

Pharmacokinetics/Genotype Associations for Major Cytochrome P450 Enzymes in Native and First- and Third-generation Japanese Populations: Comparison With Korean, Chinese, and Caucasian Populations

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Application of foreign clinical data across geographic regions can accelerate drug development. Drug disposition can be variable, and identification of factors influencing responsible pharmacokinetic/pharmacogenomic approaches could facilitate the universal application of foreign data and reduce the total amount of phase III clinical trials evaluating risks in different populations. Our objective was to establish and compare genotype (major cytochrome P450 (CYP) enzymes)/phenotype associations for Japanese (native and first- and third-generation Japanese living abroad), Caucasian, Chinese, and Korean populations using a standard drug panel. The mean metabolic ratios (MRs) for the four ethnic groups were similar except for a lower activity of CYP2D6 in Caucasians and CYP2C19 in Asians. Genotype, not ethnicity, impacted the MR for CYP2C9, CYP2C19, and CYP2D6; neither affected CYP1A2, CYP2E1, and CYP3A4/5 activities. We conclude that equivalent plasma drug concentrations and metabolic profiles can be expected for native Japanese, first- and third-generation Japanese, Koreans, and Chinese for compounds handled through these six CYP enzymes.

Accumulating data implicates ethnicity as a factor that influences drug response¹ and disease state.² Further evidence will be required to determine whether these differences are simply reflections of varying genotype distributions. Similarly, our ability to accurately predict pharmacokinetic parameters for drugs across different ethnic populations is confounded by an absence of data on the influence of intrinsic factors on the variability of drug metabolizing enzyme and transporter activities.

It is recognized that a better understanding of the influence of ethnicity on drug response could rapidly speed access to new medicines for patients in East Asian countries, including Japan, the world's second largest market after the United

States (http://www.imshealth.com/web/content/0,3148,64576068_63872702_70261002_70960269,00.html). Specifically, the International Conference on Harmonization–E5 guidelines “Ethnic Factors in the Acceptability of Foreign Clinical Data”³ have been established with the objective that global phase III studies should be able to enroll Asians from all over the world, not just those who reside within their native countries, and that these studies should meet the native country's requirement for registration. A comparison of cytochrome P450 (CYP) activities among Asian ethnicities provides an approach toward this aim. Clearance of the majority of drugs is determined by CYP enzymes, and drug response and tolerance are commonly related to plasma exposure.

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The CYPs most commonly listed as contributors to drug clearance are *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, and *CYP3A4/5*.⁴ Distribution and frequency of polymorphisms in genes encoding CYP enzymes are well-established in the ethnicities investigated in this study. Convincing evidence for functionally relevant polymorphisms exists for *CYP2C9*,⁵ *CYP2C19*,^{6,7} and *CYP2D6*,⁸ but not for *CYP3A4/5*,^{9,10} *CYP1A2*,¹¹ or *CYP2E1*.¹² We are aware of no previous attempts to conduct a large-scale (>50 subjects/group) comparison of the metabolic ability of the major CYPs, incorporating ethnicity and genotype, among East Asian populations.

The objectives of the current study were to use a panel of specific CYP probe substrates to: (i) evaluate and compare the metabolic activities of *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, and *CYP3A4/5* in native Japanese; (ii) determine whether first- and/or third-generation Japanese can serve as surrogates for native Japanese in pharmacokinetic bridging studies as outlined in the International Conference on Harmonization-E5 guidelines; (iii) compare CYP activities among native Japanese, Chinese, Korean, and Caucasian populations to evaluate the effect of intrinsic factors on phenotype; and (iv) to associate CYP activities in each population with known functional gene polymorphisms for each enzyme.

RESULTS

CYP enzyme activity

More than 600 subjects received a series of specific probe substrates of *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, or *CYP3A4/5* either orally or intravenously and metabolic activity for each enzyme was determined based on the MR from blood or urine (Table 1). The parent and metabolite concentration data obtained after i.v. administration of midazolam for the first-generation Japanese subjects from one clinical site was significantly ($P < 0.05$) different from the other clinical sites. A systematic error could not be determined and thus, these data for all subjects were excluded from any analysis.

Comparing the native Japanese population with the other two Japanese populations, there were no significant differences ($P < 0.05$) in mean MRs for four of six enzymes evaluated. Specifically, mean *CYP1A2*, *CYP2C9*, *CYP2C19*, and *CYP3A4/5* activities were not different between native Japanese and first- or third-generation Japanese (Table 2). For *CYP2D6*, the mean MR for native and first-generation Japanese were not different

($P > 0.05$); however, the mean MR for third-generation Japanese was 97% higher than native Japanese and the difference was statistically significant ($P < 0.05$). For *CYP2E1*, there were small ($\leq 25\%$) but statistically significant ($P < 0.05$) decreases in mean MR comparing the native Japanese group to either the first- or third-generation Japanese group.

Comparisons of the mean MRs among Asian populations (native Japanese with either the Korean or Chinese) yielded similar results to comparisons among Japanese populations. Contrasting native Japanese with the Korean population, the mean MRs were significantly different ($P < 0.05$) for three enzymes, *CYP1A2*, *CYP2C9*, and *CYP2E1* (Table 2), with mean differentials ranging only from -29% for *CYP2E1* to $+21\%$ for *CYP2C9*. A similar comparison between native Japanese and Chinese participants demonstrated that mean activity was significantly different ($P < 0.05$) for four enzymes: *CYP1A2*, *CYP2C9*, *CYP2D6*, and *CYP2E1* (Table 2) with mean MR changes ranging from -17% for *CYP2E1* to $+125\%$ for *CYP2D6*.

Comparing native Japanese with the Caucasian population, significant differences ($P < 0.05$) in the mean MRs were observed for each CYP (*CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP2E1*) with the exception of *CYP3A4* (Table 2). Differences among means ranged from -67% for *CYP2C9* to $+77\%$ for *CYP2D6*.

Comparing mean MRs among the six populations, it is immediately apparent that CYP activities are more similar among the Japanese groups than to other populations, Asian or Caucasian. Disregarding *CYP2E1* data, for which all populations differed from the native Japanese, only one comparison yielded a significant ($P < 0.05$) difference. Korean and Chinese mean MRs were the next most similar with three and four significant ($P < 0.05$) differences, respectively. Finally, Caucasians were the least like native Japanese and differed significantly ($P < 0.05$) for five of six enzyme activities measured.

Genotypic data

Allele and genotype single-nucleotide polymorphism frequencies were calculated and compared according to populations (Table 3). Greater than 98% of the markers across ethnic groups met the assumptions of the Hardy–Weinberg equilibrium ($P > 0.05$). Eight loci spread over five CYP genes (*CYP1A2*, *CYP2C19*, *CYP2D6*, and *CYP3A4/5*) proved non-polymorphic. In general, allele frequencies were similar across Asian populations, but often differed markedly from Caucasian frequencies at specific loci.

Table 1 Treatment schedule and collection of blood and urine from patients on multicocktail study

Drug	Administration route	Day 1	Day 2	Day 3	Collection	Times
Midazolam	p.o.	2 mg			Blood	0, 2, 3, 4 h
Caffeine	p.o.		93 mg ^a		Blood	0, 2, 3, 4 h
Omeprazole	p.o.		20 mg		Blood	0, 2, 3, 4 h
Dextromethorphan	p.o.		30 mg		Urine	0–12 h
Chlorzoxazone	p.o.		200 mg ^b		Blood	0, 2, 3, 4 h
Midazolam	i.v.			1 mg	Blood	0, 2, 3, 4 h
Losartan	p.o.			50 mg	Urine	0–12 h

^a1st- and 3rd-generation Japanese and Caucasian subjects were given 100 mg caffeine due to formulation differences; ^b1st- and 3rd-generation Japanese and Caucasian subjects were given 250 mg chlorzoxazone due to formulation differences.

