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Research Paper

Combination Analysis in Genetic Polymorphisms of Drug-Metabolizing Enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A5 in the Japanese Population

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Abstract

The Cytochrome P450 is the major enzyme involved in drug metabolism. CYP enzymes are responsible for the metabolism of most clinically used drugs. Individual variability in CYP activity is one important factor that contributes to drug therapy failure. We have developed a new straightforward TaqMan PCR genotyping assay to investigate the prevalence of the most common allelic variants of polymorphic CYP enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A5 in the Japanese population. Moreover, we focused on the combination of each genotype for clinical treatment. The genotype analysis identified a total of 139 out of 483 genotype combinations of five genes in the 1,003 Japanese subjects. According to our results, most of subjects seemed to require dose modification during clinical treatment. In the near future, modifications should be considered based on the individual patient genotype of each treatment.

Key words: Cytochrome P450, TaqMan-PCR, dried saliva, Japanese population, Genetic analysis.

Introduction

Cytochrome P450 (CYP) enzymes constitute the major metabolizing enzyme system in humans, and are responsible for the metabolism of exogenous compounds, including many drugs, carcinogens, mutagens and alcohols [1,2]. Generally, the CYP enzymes represent 70–80% of phase I metabolism and are responsible for the biotransformation of lipophilic drugs to polar metabolites, which can be excreted by the kidneys [3]. The human hepatic CYP system consists of more than 30 related isoenzymes with different, sometimes overlapping substrate specificities [4]. A major cause of inter-individual variability of drug pharmacokinetics is the genetic polymorphism of metabolizing enzymes [5]. In many cases, *CYP* polymorphism has a major variable affecting drug plasma concentration, drug detoxification, and drug activation in the case of a prodrug [6]. Several of the major genetic polymorphisms affecting drug-metabolizing enzyme activity of potential clinical relevance are those related to the CYP450 enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A5.

CYP1A2 is one of the drug-metabolizing enzymes of major importance, as it catalyzes the phase I metabolism of a broad range of different drugs, including caffeine, lidocaine, theophylline, and propranolol [7, 8]. *CYP2C9* is involved in the metabolism of many clinically important drugs, including (*S*)-warfarin, losartan and phenytoin [9, 10]. Ethnic distributions of the *CYP2C9* polymorphisms are clinically significant in anticoagulation therapy because

CYP2C9 is the major P450 that inactivates the active *S*-enantiomer of warfarin [6]. CYP2C19 catalyzes the metabolism of many commonly used drugs, including omeprazole [11], phenytoin [12], and diazepam [13]. Most poor metabolization of CYP2C19 is attributable to the *CYP2C19*2* and *3 alleles. Proton pump inhibitors, which are used for the treatment of gastric acid-related disease, are mainly metabolized by CYP2C19 in the liver [14]. CYP2D6 is responsible for the metabolism of approximately 20 to 25% of all marketed drugs [15]. More than 50 clinically important drug substrates of CYP2D6 have been identified [16], including tamoxifen [17], codeine [18] and dextromethorphan [19]. *CYP2D6* is highly polymorphic. Variant alleles of *CYP2D6* are classified on the basis of enzymatic activities. *CYP2D6* has complex alleles such as *CYP2D6*36-*10* Tandem-type [20]. However, these two alleles *10 and *5 are considered to be sufficient as a minimum genetic information before personalizing medication based on genotype [21,22]. *CYP2D6*5* has been reported previously [23]. CYP3A4/5 metabolize a broad range of structurally diverse therapeutic compounds, including tacrolimus [24,25] and midazolam [26]. Since the minor allele frequencies of CYP3A4 SNPs are low in the Japanese population, we focused on the *CYP3A5* gene. The *CYP3A5*3* allele is the most frequently occurring allele of *CYP3A5* and contains a single nucleotide polymorphism (SNP) that introduces a frame shift during translation, resulting in a truncated, non-functional protein [27].

