to the manufacturer’s instructions, and was mounted on the OligoArrayR which was then incubated for 30 min at room temperature. Each slide was washed twice with coloring buffer (Nissinbo 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 OligoArrayR and the assembly was then incubated for 30 min at room temperature. The OligoArrayR 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 χ²<3.84. InStat (GraphPad software, CA, USA) was used for statistical calculations.

Results

The CYP2C9*2 (416 C>T, exon 3) and CYP2C9*3 (1061 A>C, exon 7) variants were determined by the OligoArrayR 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 (χ²<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/
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 3.03; 95% CI 1.5 – 6.1), compared with Ashkenazi Jews (0.85 / 0.15), Yemenite Jews (0.849 / 0.151). Thus, The CYP2C9*2 (416 C>T, exon 3) and CYP2C9*3 (1061 A>C, exon 7) variants were rare in the Japanese population, and showed different frequencies in the four Jewish ethnic groups examined.

Discussion

The purpose of this study was to evaluate ethnic differences of the CYP2C9*2 (Arg144Cys, exon 3) and CYP2C9*3 (Ile359Leu, exon 7) variants. An OligoArrayR method was developed for screening multiplexed genotyping of the SNPs for the CYP2C9 gene. That genotyping by this OligoArrayR method was highly accurate verified by a fluorescent dye-terminator cycle sequencing method. Ethnic differences in the frequencies of the CYP2C9*2 (416 C>T, exon 3) and CYP2C9*3 (1061 A>C, exon 7) variants were found in Japanese in comparison with the four Israeli populations.

Our observations indicate that the CYP2C9*2 (416 C>T, exon 3) variant was absent in the Japanese group, in agreement with previous studies (Nasu et al., 1997; Kimura et al., 1998; Hiratsuka et al., 1999). Moreover, we observed different frequencies for the CYP2C9*2 (416 C>T, exon 3) variant in the four Jewish ethnic groups that were included in the present study, being 2-fold (OR 2.13) more frequent among Libyan Jews compared with Ashkenazi Jews. The CYP2C9*3 (1061 A>C, exon 7) variants determination also showed clear differences in the frequencies among the various ethnic groups studied, being the most frequent among Libyan Jews compared with Ashkenazi Jews. The CYP2C9*3 (1061 A>C, exon 7) variants determination also showed clear differences in the frequencies among the various ethnic groups studied, being the most frequent among the Libyan Jews (OR 3.03). However, the differences in the CYP2C9*3 (1061 A>C, exon 7) variant were not as large as for the CYP2C9*2 allele. This suggests that the CYP2C9*2 (416 C>T, exon 3) variant appeared more recently in the course of human history.

These differences indicate that members of certain Israeli ethnic groups might be at higher risk for adverse drug reactions.

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Fig. 1. The schematic procedure for hybridization and color development of the oligonucleotide based DNA array method (OligoArrayR).

Fig. 2. Representative genotyping of the OligoArrayR system.
when treated with common drugs that are metabolized by P-450 2C9, such as warfarin and losartan. Further studies will be required to more fully characterize the ethnic differences in CYP2C9 genotypes and their postulated clinical relevance. This will involve the use of larger numbers of donors and additional ethnic groups. The OligoArray® method was found to be convenient for the reliable genotyping of CYP2C9, and has the potential for routine use, after appropriate modification in the clinic for detecting susceptible individuals prior to drug treatment decisions.

It is important to understand how variations in molecular function conferred by a single nucleotide polymorphism in one or more genes that modulate drug actions cause variability in drug response and in drug safety. The incorporation of genetic information into the routine prescribing of drugs may become standard to avoid serious toxicity with narrow-therapeutic index drugs such as warfarin, and to maximize the likelihood of a positive response in the individual patient (Evans and Relling, 1999; Iqbal, 2002; Gurwitz and Weizman, 2004; Frueh and Gurwitz, 2004). CYP2C9 variants that drastically decrease the catalytic activity of the enzyme occur frequently in individuals. Subjects that are homozygous for CYP2C9*3 display striking elevations in S-warfarin plasma concentrations, and as a result, the effects of warfarin can be greatly magnified with standard dosages (Evans and Johnson, 2001). Up to 80% of the carriers of a single copy of the CYP2C9*3 variant may need a low initial loading dose of the anticoagulant drug acenocoumarol (Hermida et al., 2002). CYP2C9*2 and CYP2C9*3 polymorphisms are associated with an increased risk of excess anticoagulation and bleeding events among patients who received an anticoagulant drug warfarin in clinical setting (Higashi et al., 2002; Takahashi et al., 2003). Screening for CYP2C9 variants may therefore allow clinicians to develop dosing protocols and surveillance techniques for reducing the risk of adverse drug reactions in patients receiving warfarin or acenocoumarol for anticoagulant therapy.

Ethnic differences in the CYP2C9 allele have been previously elucidated (Nasu et al., 1997). Yasar et al. reported that the frequencies of the CYP2C9*1, CYP2C9*2 and CYP2C9*3 alleles in a Swedish population were 0.819, 0.107, and 0.074, respectively (Yasar et al., 1999). Gaedigk et al. reported that the 2C9*2 variant appears to be absent in Chinese and Inuit populations, but was present in Native Canadian Indian and Caucasian subjects at frequencies of 0.03 and 0.08–0.15, respectively (Gaedigk et al., 2001). The CYP2C9*3 variant was not detected in the Inuit group, but occurred in the Native Canadian Indian group at a frequency comparable to that of other ethnic groups. This group of Inuit individuals represents the first population in which no CYP2C9*2 or CYP2C9*3 variants have been detected so far. In the present study, the CYP2C9*2 variant was absent, and the frequency of the CYP2C9*3 variant was low in the Japanese population studied compared with those for the four Israeli populations included in the present study. Hardy–Weinberg equilibrium is usually applied to assess the proper distribution of genotypes or the verification of a hypothesis. We verified the reliability of our DNA samples by a Hardy–Weinberg test (Table 2). The Hardy–Weinberg test indicated equilibrium ($\chi^2 < 3.84$) in all populations. Collectively, these findings suggest that both the CYP2C9*2 and CYP2C9*3 variants appear to be relatively rare in Chinese, Japanese, and Inuit populations compared with Caucasian populations.

The development of genomic science raises the possibility that this approach could be much more widely applied to therapeutics. Several methods have been reported for CYP2C9 genotyping using PCR-RFLP techniques and the Rapid Thermal Cycler method (Gaedigk et al., 2001; Yasar et al., 1999; Hiratsuka et al., 1999). Wen et al. reported the rapid detection of the known SNPs of CYP2C9 using an oligonucleotide microarray (Wen et al., 2003). A reliable and inexpensive method for CYP2C9 genotyping will be required for its routine use in the clinic. In the present study, an OligoArray® method was found to be both reliable and cost-
effective for CYP2C9 genotyping and has the potential for clinical applications.

The ultimate goal of pharmacogenomics is to use genetic information to deliver personalized medicine, “the right drug at the right dose to the right patient at the right time” (Evans and Relling, 1999). In the future, one could envision an individualized therapeutic approach based on genotyping for specific sets of polymorphisms.

Acknowledgements

This study was supported by a Grant-in-aid for Scientific Research (No. 15590768) in Japan Society for the Promotion of Science, the Test of Fostering Potential of Japan Science and Technology Corporation, and Open Research Translational Research Center Project, Advanced Medical Science Center, Iwate Medical University.

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