Table 2 Mean phenotyping indices and comparisons with the native Japanese group

Enzyme	Phenotypic index	Ethnicity group	n	Least-square mean (range)	Least-square mean ratio (comparison group vs. native Japanese)		
					Ratio	95% confidence interval (CI)	Adjusted P-value
CYP1A2	Paraxanthine/caffeine	Native Japanese	100	0.24 (0.08, 0.45)	—	—	—
		1st-generation Japanese	105	0.23 (0.10, 0.57)	0.98	(0.90, 1.07)	1
		3rd-generation Japanese	83	0.22 (0.11, 0.47)	0.92	(0.84, 1.01)	0.3274
		Korean	100	0.18 (0.10, 0.38)	0.76	(0.69, 0.82)	<0.0001
		Chinese	101	0.34 (0.15, 0.69)	1.41	(1.29, 1.54)	<0.0001
		Caucasian	143	0.31 (0.08, 0.84)	1.29	(1.19, 1.40)	<0.0001
CYP2C9	Losartan/E-3174	Native Japanese	100	0.44 (0.21, 19.47)	—	—	—
		1st-generation Japanese	99	0.49 (0.17, 1.32)	1.12	(1.00, 1.26)	0.245
		3rd-generation Japanese	82	0.44 (0.17, 1.41)	1	(0.88, 1.13)	1
		Korean	99	0.53 (0.30, 1.63)	1.21	(1.07, 1.36)	0.0083
		Chinese	100	0.62 (0.26, 3.38)	1.43	(1.27, 1.60)	<0.0001
		Caucasian	139	0.63 (0.20, 12.50)	1.43	(1.29, 1.60)	<0.0001
CYP2C19	Omeprazole/ 5-hydroxyomeprazole	Native Japanese	100	2.06 (0.34, 37.47)	—	—	—
		1st-generation Japanese	103	1.79 (0.40, 36.29)	0.87	(0.65, 1.16)	1
		3rd-generation Japanese	83	2.52 (0.38, 27.12)	1.22	(0.89, 1.67)	1
		Korean	93	2.04 (0.51, 32.87)	0.99	(0.73, 1.34)	1
		Chinese	98	1.86 (0.39, 37.19)	0.9	(0.67, 1.22)	1
		Caucasian	143	0.68 (0.16, 22.48)	0.33	(0.25, 0.43)	<0.0001
CYP2D6	Dextromethorphan/ dextrorphan	Native Japanese	100	0.00 (0.00, 0.09)	—	—	—
		1st-generation Japanese	105	0.01 (0.00, 1.40)	1.71	(1.12, 2.62)	0.0659
		3rd-generation Japanese	83	0.01 (0.00, 0.20)	1.97	(1.26, 3.09)	0.0158
		Korean	100	0.00 (0.00, 0.40)	1.24	(0.81, 1.91)	1
		Chinese	101	0.01 (0.00, 0.11)	2.25	(1.47, 3.44)	0.0011
		Caucasian	142	0.01 (0.00, 7.08)	1.77	(1.19, 2.63)	0.0229
CYP2E1	6-Hydroxychlorzoxazone/ chlorzoxazone	Native Japanese	100	0.03 (0.01, 0.11)	—	—	—
		1st-generation Japanese	105	0.02 (0.00, 0.09)	0.75	(0.68, 0.82)	<0.0001
		3rd-generation Japanese	83	0.02 (0.01, 0.06)	0.81	(0.73, 0.90)	0.0004
		Korean	100	0.02 (0.01, 0.04)	0.71	(0.65, 0.78)	<0.0001
		Chinese	101	0.02 (0.01, 0.06)	0.83	(0.76, 0.92)	0.0013
		Caucasian	143	0.02 (0.01, 0.09)	0.87	(0.80, 0.96)	0.0164
CYP3A4	1-Hydroxymidazolam/ midazolam (p.o.)	Native Japanese	100	0.45 (0.16, 1.36)	—	—	—
		1st-generation Japanese	105	0.48 (0.14, 1.76)	1.08	(0.98, 1.20)	0.6407
		3rd-generation Japanese	84	0.43 (0.22, 1.03)	0.96	(0.87, 1.07)	1
		Korean	100	0.40 (0.12, 1.00)	0.89	(0.80, 0.98)	0.1048
		Chinese	101	0.45 (0.15, 1.14)	1	(0.91, 1.11)	1
		Caucasian	143	0.41 (0.17, 1.35)	0.93	(0.84, 1.02)	0.5409

Total mean allele frequency differences between native Japanese and each of the five other populations are summarized in **Table 4**. No single locus showed a significant difference ($P < 0.05$) in mean allele frequency between the native Japanese and the first-generation Japanese (data not shown). One significant

difference ($P < 0.05$) in mean allele frequency was observed at the *CYP1A2* C(-163)A locus between the native and the third-generation Japanese; however, this is within the range of what would be expected by chance alone owing to the relatively large number of comparisons performed (**Table 4**). Caucasian subjects

Table 3 Allelic frequencies (*n*) of genotypes in six groups including four ethnic groups (native Japanese, 1st-generation Japanese, 3rd-generation Japanese, Korean, Chinese, and Caucasian)

Gene	Allele	DNA change	Native Japanese	1st-generation Japanese	3rd-generation Japanese	Korean	Chinese	Caucasian
CYP1A2	*1C	G(-3860)A	0.319 (160)	0.269 (184)	0.292 (152)	0.296 (186)	0.282 (200)	0.010 (194) ^a
	*1D	(-2464)delT	0.506 (160)	0.430 (184)	0.455 (152)	0.468 (186)	0.535 (200)	0.088 (194) ^a
	*1E	T(-739)G	0.019 (160)	0.032 (184)	0.019 (152)	0.054 (186)	0.104 (200) ^a	0.041 (194)
	*1K	C(-729)T	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.015 (194)
	*1F	C(-163)A	0.700 (160)	0.608 (184) ^a	0.591 (152)	0.661 (186)	0.693 (200)	0.737 (194)
	*2	C63G	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
	*1H	A2025C	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
	*3	G2385A	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
	*4	A2499T	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
	*5	G3497A	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
	*7	G3534A	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
	*6	C5090T	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.010 (194)
	*1B	T5347C	0.813 (160)	0.823 (184)	0.864 (152)	0.817 (186)	0.847 (200)	0.356 (194) ^a
CYP2C9	*2	C430T	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.133 (284) ^a
	*3	A1075C	0.035 (200)	0.048 (210)	0.012 (164)	0.035 (200)	0.025 (200)	0.056 (284)
CYP2C19	*2	G681A	0.345 (200)	0.262 (210)	0.331 (164)	0.250 (200) ^a	0.297 (200)	0.136 (284) ^a
	*3	G636A	0.090 (200)	0.100 (210)	0.133 (164)	0.080 (200)	0.035 (200) ^a	0.000 (284) ^a
	*4	1AG	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.003 (284)
	*5	C1297T	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.000 (284)
	*17	C(-806)T	0.005 (200)	0.010 (210)	0.012 (164)	0.015 (200)	0.005 (200)	0.201 (284) ^a
CYP2D6	*2	C2850T	0.155 (200)	0.144 (210)	0.183 (164)	0.175 (200)	0.160 (200)	0.328 (284) ^a
	*2	G4180C	0.590 (200)	0.582 (200)	0.549 (164)	0.675 (200)	0.680 (200)	0.525 (284)
	*3	2549 A>Del	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.021 (284) ^a
	*4	G1846A	0.005 (200)	0.005 (210)	0.000 (164)	0.005 (200)	0.000 (200)	0.182 (284) ^a
	*5	Gene Deletion	0.070 (200)	0.071 (210)	0.048 (164)	0.075 (200)	0.064 (200)	0.017 (284) ^a
	*6	1707T>Del	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.021 (284) ^a
	*7	Az935C	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.000 (284)
	*8	G1758T	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.000 (284)
	*10	C100T	0.435 (200)	0.433 (210)	0.373 (164)	0.505 (200)	0.530 (200)	0.196 (284) ^a
	*14	G1758A	0.005 (200)	0.000 (210)	0.000 (164)	0.030 (200)	0.005 (200)	0.000 (284)
	*18	4125-4133 insGTGCCCACT	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.000 (284)
	*21	2573insC	0.005 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.005 (200)	0.000 (284)
	*36	Exon 9 2D6/2D7 gene conversion	0.000 (200)	0.000 (210)	0.012 (164)	0.020 (200)	0.000 (200)	0.000 (284)
X2	Duplication	0.060 (200)	0.029 (210)	0.049 (164)	0.020 (200)	0.020 (200)	0.042 (284)	
CYP2E1	*5	G(-1293)C	0.206 (160)	0.177 (184)	0.208 (152)	0.188 (186)	0.228 (200)	0.041 (194) ^a
CYP3A4	*1B	A(-392)G	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.026 (194)
	*2	T15713C	0.000 (160)	0.000 (184)	0.000 (152)	0.000 (186)	0.000 (200)	0.000 (194)
CYP3A5	*1B	G(-86)A	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.017 (284)
	*1C	C(-74)T	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.017 (284)
	*3	A6986G	0.780 (200)	0.810 (210)	0.741 (164)	0.755 (200)	0.723 (200)	0.955 (284) ^a
	*6	G14690A	0.000 (200)	0.000 (210)	0.000 (164)	0.000 (200)	0.000 (200)	0.000 (284)

^aSignificantly different ($P < 0.05$) mean allelic frequency compared with native Japanese mean.

Table 4 Significant differences (expected and observed) in mean allele frequencies between native Japanese and 1st-generation Japanese, 3rd-generation Japanese, Korean, Chinese, and Caucasian populations

Native Japanese vs.	Expected number of significant results by chance		Observed number of significant results	
	<0.05	<0.01	<0.05	<0.01
1st-generation Japanese	1.85	0.37	0	0
3rd-generation Japanese	1.85	0.37	1	0
Korean	1.85	0.37	1	0
Chinese	1.85	0.37	2	1
Caucasian	1.85	0.37	15	13

had the highest number of markers showing significantly different ($P < 0.05$) mean allele frequencies when compared with native Japanese. Korean and Chinese populations had only one and two markers at $P < 0.05$, respectively, with mean frequencies significantly different from native Japanese.