The previous study reported on the enzyme activities and genotype associations of *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, and *CYP3A4/5* to evaluate the effect among native Japanese, Chinese, Koreans, and Caucasians, using caffeine, midazolam, omeplazole, dextromethorphan, chlozoxazone and losartan as panels of specific CYP probe substrates [28]. Furthermore, other studies have reported on population and combination analysis of genetic polymorphism for *CYP* genes in Greek subjects [29] and Chinese subjects [30].

The purpose of the present study was to investigate the prevalence of the most common allelic variants of *CYP1A2*1C*, *CYP2C9*3*, *CYP2C19*2, *3*, *CYP2D6*10* and *CYP3A5*3*, as well as the number and frequencies of individuals with genotypes predictive of extensive metabolizers (EM), poor metabolizers (PM), intermediate metabolizers (IM), and the phenotypic status in a representative sample of the Japanese population. Although a large number of studies have been carried out on each genotype analysis, no study has ever attempted to analyze the combination of many genotypes. Therefore we focused on the combination of each genotype for clinical treatment.

Genetic variation in drug metabolism could cause therapeutic failures, adverse drug effects or even fatal drug intoxications [15]. The development of molecular methods for genotyping could provide researchers with the tools to pinpoint the genetic differences between individuals and in some cases give the prescribing clinician a means to improve the pharmacotherapeutic regimen of each patient on the basis of the genetic profile, thus reducing harmful side-effects or inadequate drug response [29]. Despite the advantages of *CYP* genotyping in drug therapy, currently it is rarely performed in clinical practice. Our results contribute to the overall knowledge of the Japanese distribution of the genetically controlled metabolism of several important drugs and may help in the optimization of pharmacotherapy in ethnically Japanese subjects.

Materials and methods

Human Genomic DNA Samples: A total of 1,017 non-related Japanese subjects participated in each genotype determination of the allele frequencies of *CYP1A2*1C, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2D6*10* and *CYP3A5*3*. The saliva of the healthy Japanese volunteers was analyzed by TaqMan PCR (Applied Biosystems, Foster City, CA) assay. The Medical Ethics Committee of Mukogawa Women's University approved the study protocol. Informed consent was obtained from all subjects. The subjects were asked to rub their inner cheek surfaces against their teeth and to collect saliva in small plastic tubes. Then ca. 50 µL of the saliva were put on pieces of water-soluble paper (Nippon Paper Papylia Co., Ltd., Mishima Dissolve Paper, 60MDP), and were dried for one hour at room temperature.

Detection of the *CYP* genotyping by TaqMan PCR assay: SNPs of each gene were genotyped by TaqMan assay on ABI 7300 Real Time PCR System (Applied Biosystems). The TaqMan assay was performed with a 20 µL reaction volume and consisted of 10 µL of Thunderbird probe qPCR Mix (TOYOBO), 0.4 µL of 50×ROX reference dye (TOYOBO), 1 µL of 20×each TaqMan probe & each Primer Mix, 2 µL of 2×PCR Buffer for KOD FX Neo (TOYOBO), and 6.6 µL of distilled water. The dried saliva was punched with a 1.2 mm diameter disk and put into the reaction mixture directly without DNA extraction. The thermal cycling process was performed according to the Applied Biosystems PCR conditions; 2 min at 50°C, 10 min at 95°C, 40 cycles of denaturation at 95°C for 15s, and annealing and extension at 60°C for 1 min. The results were analyzed by ABI Prism 7300 SDS software. TaqMan probe assay IDs indicate that CYP1A2*1C: C__15859191_30, CYP2C9*3: C___27104892_10, CYP2C19*2: C__25986767_70,

CYP2C19*3: C__27861809_10, CYP2D6*10: C__11484460_40, CYP3A5*3: C__26201809_30.

Method validation: The TaqMan assay was validated for system accuracy. The method validation was performed by the PCR-RFLP method using 219 samples. The PCR-RFLP protocols were shown in Supplementary Material: Table S1.