Genotype determinations for each CYP were generated from the sums of the single allele genotypes using the standard nomenclature convention provided by the CYP allele homepage (<http://www.imm.ki.se/CYPalleles/>). Genotype frequencies (Table 5) were similar for all three Japanese populations with the exception of *CYP1A2*. Consistent with findings at the single-allele level, there are differences between the **1B* and **1F* frequencies between native Japanese and third-generation Japanese.

CYP enzyme activity–genotype associations

When enzyme activity data are partitioned by single-allele genotype, the enzyme activity–genotype distributions are nearly identical for the three Japanese populations. As an example, *CYP3A4/5* MRs are plotted by genotype for native, first-generation, and third-generation Japanese and demonstrate complete overlap and very similar ranges of MR (Figure 1). Scatter plots for these three populations with each of the other five CYP enzymes showed similar overlap in MRs and ranges (not shown).

CYP enzyme activity–genotype associations for the four ethnic groups and statistical comparison of means are presented in Figures 2–7. For *CYP1A2*, genotype differences within and between groups are not reflected in MR as the ranges for each genotype overlap almost completely. However, some differences among populations are apparent. The **1F* allele appears more prevalent in Caucasians (Figure 2a). Another apparent trend is that Chinese participants for a number of genotypes appear shifted to the right when compared with Korean individuals indicating a greater mean MR in the Chinese population. Compared with native Japanese, mean Chinese MRs were significantly ($P < 0.05$) higher for three genotypes; whereas mean MRs for Koreans were significantly ($P < 0.05$) lower for three specific genotypes (Figure 2b and Table 3).

For *CYP2C9*, a substantial overlap between the groups is observed (Figure 3a). The **2* allele is exclusive to Caucasians and yields a slight reduction in the mean MR as previously

demonstrated. The **3,*3* genotype yielded a “poor metabolizer” (PM) phenotype, and a PM outlier (native Japanese) was also observed for the **1,*3* genotype (Figure 3a). Mean MRs for the **1,*1* genotype within the six populations clustered closely in the range commonly associated with extensive metabolizers (Figure 3b). Differences in MR means for this genotype were significant ($P < 0.05$) for four populations; however, the absolute value of these differences was exceedingly small.

The scatter plot of *CYP2C19* activity by genotype demonstrated a bimodal response (Figure 4a). When associated with a wild-type allele, the **2* and **3* alleles result in a decreased mean MR, and when homozygous **2,*2*, **3,*3* or **2,*3*, result in a PM phenotype. Among Asian populations, only the Korean mean MR for the **1,*1* genotype was significantly ($P < 0.05$) different (Figure 4b).

While the Asian populations overlap greatly in phenotype, Caucasians showed a significantly ($P < 0.05$) lower mean MR, indicating a substantially higher metabolic activity than the other ethnic groups as long as one wild-type allele was present (Table 2; Figure 4b). The **17* allele proved to be a predominantly Caucasian allele and was associated with the highest range of metabolic activity. *CYP2C19* **1,*17* heterozygotes had a significantly lower MR than *CYP2C19* **1,*1* homozygotes ($P < 0.05$). Additionally, individuals with a **3,*3* genotype demonstrated a wide MR ranging from extensive metabolizer to PMs.

The distribution for *CYP2D6* is complex, with a large number of single-nucleotide polymorphisms interrogated; however, there is an indication of a trimodal distribution commonly referred to as the ultra-rapid,¹³ extensive metabolizer, and PM phenotypes (Figure 5a). There is no clear indication of borders for an intermediate phenotype. The **10,*10* phenotype is often identified as the class prototype for a proposed intermediate phenotype grouping but is totally contained within the MR range of the **1,*1* genotype.

When compared with native Japanese, mean MRs were significantly ($P < 0.05$) lower for first- and third-generation Japanese for two genotypes **1,*10* and **2,*10* (Figure 5b). The mean MR for third-generation Japanese and Koreans also differed significantly ($P < 0.05$) from the native Japanese mean for the **1,*1* genotype.

The **4* allele was primarily, but not exclusively, a Caucasian allele whereas, the **10* allele was exclusive to Asian populations. Two outliers were identified: one native Japanese and one Korean individual with the **5,*14* genotype with a higher than expected MR (Figure 5a).

The phenotype–genotype distribution for *CYP2E1* showed complete overlap among groups with no substantial influence of genotype on MR (Figure 6a). Although mean MRs for all populations were significantly ($P < 0.05$) different from the native Japanese mean (Table 3), the absolute differences between means are very small and probably reflect the large sample size and high precision of the phenotype assays. Additionally, the separation of the native Japanese mean MR from each of the other populations is also demonstrated in Figure 6b for the **1,*1* genotype.

Finally, for *CYP3A5*, the MRs for all genotypes often fell within the range determined for homozygous wild-type (**1,*1*) individuals despite being the low-frequency allele (Figure 7a).

Table 5 Genotype frequencies observed for *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4/5* polymorphisms in native and 1st- and 3rd-generation Japanese, Korean, Chinese, and Caucasian populations

Gene	Genotype	Native Japanese	1st-generation Japanese	3rd-generation Japanese	Korean	Chinese	Caucasian
<i>CYP1A2</i>	*1B,*1B	0.0750	0.1183	0.2237	0.1075	0.0900	0.0619
	*1B,*1C,*1D,*1F AND *1B	0.1750	0.1720	0.1974	0.2043	0.1600	0.0206
	*1B,*1C,*1D,*1F AND *1B,*1C,*1D,*1F	0.1125	0.0968	0.0921	0.0860	0.0600	—
	*1B,*1C,*1D,*1F AND *1B,*1D,*1E,*1F	0.0125	—	0.0132	0.0215	0.0400	—
	*1B,*1C,*1D,*1F AND *1F	0.1375	0.0968	0.0921	0.1290	0.1000	—
	*1B,*1D,*1E,*1F AND *1B	0.0125	0.0538	—	0.0430	0.0600	0.0206
	*1B,*1D,*1E,*1F AND *1B,*1D,*1E,*1F	—	—	0.0132	—	0.0300	—
	*1B,*1D,*1E,*1F AND *1F	0.0125	0.0108	—	0.0323	0.0200	0.0412
	*1B,*1D,*1E,*1F AND *1K	—	—	—	—	—	0.0206
	*1B,*1D,*1F AND *1B	0.1500	0.1505	0.0921	0.0860	0.0900	0.0206
	*1B,*1D,*1F AND *1B,*1C,*1D,*1F	0.0875	0.0753	0.0789	0.0645	0.1400	—
	*1B,*1D,*1F AND *1B,*1D,*1E,*1F	—	—	—	0.0108	0.0300	—
	*1B,*1D,*1F AND *1B,*1D,*1F	—	—	0.0263	0.0215	0.0100	—
	*1B,*1D,*1F AND *1F	0.1000	0.0323	0.0658	0.0323	0.0100	0.0412
	*1B,*1D,*1F AND *1F,C(-730)T	—	—	—	—	—	0.0103
	*1B,*1F	0.1125	0.1720	0.0921	0.1183	0.1200	0.3196
	*1B,*1F AND *1B	—	—	—	0.0108	0.0100	—
	*1B,*1F AND *1F	0.0125	—	—	0.0108	—	0.0103
	*1B,*1F AND *6	—	—	—	—	—	0.0206
	*1F,*1F	—	0.0215	0.0132	0.0215	0.0300	0.4124
<i>CYP2C9</i>	*1,*1	0.9300	0.9048	0.9756	0.9300	0.9500	0.6690
	*1,*2	—	—	—	—	—	0.1972
	*1,*3	0.0700	0.0952	0.0244	0.0700	0.0500	0.0845
	*2,*2	—	—	—	—	—	0.0282
	*2,*3	—	—	—	—	—	0.0141
	*3,*3	—	—	—	—	—	0.0070
<i>CYP2C19</i>	*1,*1	0.3100	0.4190	0.2439	0.4000	0.4300	0.4014
	*1,*17	0.0100	0.0095	0.0122	0.0300	0.0100	0.3239
	*1,*2	0.3900	0.3143	0.3902	0.3600	0.4300	0.1901
	*1,*3	0.1000	0.0952	0.1707	0.1200	0.0400	—
	*17,*17	—	—	—	—	—	0.0070
	*2,*17	—	—	—	—	—	0.0563
	*2,*2	0.1300	0.0762	0.0976	0.0500	0.0600	0.0141
	*2,*3	0.0400	0.0571	0.0610	0.0400	0.0300	—
	*3,*17	—	0.0095	0.0122	—	—	—
	*3,*3	0.0200	0.0190	0.0122	—	—	—
*4,*17	—	—	—	—	—	0.0070	
<i>CYP2D6</i>	*1 X2,*5	—	—	—	—	0.0100	—
	*1,*1	0.1500	0.1333	0.2317	0.0600	0.0700	0.1831
	*1,*1 X2	0.0100	0.0095	—	0.0100	—	—
	*1,*10	0.2700	0.3429	0.2439	0.3000	0.2800	0.0070
	*1,*10 X2	0.0200	0.0095	0.0122	—	0.0100	—
	*1,*2	0.0800	0.1143	0.1098	0.1000	0.0900	0.2676

Table 5 continued on next page

Table 5 (continued)

Gene	Genotype	Native Japanese	1st-generation Japanese	3rd-generation Japanese	Korean	Chinese	Caucasian
	*1,*2 X2	0.0100	—	0.0122	0.0100	—	0.0282
	*1,*21	0.0100	—	—	—	—	—
	*1,*3	—	—	—	—	—	0.0211
	*1,*36	—	—	0.0122	0.0100	—	—
	*1,*4	0.0100	0.0095	—	—	—	0.1127
	*1,*4 X2	—	—	—	—	—	0.0070
	*1,*5	0.0500	0.0286	0.0122	0.0400	0.0400	0.0141
	*1,*6	—	—	—	—	—	0.0211
	*10,*10	0.1800	0.1143	0.1220	0.1900	0.2600	—
	*10,*10 X2	0.0100	0.0095	—	—	—	—
	*10,*14	—	—	—	—	0.0100	—
	*10,*36	—	—	—	0.0200	—	—
	*2 X2,*5	0.0100	—	—	—	—	—
	*2,*10	0.1100	0.1048	0.0976	0.1000	0.1200	0.0070
	*2,*10 X2	—	—	0.0122	—	—	—
	*2,*14	—	—	—	0.0300	—	—
	*2,*2	—	0.0190	0.0244	0.0100	0.0200	0.0775
	*2,*2 X2	—	—	0.0122	—	—	—
	*2,*21	—	—	—	—	0.0100	—
	*2,*3	—	—	—	—	—	0.0141
	*2,*36	—	—	0.0122	—	—	—
	*2,*4	—	—	—	—	—	0.1197
	*2,*4 X2	—	—	—	—	—	0.0070
	*2,*5	0.0300	0.0095	0.0244	0.0300	0.0200	0.0211
	*2,*6	—	—	—	—	—	0.0211
	*3,*4	—	—	—	—	—	0.0070
	*4,*10	—	—	—	—	—	0.0141
	*4,*36	—	—	—	0.0100	—	—
	*4,*4	—	—	—	—	—	0.0493
	*5,*10	0.0400	0.0857	0.0610	0.0600	0.0600	—
	*5,*14	0.0100	—	—	0.0200	—	—
	*5,*5	—	0.0095	—	—	—	—
CYP3A4/3A5	*1,*1	0.0500	0.0571	0.0732	0.0600	0.0700	—
	*1,*3	0.3400	0.2667	0.3659	0.3700	0.4100	0.0775
	*1B AND *3,*3	—	—	—	—	—	0.0352
	*1C AND *3,*3	—	—	—	—	—	0.0282
	*1C,*3 OR *1,*1C AND *3	—	—	—	—	—	0.0070
	*3,*3	0.6100	0.6762	0.5610	0.5700	0.5200	0.8521

Two significant ($P < 0.05$) differences in mean MR for the *3,*3 genotype were noted for the Korean and Caucasian populations when compared with the native Japanese (Figure 7b). While statistically significant, the differences among all means determined for the *3,*3 genotype were exceedingly small. The *1B and *1C loci were polymorphic only in the Caucasian population and the *6 loci was not polymorphic in any population (Figure 7a and Table 3). CYP3A4 polymorphisms are

not included in this scatter diagram because the *1B loci was polymorphic only in Caucasians and the *2 locus was not polymorphic in any population (Table 3).

DISCUSSION

This large, multicenter study was initiated with multiple objectives: specifically, to compare metabolic activities of the major CYP enzymes first among Japanese (native and expatriated),

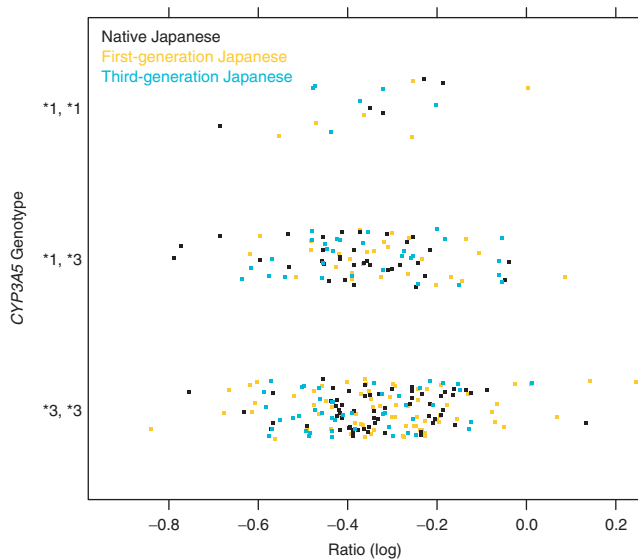


Figure 1 Plot of *CYP3A4/5* genotype vs. phenotype (1-hydroxymidazolam/midazolam ratio) for native, first-generation, and third-generation Japanese groups. Metabolic activity for individuals increases from left to right.

then among Asian and Caucasian populations, and finally to associate metabolic activity with genetic polymorphisms in an effort to understand and predict the phenotypic variation observed. We found essentially identical phenotypic distributions among the Japanese populations or when native Japanese were compared to the Korean and Chinese populations. A few exceptions were noted and are discussed below; however, in comparison with population variability in measurements, the relatively small mean differences were not deemed clinically relevant. The Asian populations displayed similar allele profiles for the six CYP genes and the genotypes exhibited in *CYP2C9*, *CYP2C19*, and *CYP2D6* clearly influenced MRs. Caucasians differed most often from the native Japanese mean phenotype and in allele or genotype frequencies. Importantly, similar genotypes yielded similar metabolic activities and these determinations were independent of ethnicity and for Japanese populations, geographic location.

Although differences in mean MRs were noted when comparing native Japanese with the first- and third-generation populations, overall the similarities are much more striking (Table 2; Figures 2b–7b). Statistically significant differences ($P < 0.05$) were noted for a number of the comparisons including *CYP2D6* (native vs. third-generation) and *CYP2E1* where the least-square mean ratios were lower in first- and third-generation populations. It should be noted that the *CYP2D6* mean in native Japanese was significantly higher than means for the other five populations. However, the magnitude of the mean differences was relatively small compared to variability within the populations. The observed significant difference between native and third-generation Japanese (but not first-generation) may also be the product of a very narrow range of *CYP2D6* metabolic activities (0.0003–0.0945) measured for the native population. Exceedingly small variability surrounding the mean component ratio for the *CYP2D6* genotype, as

well as the relatively large sample population for the Japanese populations, yielded a statistically different, yet unimpressive, difference between means.

To our knowledge, this is the largest study undertaken to understand genotype and ethnicity influences on *in vivo* CYP activity. The relatively large number of subjects per ethnicity (~100) recruited for this study provides adequate power and confidence in detecting clinically significant differences in activity¹⁴ for the relevant enzymes across the ethnicities. In general, genotype influenced CYP enzyme activities independent of ethnicity. Since CYPs are the major determinants of clearance for the majority of drugs, this finding has significant implications for clinical drug development across Asian populations, and clearly favors bridging studies for registration in Japan. The large number of subjects in this study also provides a more realistic measure of variability in the general population than previously available from smaller studies. For example, even though the *CYP2D6* *10 allele in Asian populations has been associated with lower enzyme activity than the wild type,¹⁵ the range of variability in *CYP2D6* enzyme activity in those homozygous for the *CYP2D6* *1 alleles overlaps the range of variability for those with one or two copies of the *CYP2D6* *10 allele. Taking this theme a step further, it could be that only those Caucasians who are homozygous for the *CYP2D6* *4 allele are true outliers with regard to the *CYP2D6* phenotype. Similar arguments could also be applied to genotypic influences for *CYP2C9* and *CYP2C19*, in that other extrinsic factors may be equal or greater contributors to variability than genotype on enzyme activity, unless genotype has a very severe effect on enzyme activity.

The relationship between the *CYP2C19* *17 polymorphisms (–806 and –3402) and potential ultrarapid omeprazole metabolism reported by Sim and others¹⁶ was investigated in all populations. Complete linkage disequilibrium between –806 and –3402 was found in all samples, and no other *CYP2C19* polymorphisms were detected on *CYP2C19* *17 alleles. Allele frequencies for Chinese were lower (0.5%) than those reported by Sim (4.4%). However, the allele frequencies were similar to those previously found for Caucasians. The Korean population had an allele frequency (1.5%) very similar to that in Japanese populations (0.9%) and to those reported by Fukushima-Uesaka and others.¹⁷ We found only one Caucasian homozygous for *CYP2C19* *17; this was lower than the Hardy–Weinberg expected frequency for homozygous variants ($P < 0.05$). One individual had an MR of 0.51 for omeprazole, which was higher than the mean MR (0.245) found in Caucasians for omeprazole metabolism reported by Sim.¹⁶ Mean MRs for heterozygous *CYP2C19* *17 Caucasians (0.61) were similar to those seen in each of the heterozygous Asian samples (0.74). This study confirms *CYP2C19* *17 as a rapid metabolizer allele compared to *CYP2C19* homozygous wild-type individuals.