Results and discussion

Pharmacogenetic research has gained enormous momentum, with recent advances in molecular genetics and genome sequencing [31]. CYP enzymes metabolize more than 80% of drugs used in clinical settings [32]. Since five *CYP* genes, *CYP1A2, CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP3A5* are medically significant, genotyping them could help medical treatment and optimization of drug therapy. In particular, most Japanese have mutations in these genes compared to Caucasians and it is already known that deficiencies of these genes may result in a need for dose adjustment. Genetic variation in drug metabolism can lead to therapeutic failures, adverse drug effects or even fatal drug intoxications [15].

The allele frequencies of the analyzed five genes were shown in Table 1. These results gave us a similar distribution to the previous report [28]. It appears that the frequencies of these alleles in Japanese individuals are more polymorphic than in Caucasian individuals. Deviations from Hardy-Weinberg equilibrium were not statistically significant in the individual polymorphisms except for *CYP2D6*10* (CYP1A2; χ^2=0.42, CYP2C9;χ^2=0.76, CYP2C19*2; χ^2 =1.64, CYP2C19*3; χ^2 =0.61, CYP2D6; χ^2 =55.89, CYP3A5; χ^2 =0.35, df = 1, P > 0.05). The genotype frequency of *CYP2D6*10* was not in Hardy-Weinberg equilibrium, partly because the extreme polymorphisms of *CYP2D6* gene, such as deletions and duplications, had been observed in the Japanese population [17, 20].

Table 1. Allele frequencies of *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP3A5*(n=1017).

Gene	Variant allele	This study	Japanese[28]	Caucasian[28]
CYP1A2	**1C*	0.234	0.319	0.010[a]
CYP2C9	**3*	0.027	0.035	0.056[a]
CYP2C19	**2*	0.295	0.345	0.136[a]
	**3*	0.111	0.090	0.000[a]
CYP2D6	**10*	0.427	0.435	0.196[a]
CYP3A5	**3*	0.762	0.780	0.955[a]

[a] Significantly different (P< 0.05) mean allelic frequency compared with this study mean (Pearson's chi-square test).

The results of the TaqMan assay were completely consistent with those of the PCR-RFLP method (data not shown). The high accuracy of the results of the two different experiments proves that the TaqMan PCR assay was a simple and reliable genotyping method. Since we used a sample of dried saliva on water-soluble papers, we did not require DNA extraction and it was possible to put the sample directly into the reaction tubes [33]. Furthermore, this simple sampling method can minimize contaminants during the experiment.

The genotype distributions of five genes were shown in Table 2. None of these subjects was found to be homozygous for the *CYP2C9*3* allele. The fourteen subjects could not define *CYP2D6* genotype. The polymorphism of these subjects seems to be homozygous for *CYP2D6*5*, because this polymorphism is the deletion mutant. The previous reports suggested that the *CYP2D6*5* allele was about 6% [34]. The *CYP2D6*1/*5* and **5/*10* were included in **1/*1* and **10/*10*, respectively. In the future, detailed analysis of *CYP2D6*5* genotype will have to be carried out using our newly developed long PCR method [23].

Table 2. Genotype frequencies of *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP3A5* in the Japanese population (n=1017).

Gene	Genotypes	Phenotypes	Number of subjects	Observed frequency (%)
CYP1A2	**1A/*1A*	EM	593	58.3
	**1A/*1C*	IM	372	36.6
	**1C/*1C*	PM	52	5.1
CYP2C9	**1/*1*	EM	963	94.7
	**1/*3*	IM	54	5.3
	**3/*3*	PM	0	0
CYP2C19	**1/*1*	EM	345	33.9
	**1/*2*	IM	377	37.1
	**1/*3*	IM	142	14.0
	**2/*2*	PM	80	7.9
	**2/*3*	PM	63	6.2
	**3/*3*	PM	10	1.0
CYP2D6	**1/*1*	EM	387	38.1
	**1/*10*	IM	375	36.9
	**10/*10*	IM	241	23.7
	Undefined		14	1.4
CYP3A5	**1/*1*	EM	61	6.0
	**1/*3*	IM	362	35.6
	**3/*3*	PM	594	58.4