The observations of this study are consistent with the general consensus in the literature that the polymorphisms assessed for *CYP1A2* and *CYP2E1* do not have functional relevance on enzyme activity and do not differentiate the Asian populations investigated. It is also generally accepted that the *CYP3A5* polymorphisms investigated do not influence midazolam

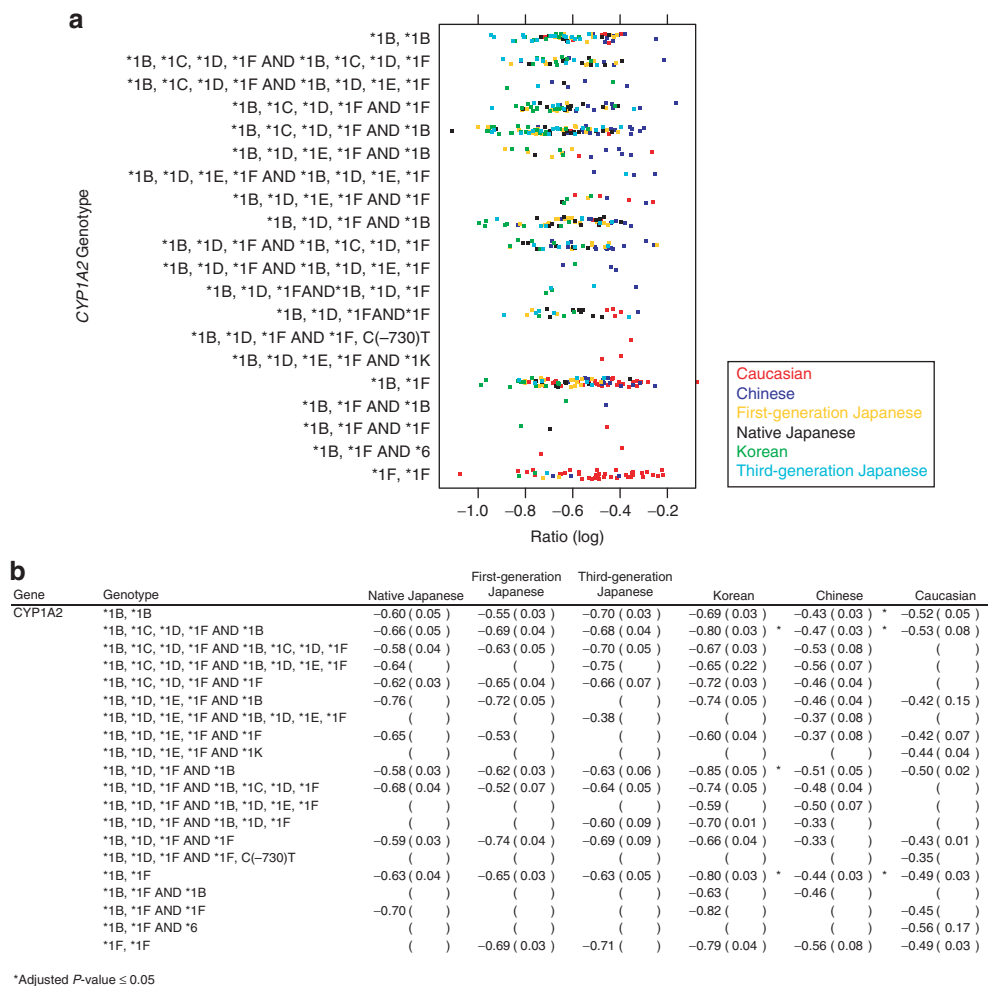


Figure 2 (a) Plot of *CYP1A2* genotype vs. phenotype (paraxanthine/caffeine ratio) for the four ethnic groups (Japanese, Korean, Chinese, and Caucasian). Metabolic activity for individuals increases from left to right. (b) Mean (\pm s.e.) metabolic ratios for *CYP1A2* alleles. Asterisks identify means that are significantly different ($P < 0.05$) from the native Japanese mean.

pharmacokinetics.¹⁸ This could possibly be explained by a minor role for *CYP3A5* in drug metabolism. However, the influence of *CYP3A5* polymorphisms on tacrolimus dosage requirements is compelling¹⁹ and thus the role of *CYP3A5* in drug metabolism in general is still equivocal.

Functional polymorphisms in *CYP2C9*, *CYP2C19*, and *CYP2D6* influence dosage requirements of some drugs; therefore, genotyping offers a potential benefit to patients in terms of increased efficacy and decreased adverse events. Since there is the potential for pharmacokinetic outliers in phase I and phase II studies in clinical development, the ability to prospectively genotype may offer insights in optimizing the value of medicines in drug development. However, one caveat to routine screening in these early stages of clinical development is the small numbers of subjects, which may not offer the conclusive evidence required to make decisions on which sub-populations to favor in the later stages of drug development and postmarket. The lack of the effect of *CYP1A2*, *CYP2E1*, and *CYP3A5* on enzyme activity in this study brings into question the value of routine genotyping in these genes. However, the results in

this study support genotyping activities for *CYP2C9*, *CYP2C19*, and *CYP2D6*.

The acceptability of foreign clinical data to a new region continues to be a challenging dilemma in spite of the issuance of the International Conference on Harmonization-E5 guidance.³ This guidance requires a comparison of the pharmacokinetics from one region to another as one of the criteria for bridging of foreign clinical data. Usually a phase I comparison study enrolling both Asian patients and Caucasians is used to meet this requirement.³ The comparisons made in these studies can lead to a better understanding of the potential intrinsic or extrinsic ethnic differences such as diet and lifestyle factors between the two regions. Although these are relatively short-term studies, recruitment can take a considerable amount of time and effort. However, the impact of successful bridging may make it possible to enroll patients simultaneously in one global protocol rather than evaluating regions sequentially. Additionally, it may be possible to use the clinical data from first- and third-generation populations of each group, recruited outside the host country in the study in lieu of a separate study

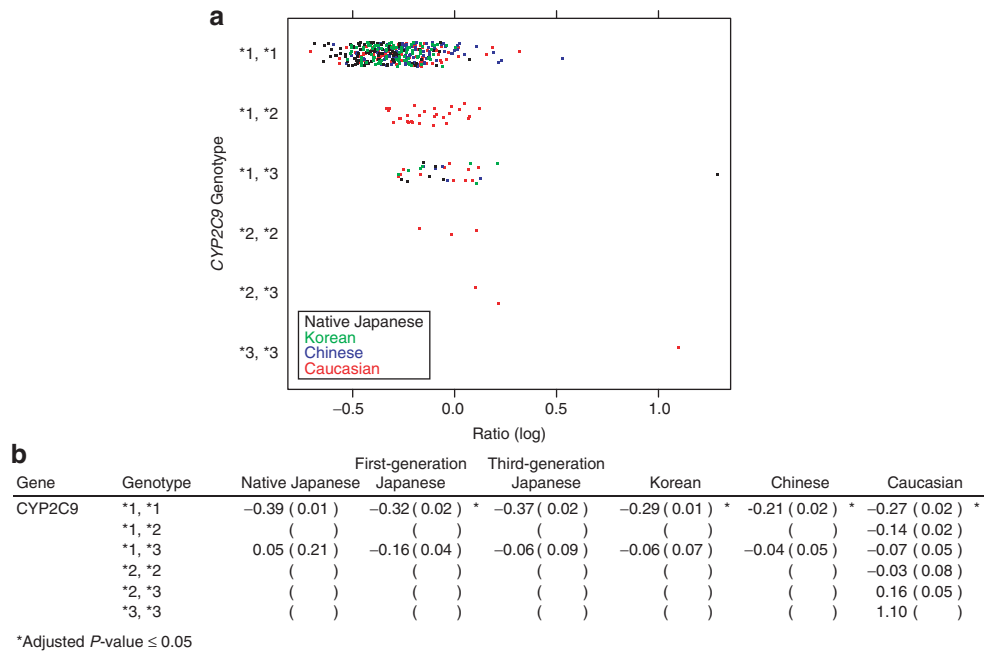


Figure 3 (a) Plot of *CYP2C9* genotype vs. phenotype (losartan/E-3174 ratio) for the four ethnic groups (Japanese, Korean, Chinese, and Caucasian). Metabolic activity for individuals increases from right to left. (b) Mean (\pm s.e.) metabolic ratios for *CYP2C9* alleles. Asterisks identify means that are significantly different ($P < 0.05$) from the native Japanese mean.