In general, the dosage of the drug would be in accordance with the mode values of the genotype, which are *CYP1A2*1A/*1A*, *CYP2C9*1/*1*, *CYP2C19*1/*2*, *CYP2D6*1/*1* and *CYP3A5*3/*3*, in each drug metabolic enzyme. However, the dosage seems to be set and does not appear to produce ad-

verse effects in clinical studies. In other words, most Japanese have be administered under lower recommended dosage of drugs. We focused on the combination of each genotype for clinical treatment, because a large number of studies have been performed on each genotype analysis, and no study has ever attempted to analyze the combination of many genotypes. The genotype analysis identified a total of 139 out of 483 genotype combinations of five genes in the 1,003 Japanese subjects (except for CYP2D6 undefined fourteen subjects), which is shown in Supplementary Material: Table S2. Most of these combinations appeared in less than 1% of all samples. The thirty combinations with frequencies greater than or equal to 1% were used as a focus group, making up 68.0% of all samples shown in Table 3. The highest frequency was 5.1%. These results suggest that there are varieties of combinations in the Japanese populations. The dose modifications should be considered based on individual patient genotype for clinical treatment. Moreover, the genetic analysis of these five *CYP* genes would be very helpful for drug treatment in clinical studies.

Table 3. The combined genotype frequency of *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP3A5* in the Japanese population.

	Genotype						
No.	*CYP1A2*	*CYP2C9*	*CYP2C19*	*CYP2D6*	*CYP3A5*	Subject	%
1	*1A/*1A	*1/*1	*1/*1	*1/*1	*1/*3	33	3.3%
2	*1A/*1A	*1/*1	*1/*1	*1/*1	*3/*3	44	4.4%
3	*1A/*1A	*1/*1	*1/*1	*1/*10	*1/*3	23	2.3%
4	*1A/*1A	*1/*1	*1/*1	*1/*10	*3/*3	25	2.5%
5	*1A/*1A	*1/*1	*1/*1	*10/*10	*1/*3	17	1.7%
6	*1A/*1A	*1/*1	*1/*1	*10/*10	*3/*3	25	2.5%
7	*1A/*1A	*1/*1	*1/*2	*1/*1	*1/*3	35	3.5%
8	*1A/*1A	*1/*1	*1/*2	*1/*1	*3/*3	37	3.7%
9	*1A/*1A	*1/*1	*1/*2	*1/*10	*1/*3	27	2.7%
10	*1A/*1A	*1/*1	*1/*2	*1/*10	*3/*3	51	5.1%
11	*1A/*1A	*1/*1	*1/*2	*10/*10	*1/*3	18	1.8%
12	*1A/*1A	*1/*1	*1/*2	*10/*10	*3/*3	30	3.0%
13	*1A/*1A	*1/*1	*1/*3	*1/*1	*3/*3	21	2.1%
14	*1A/*1A	*1/*1	*1/*3	*1/*10	*1/*3	11	1.1%
15	*1A/*1A	*1/*1	*1/*3	*1/*10	*3/*3	17	1.7%
16	*1A/*1A	*1/*1	*1/*3	*10/*10	*3/*3	10	1.0%
17	*1A/*1A	*1/*1	*2/*2	*1/*1	*3/*3	11	1.1%
18	*1A/*1A	*1/*1	*2/*2	*1/*10	*3/*3	11	1.1%
19	*1A/*1C	*1/*1	*1/*1	*1/*1	*1/*3	14	1.4%
20	*1A/*1C	*1/*1	*1/*1	*1/*1	*3/*3	37	3.7%
21	*1A/*1C	*1/*1	*1/*1	*1/*10	*1/*3	13	1.3%
22	*1A/*1C	*1/*1	*1/*1	*1/*10	*3/*3	27	2.7%
23	*1A/*1C	*1/*1	*1/*1	*10/*10	*3/*3	12	1.2%
24	*1A/*1C	*1/*1	*1/*2	*1/*1	*1/*3	11	1.1%
25	*1A/*1C	*1/*1	*1/*2	*1/*1	*3/*3	29	2.9%
26	*1A/*1C	*1/*1	*1/*2	*1/*10	*1/*3	18	1.8%
27	*1A/*1C	*1/*1	*1/*2	*1/*10	*3/*3	26	2.6%
28	*1A/*1C	*1/*1	*1/*2	*10/*10	*1/*3	10	1.0%
29	*1A/*1C	*1/*1	*1/*2	*10/*10	*3/*3	27	2.7%
30	*1A/*1C	*1/*1	*1/*3	*1/*10	*3/*3	12	1.2%
total						682	68.0%