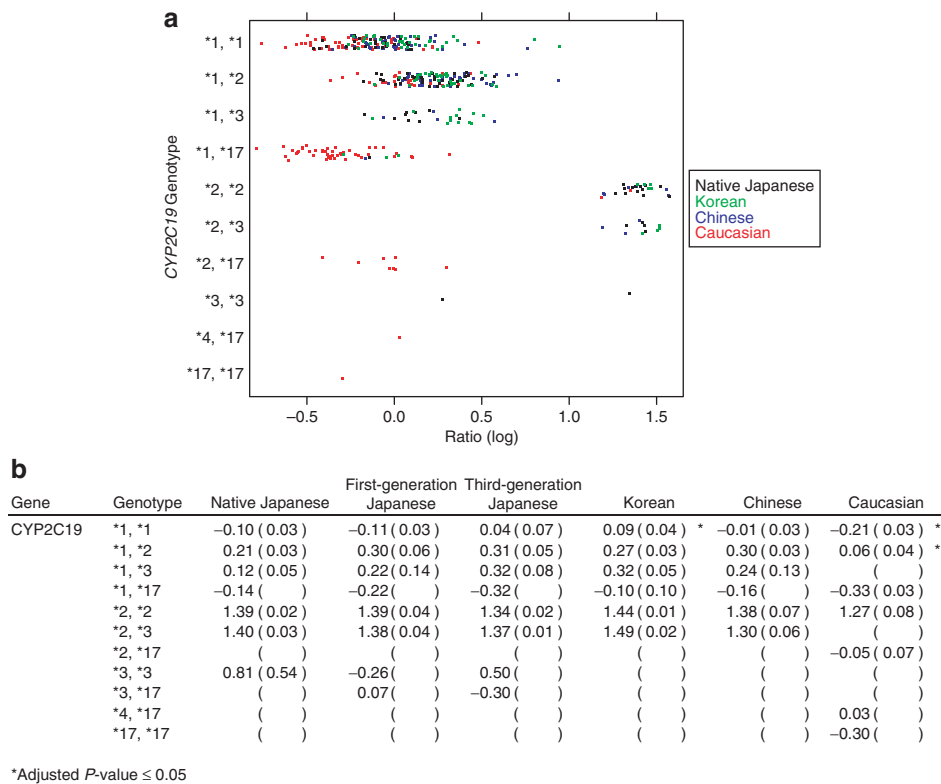


Figure 4 (a) Plot of *CYP2C19* genotype vs. phenotype (omeprazole/5-hydroxyomeprazole ratio) for the four ethnic groups (Japanese, Korean, Chinese, and Caucasian). Metabolic activity for individuals increases from right to left. (b) Mean (\pm s.e.) metabolic ratios for *CYP2C19* alleles. Asterisks identify means that are significantly different ($P < 0.05$) from the native Japanese mean.

in the foreign region. Both of these options could lead to a more efficient phase III clinical program that would provide time and cost savings for registration of the drug in the new region.

The data presented herein suggest that the bridging guidance can be simplified for drugs that are metabolized by the major CYPs. The comparison data of the four ethnic populations presented demonstrate that drugs that are metabolized by *CYP1A2*,

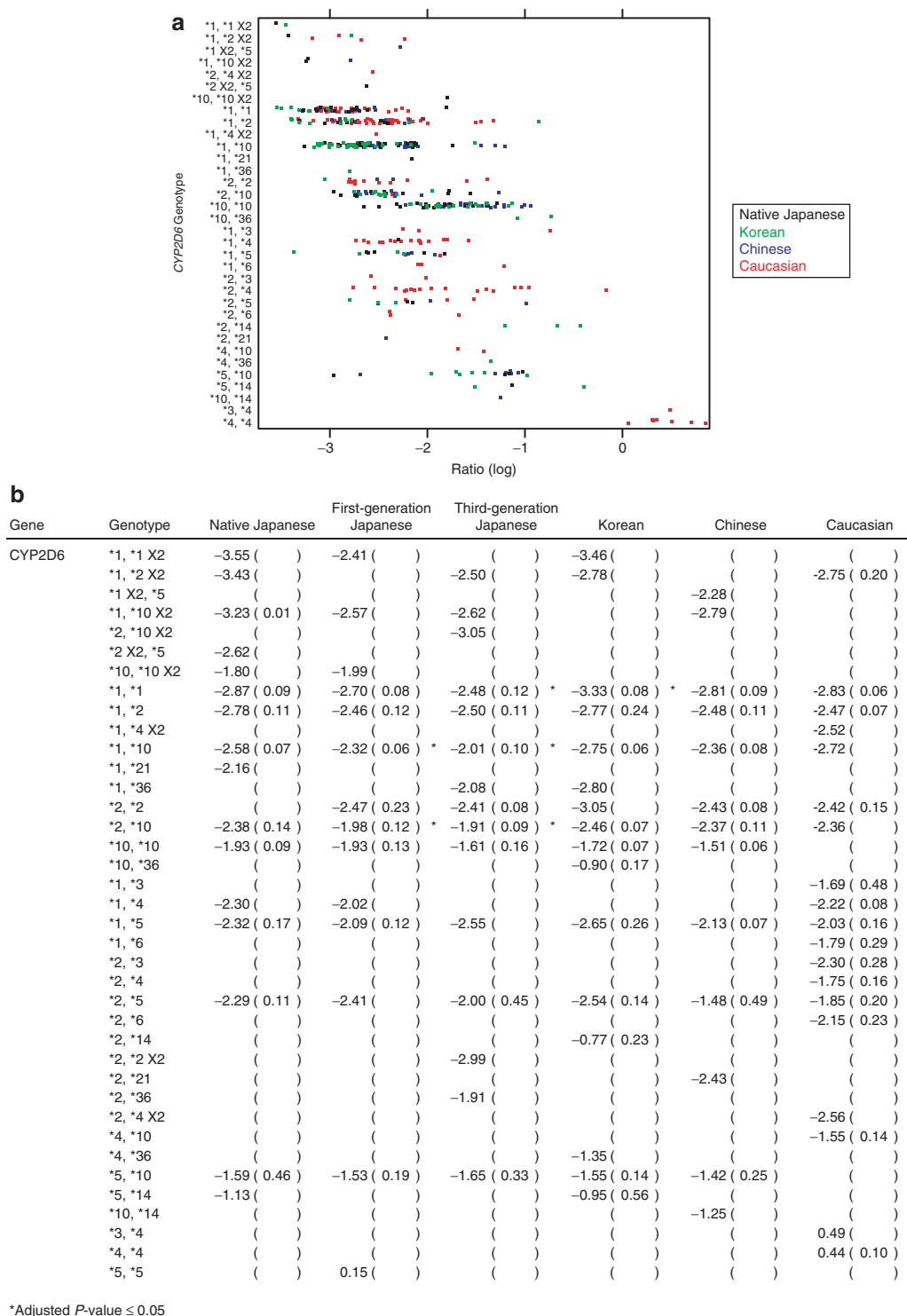


Figure 5 (a) Plot of *CYP2D6* genotype vs. phenotype (dextromethorphan/dextrorphan ratio) for the four ethnic groups (Japanese, Korean, Chinese, and Caucasian). Metabolic activity for individuals increases from right to left. (b) Mean (\pm s.e.) metabolic ratios for *CYP2D6* alleles. Asterisks identify means that are significantly different ($P < 0.05$) from the native Japanese mean.

CYP2E1, and *CYP3A4/5* are essentially independent of ethnicity and known genotypes. Thus, the plasma drug concentration data determined in one region should be similar to that of the new region. In most instances, a formal pharmacokinetic bridging study would not add significant information, allowing scarce resources to be efficiently reassigned. For drugs that

are metabolized by *CYP2C9*, *CYP2C19*, and *CYP2D6*, identical genotypes were associated with similar MRs. For example, the MRs for *CYP2C19* *2, *2 and *CYP2C19* *2, *3 were similar among the ethnic groups suggesting that genotype not ethnicity, drives the MR. Thus, for *CYP2C9*, *CYP2C19*, and *CYP2D6*, the concentration data generated from one region should be similar to

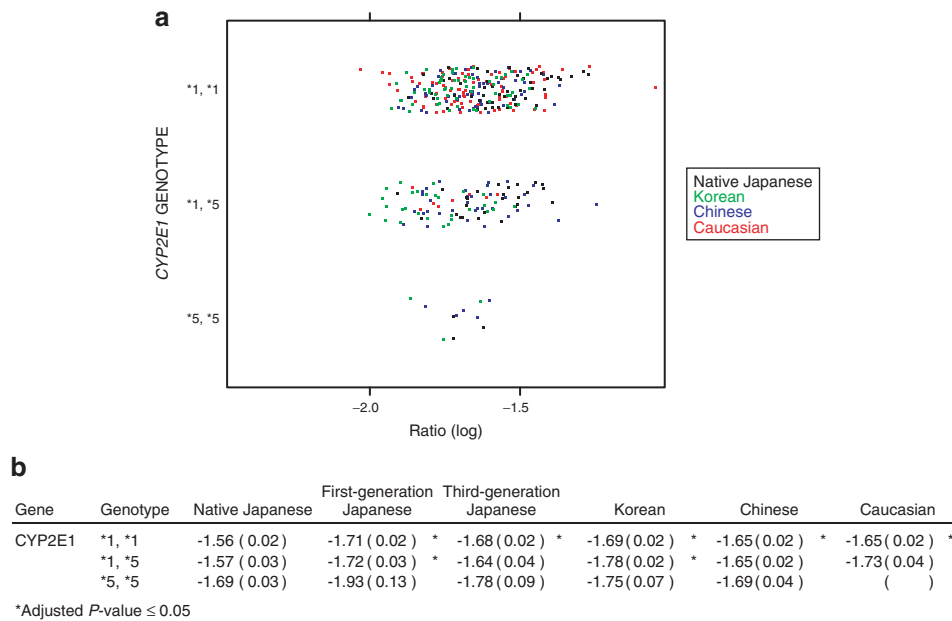


Figure 6 (a) Plot of *CYP2E1* genotype vs. phenotype (6-hydroxychlorzoxazone/chlorzoxazone ratio) for the four ethnic groups (Japanese, Korean, Chinese, and Caucasian). Metabolic activity for individuals increases from left to right. (b) Mean (\pm s.e.) metabolic ratios for *CYP2E1* alleles. Asterisks identify means that are significantly different ($P < 0.05$) from the native Japanese mean.

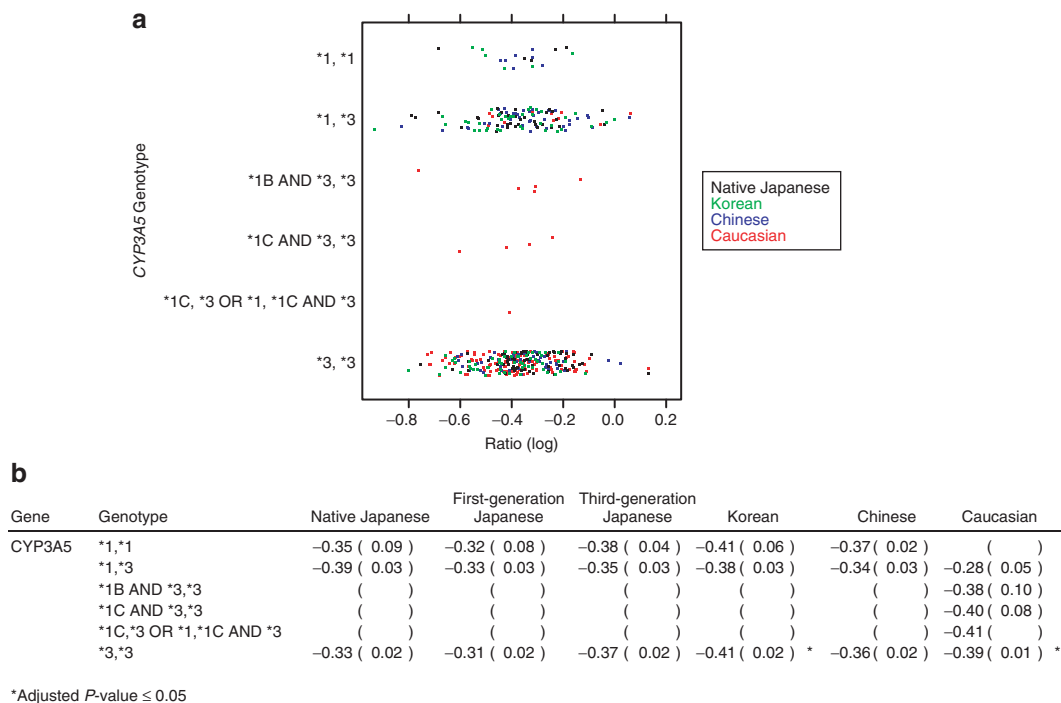


Figure 7 (a) Plot of *CYP3A5* genotype vs. phenotype (1-hydroxymidazolam/midazolam ratio) for the four ethnic groups (Japanese, Korean, Chinese, and Caucasian). Metabolic activity for individuals increases from left to right. (b) Mean (\pm s.e.) metabolic ratios for *CYP3A5* alleles. Asterisks identify means that are significantly different ($P < 0.05$) from the native Japanese mean.

another region as long as a wide range of genotypes were evaluated in the initial region. If this can be accomplished, then a formal pharmacokinetic bridging study may not be necessary. For compounds with narrow therapeutic windows, or potential for serious adverse events, genotyping of individuals irrespective of ethnicity is likely to optimize patient selection and treatment.

While this study is among the most comprehensive that focused on the considerations of pharmacokinetic bridging studies, there were a number of limitations. Extrapolation to compounds other than the six substrate probes, utilizing single or mixed pathways, has not been examined. In addition, this study is not all-inclusive, representing all the compounds metabolized

Table 6 Demographic data for six populations of healthy, male, non-smoking subjects

	Native Japanese	1st-generation Japanese ^a	3rd-generation Japanese ^b	Korean	Chinese	Caucasian
Subjects	100	105	84	100	101	143
Age (years)	22.9 ± 2.3 ^c	26.1 ± 5.5	36.5 ± 7.3	23.5 ± 3.1	26.3 ± 6.4	29.9 ± 7.7
Height (cm)	171.9 ± 5.4	172.7 ± 6.4	169.3 ± 5.9	173.4 ± 5.9	171.0 ± 6.3	180.0 ± 6.4
Weight (kg)	63.8 ± 7.6	65.8 ± 9.4	72.6 ± 8.5	68.8 ± 7.5	66.1 ± 8.7	78.8 ± 9.2

^a1st-generation Japanese, both parents of Japanese descent and subjects themselves born in Japan but lived outside of Asia for at least 3 months but no more than 5 years;

^b3rd-generation Japanese, both parents of Japanese descent with at least one parent born outside of Japan and the subjects themselves born outside of Japan and living outside of Asia; ^cmean ± s.d.

Table 7 DNA primers, probes, and PCR conditions for TaqMan allelic discrimination assays

SNP	DNA change	PCR forward primer 5' → 3'	PCR reverse primer 5' → 3'	TaqMan probes ^a 5' → 3'	PCR conditions
CYP2C19 *4	A1G	AAGCTCACGGTTGTC TTAACAAGAG	GAGAGACAGAGCACAA GGACCAC	VIC-CTTCA A TGGATCCTTTT-MGB; 6FAM-CTTCA G TGGATCCTT-MGB	50C - 2', 95C - 10'(1); 95C - 15', 64 - 1'(40)
CYP2C19 *5	C1297T	TGAGGAGTAACTTCT CCCTATGTTTGT	GGTCAGGAATAAAAACA GCTCCAT	VIC-AGGAAAA C GGATTGT-MGB, 6FAM-CAGGAAAA T GGATTG-MGB	50C - 2', 95C - 10'(1); 92C - 15', 60 - 1'(40)
CYP2D6 *10	C100T	ACCTGATGCACCGGCG	GGCAGTGGCAGGGGG	VIC-ACGCTAC C CACCAGG-MGB; 6FAM-CACGCTAC T CACCAGG - MGB	50C - 2', 95C - 10'(1); 95C - 15', 64 - 1'(40)
CYP2E1 *5	G(-1293)C	AAAACCAGAGGGAAG CAAAGG	CCACATAAGCAAGTCATT GGTTGT	TET-ACCTAACACTGCA C CTCTC CTGAACCAA-TAMRA; 6FAM-ACCTAACACTGCA G CTCTC CTGAACCAA-TAMRA	50C - 2', 95C - 10'(1); 95C - 15', 62 - 1'(40)
CYP3A4 *1B	A(-392)G	AAGATCTGTAGGTG TGGCTTGTG	AGTGGAGCCATTGGCA TAAAA	TET-AAATCGCCTCTCT T CTG CCCTTGTCT-TAMRA; 6FAM-AAATCGCCTCTCT C CTG CCCTTGT-TAMRA	50C - 2', 95C - 10'(1); 95C - 15', 62 - 1'(40)
CYP3A5 *1B	G(-86)A	TTTCAGCAGCTTG GCTGAAGA	TGCTGTTGCTGGGCTGTT	VIC-CCTGGAGCTT C CCT-MGB; 6FAM-CTGGAGCTT T CTG-MGB	50C - 2', 95C - 10'(1); 95C - 15', 64 - 1'(40)
CYP3A5 *1C	C(-74)T	TTTCAGCAGCTT GGCTGAAGA	GTTCTGTGAGTCTTCC TTTTAGCTGA	VIC-AAGCTCCAGG C AAA-MGB; 6FAM-AGCTCCAGG T AAAC-MGB	50C - 2', 95C - 10'(1); 95C - 15', 60 - 1'(40)
CYP3A5 *3	A6986G	ACCCAGCTTAACGA ATGCTCTACT	GAAGGGTAATGGGTCC AAACAG	VIC-TTTGTCTTTCA A TCTCTT-MGB; 6FAM-TGTCTTTCA G TATCTCTT-MGB	50C - 2', 95C - 10'(1); 95C - 15', 64 - 1'(40)
CYP3A5 *6	G14690A	AACAATCCACAAG ACCCCTTTG	TGAGAGAAATAATGGATCTA AGAAACCA	VIC-AGCACTAA G AAGTTC-MGB; 6FAM-AGCACTAA A AAGTTC-MGB	50C - 2', 95C - 10'(1); 95C - 15', 60 - 1'(40)

^aBolded/underlined nucleotides indicate position of single-nucleotide polymorphism (SNP) in TaqMan probe.

by CYP enzymes. Since no pharmacodynamic responses were measured in this study, it is not possible to infer pharmacodynamic-pharmacogenetic relationships. Other enzymes and transporters (such as UDP-glucuronosyltransferases) involving drug metabolism were not evaluated in this study.