Our result indicated 5% PM (**1C/*1C*) and 58% EM (**1A/*1A*) for *CYP1A2*, and indicated that these subjects require either dose increments or dose reductions. Nakajima *et al.* reported that the point mutation of *CYP1A2*1C* caused a significant decrease of CYP1A2 activity measured by the rate of caffeine 3-demethylation in Japanese smokers [35]. *CYP2C9*2* and *CYP2C9*3*, the two most common variant alleles, exhibit largely reduced enzymatic activities. The Japanese subjects with the *CYP2C9*1/*3* and *CYP2C9*3/*3* genotypes have lower warfarin doses than those with the *CYP2C9*1/*1* genotype [36]. Our result indicated 5.3% IM (**1/*3*) for *CYP2C9*, and indicated that these subjects would require dose modifications.

For the *CYP2C19* gene, there were many reports on the association between genes and clinical doses. Furuta *et al*. have reported that cure rates by lansoprazole therapy were 84.6% in PMs, 67.9% in IMs, and 45.8% in EMs [11]. The report also indicated that the mean values for the areas under the plasma - a concentration-time curve are about 13 times as high as those of the EM group. These clinical data would be useful for drug therapy in future studies. For lansoprazole therapy, our results indicated IM (**1/*2*, **1/*3*) and EM (**1/*1*) for *CYP2C19*, and indicated that these subjects required dose modifications.

According to the previous report with Tamoxifen therapy, the patients who have one or no normal allele of *CYP2D6* required increased dose adjustment [17]. Since tamoxifen is a pro-drug, it is impossible to achieve the expected effect on a patient who has a **10* allele. The 60.5% subjects (**1/*10*, **10/*10*) may require dosage adjustment in Japanese subjects for the *CYP2D6* gene.

In our study, the *CYP3A5*3* allele was found in abundance in the Japanese population with an indicated 76.2%. Tacrolimus has a low therapeutic index with a wide range of side effects and large interindividual variability in its pharmacokinetics, particularly in the dose required to reach target trough blood concentrations, thus necessitating routine therapeutic drug monitoring in clinical practice [6]. Interestingly, Tsuchiya *et al*. reported that the *CYP3A5*1* allele required a higher daily tacrolimus dose as compared to those of the *CYP3A5*3/*3* genotype in kidney transplant recipients [37]. According to our results, 41.6% EM and IM (**1/*1*, **1/*3*) subjects would require dose adjustment or change of drug in Japanese subjects. Although a few attempts have been made with *CYP3A5*, it would be valuable to examine the subjects more closely in clinical settings.

This study is the first to investigate a population data that includes more than a thousand subjects and the combined genotypes of five important genes,

which were *CYP* genes, *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP3A5*, all highly polymorphic genes in the Japanese population. Prospective clinical trials that evaluate the utility of genotyping and individualized medicine are critical in guiding clinical practice of individualized drug therapy [6].

Molecular genetic analysis could open new doors for targeted drug development and drug regulation to promote effective, safe, and cost-effective individualized drug therapy. Further prospective studies, showing a direct correlation between predictive genotyping and improvement of drug efficacy, could lead to the establishment of *CYP* genotyping [2]. If we knew our genotype information before treatment started, it would be possible to predict the severe side effects in clinical treatment. In medical settings, we expect the pharmacist to be able to design drug doses in consideration of the genotypes, on the grounds that the pharmacist is an expert in drugs. In order to propose the most appropriate structure for education programs for pharmacy students, it is vital that further research and development take place. This study has sought to spearhead such research and development.

Supplementary Material

Tables S1 – S2.
http://www.medsci.org/v12p0078s1.pdf

Competing Interests

The authors have declared that no competing interest exists.

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