In summary, for drugs primarily metabolized by the CYP system, in particular *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP3A4/5*, *CYP2D6*, and *CYP2E1*, the data presented herein support two important conclusions. First, in many instances, subjects native to Japan, China, and Korea can be expected to have similar MRs for drugs primarily cleared by the major drug metabolizing CYPs. The genotype substantially influenced the phenotype for CYP2C9, CYP2C19, and CYP2D6 indicating that allele frequencies for relevant CYP gene polymorphisms, rather than ethnicity, should be considered when selecting a population for clinical trials. Secondly, Japanese volunteers or patients (native through third-generation) could be used to supplement the enrollment of native East Asians in clinical trials. We believe the data from this study provide a sound scientific foundation to begin testing approaches outside Japan.

METHODS

Additional methodology can be found in Supplemental Information.

Subjects. A total of 633 healthy, non-smoking men were enrolled in the study (Table 6).

Experimental design and samples. Volunteers were enrolled in clinical sites in Japan, the United States, Canada, the United Kingdom, Republic of Korea, and Republic of Singapore. The doses and dosing regimen (Table 1) have been employed by others²⁰⁻²² and was evaluated earlier by our group.²³

Phenotyping assays. Concentrations of the following drugs and metabolites were measured using high pressure liquid chromatography-tandem mass spectrometric methods: caffeine and paraxanthine, chlorzoxazone and 6-hydroxychlorzoxazone, midazolam and 1-hydroxymidazolam, omeprazole and 5-hydroxyomeprazole, dextromethorphan and dextrophan, losartan and E-3174. Assays were tested for selectivity against the other drugs and metabolites and no significant analytical interferences were observed.

Genotyping assays. Genomic DNA was isolated from whole blood using the QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA). Genotyping

Table 8 DNA primers and terminator mixes for matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) assays

Single nucleotide polymorphism (SNP)	DNA change	PCR forward primer 5' → 3' ^a	PCR reverse primer	MassExtend primer	Terminator mix
CYP1A2 *1B	T5347C	acgttggatgGGGACACAAC GCTGAATGGCTTCTACATC	acgttggatgGATTACAGGCC CTGCACTTGGCTAAAGCTG	CTGCGCTTCTCCATCAA	ACG
CYP1A2 *1C	G(-3860)A	acgttggatgGATGTCTCTTGATT AGAGCTGGTTATATGTGTGTTT	acgttggatgCAGATCTAAGA GGAGGAGGAGGACAAGCC	GCATGACAATTGCTTGAATC	ACG
CYP1A2 *1D	(-2464)delT	acgttggatgGATGTCTCTTGATT AGAGCTGGTTATATGTGTGTTT	acgttggatgCAGATCTAAGA GGAGGAGGAGGACAAGCC	GCCATGATTGTGGCACA	ACT
CYP1A2 *1E	T(-739)G	acgttggatgGAACCTGGA AGTAGTGGGGACAGAAA	acgttggatgGGTCAGCAC ATGCCGAGCAA	TGGGCTAGGTGTAGGGG	ACT
CYP1A2 *1F	C(-163)A	acgttggatgGAACCTGGA GCTAGTGGGGACAGAAA	acgttggatgGGTCAGCAC ATGCCGAGCAA	AAGCTCCATCTACCA TGGTCTCTG	ACT
CYP1A2 *1H	A2025C	acgttggatgTCCCACAGG AGAAGATTGTCAACCTTGT	acgttggatgGGCAGAACCG GCAGGCCTC	AGGTTCTGGTCTCTACC	ACT
CYP1A2 *1K	C(-729)T	acgttggatgGAACCTGGA GCTAGTGGGGACAGAAA	acgttggatgGGTCAGCA CATGCCGAGCAA	GCTGGGTAGCAAAGCCC	ACT
CYP1A2 *2	C63G	acgttggatgGAACCTGGA AGTAGTGGGGACAGAAA	acgttggatgGGTCAGCA CATGCCGAGCAA	GCACCCAGAATACCAGGCA	ACT
CYP1A2 *3	G2385A	acgttggatgTCCCACAGG AGAAGATTGTCAACCTTGT	acgttggatgGGCAGAA CCGGCAGGCCTC	GGAAGATCCAGAAGGAGCTG	ACT
CYP1A2 *4	A2499T	acgttggatgTCCCACAGGAG AAGATTGTCAACCTTGT	acgttggatgGGCAGAA CCGGCAGGCCTC	CCTTCTTGCCCTTACC	CGT
CYP1A2 *5	G3497A	acgttggatgGGGACACAAC GCTGAATGGCTTCTACATC	acgttggatgGATTACAGGC CCTGCACTTGGCTAAAGCTG	CCACTGGTTTACGAAGACA	ACG
CYP1A2 *6	C5090T	acgttggatgGGGACACAACG CTGAATGGCTTCTACATC	acgttggatgGATTACAGGCC TGCACCTTGGCTAAAGCTG	CTGAGTTCCGGCCTGAG	ACG
CYP1A2 *7	G3534A	acgttggatgGGGACACAACG CTGAATGGCTTCTACATC	acgttggatgGATTACAGGCC TGCACCTTGGCTAAAGCTG	TGAGGGGTATGTACTCA	ACG
CYP2C19 *17	C(-806)T	acgttggatgCAAGCCCT TAGCACCAAATCTC	acgttggatgCCATTTAACCCC CTAAAAAAACACG	GTGTCTTCTGTTCTCAAAG	ACG
CYP2D6 *2	C2850T	acgttggatgGTCACCA TCCCGGCAGA	acgttggatgACCCCGTT CTGTCCCGA	TCAATGATGAGAACCTG	ACG
CYP2D6 *2	G4180C	acgttggatgAGCTTCT CGGTGCCACT	acgttggatgGCAGGCTGG GGACTAGGTA	AAGCTCATAGGGGGATGGG	ACT
CYP2D6 *14	G1758A	acgttggatgAGAGACTCCT CGGTCTCTCGC	acgttggatgTAATGCCT TCATGGCCACGCG	CTT CTG CCC ATC ACC CAC	ACG
CYP2D6 *18	4125–4133ins GTGCCACT	acgttggatgGCATATAGCTC CCTGACGCC	acgttggatgCGGGGGCC CATGAACTTT	CTTCTCGGTGCCACTG	ACT
CYP2D6 *21	2573insC	acgttggatgGGTGGATGGT GGGGCTAAT	acgttggatgCGGCCCT GCACTGTTT	ACCCAGCCCAGCCCCCCC	ACT
CYP2D6 *36	Exon9 2D6/2D7 geneconversion	acgttggatgGCATATAGCTC CCTGACGCC	acgttggatgCGGGGGCC CATGAACTTT	GCAGCACTTCAGCTTCTC	ACT
CYP3A4 *2	T15713C	GAAGCTTTTAAGATTGAT TTTTTGGAT	GGATGAATTACATGGTG ATTTATATCTCA	TTTGATCCATTCTTTCTC	ACG

PCR, polymerase chain reaction.

^aLowercase letters indicate homogenous MassExtend 10-bp tag sequence.

was performed using TaqMan (Applied Biosystems, Foster City, CA) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (Sequenom, San Diego, CA) assays for the six CYP genes (Tables 7 and 8). The CYP2D6*5 allele gene deletion²⁴ and the CYP2D6 gene duplication were assayed using long PCRs²⁵ (Roche Diagnostics, Basel, Switzerland) with products resolved by agarose gel electrophoresis.

Data and statistical analysis. For caffeine,^{26,27} chlorzoxazone,^{21,26} omeprazole,^{20,22} and midazolam,^{28,29} area under the plasma concentration-time curves was calculated from 0 to 4 h (AUC_{0–4}) for parent and metabolite. For losartan^{22,27} and dextromethorphan,²²

accumulative urinary excretion amounts were measured over the 12-h urine collection period. Calculated AUC_{0–4} was previously shown to be predictive of the AUC_{0–∞} value for caffeine, omeprazole, midazolam, and chlorzoxazone.²³ Mean metabolic ratios (MRs) for each compound were compared by analysis of variance; multiple comparisons used Bonferroni's testing approach (Table 2).

Allele and genotype frequencies for single-nucleotide polymorphism markers were calculated and compared by group using a χ^2 test with one degree of freedom³⁰ (allele) and two df (genotype) and corresponding Fisher's exact test. Hardy–Weinberg equilibrium was tested by marker and by group.

One-way analysis of variance and single-locus extreme phenotype case-control analysis were used to analyze genetic and phenotypic data. The six MRs were analyzed using analysis of variance models. For small sample sizes, non-parametric tests (Wilcoxon-based drop in dispersion tests) were used for pair-wise comparisons^{31,32} with Bonferroni's correction for multiple comparisons.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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