

# Association of *CYP2B6*, *CYP3A5*, and *CYP2C19* Genetic Polymorphisms With Sibutramine Pharmacokinetics in Healthy Korean Subjects

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We assessed the association of *CYP2B6*, *CYP3A5*, and *CYP2C19* polymorphisms with sibutramine pharmacokinetics. Forty-six healthy male subjects were enrolled, and their *CYP2B6* (\*4 and \*6), *CYP3A5* (\*3), and *CYP2C19* (\*2, and \*3) genotypes were analyzed. After a single 15-mg dose of sibutramine was administered, plasma concentrations of sibutramine and its metabolites, M1 and M2, were measured. *CYP2B6* and *CYP3A5* polymorphisms did not affect the pharmacokinetics of sibutramine and its metabolites. However, the *CYP2C19* genotype substantially influenced plasma levels of sibutramine and its metabolites. The mean area under the curve (AUC) of sibutramine in *CYP2C19* intermediate metabolizers (IMs; \*1/\*2 or \*1/\*3) and poor metabolizers (PMs; \*2/\*2, \*2/\*3) was 18.5 and 252.2% higher, respectively, than the AUC in extensive metabolizers (EMs, \*1/\*1) ( $P < 0.001$ ). The AUC of M1 metabolite in IMs and PMs was 22.5 and 148.0% higher, respectively, than that of EMs ( $P < 0.001$ ). Our findings indicate that the *CYP2C19* genotype substantially affects the pharmacokinetics of sibutramine.

Sibutramine is a recently developed drug for weight loss with a novel mechanism of action. It is different from existing anti-obesity medications, such as the amphetamine-like drugs amphetamine, fenfluramine, and phentermine,<sup>1</sup> in that it is a potent inhibitor of reuptake of noradrenaline and serotonin (5-hydroxytryptamine), and it may also stimulate thermogenesis by activating  $\beta_3$ -adrenoceptors in brown adipose tissue.<sup>2,3</sup>

In humans, sibutramine is rapidly metabolized to an *N*-mono-desmethyl metabolite (M1; BTS 54 354) and an *N,N*-di-desmethyl metabolite (M2; BTS 54 505).<sup>4,5</sup> *In vivo* effects of sibutramine are due primarily to the actions of these two metabolites rather than of sibutramine itself.<sup>6</sup>

To date, very little information is available regarding the disposition of sibutramine, but the manufacturer's information<sup>7</sup> includes the claim that cytochrome P450 3A4 (*CYP3A4*) is involved in the metabolism of sibutramine. It was recently reported from microsomal studies that, in addition to *CYP3A4*, other CYP enzymes—including *CYP2B6*, *CYP3A5*, and, in part, *CYP2C19*—are involved in the metabolism of sibutramine.<sup>8</sup> The genes expressing these enzymes are highly polymorphic,<sup>9</sup> and their polymorphisms modulate the blood levels of substrates of each enzyme and their pharmacokinetic characteristics.<sup>9–15</sup>

Sibutramine has several undesirable side effects. Hypertension, tachycardia, dry mouth, and headache are the most commonly reported adverse reactions.<sup>16</sup> Sibutramine was introduced in the United States in 1997 and in Australia, the United Kingdom, and Italy in 2001. It was first marketed in the United States in February 1998. In 2002, it was temporarily withdrawn in Italy after 103 serious adverse reactions, including two deaths, were reported in the United Kingdom.<sup>17</sup> In the period from the drug's introduction to the US market in February 1998 through the end of September 2001, 397 serious adverse reactions were reported to the US Food Drug and Administration.<sup>18,19</sup> Of these 397 patients, 152 were hospitalized, and there were 29 deaths, of which 19 were attributed to cardiovascular etiologies.<sup>20</sup> Because the pharmacological effects of sibutramine are attributed mostly to its M1 and M2 metabolites,<sup>6</sup> the quantification of metabolic conversion of sibutramine into M1 and M2 might play a crucial role in the prediction of sibutramine's therapeutic effects and associated adverse events.<sup>5</sup> In addition, sibutramine itself is associated with cardiovascular side effects, including QT interval prolongation and cardiac arrest.<sup>21,22</sup>

On the basis of evidence that *CYP3A5*, *CYP2B6*, and *CYP2C19* are involved in the metabolism of sibutramine *in vitro*,<sup>6</sup> we hypothesized that polymorphisms of drug-metabolizing

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enzymes for sibutramine might affect the pharmacokinetics of sibutramine. In this study, we assessed the possible effects of *CYP3A5*, *CYP2B6*, and *CYP2C19* genetic polymorphisms on the pharmacokinetic characteristics of sibutramine and found that only *CYP2C19* genetic polymorphisms play a crucial role in its disposition.

## RESULTS

### Genotyping

The *CYP2B6*, *CYP3A5*, and *CYP2C19* genotypes and their allele frequencies in the participants are presented in **Table 1**. When we compared these data with those from Japanese populations,<sup>23,24</sup> no significant differences were found. We also screened for the *CYP3A4\*18* allele in this population but did not find any subjects with this allele. All observed genotype frequencies for *CYP2B6*, *CYP3A5*, and *CYP2C19* were in Hardy–Weinberg equilibrium. We screened for the \*2, \*3, and \*17 alleles of the *CYP2C19* genotype, but we did not find any subject with the \*17 allele. Therefore, the participants were divided into three groups according to *CYP2C19* genotype: homozygous extensive metabolizers (EMs; *CYP2C19\*1/\*1* ( $n = 13$ )), intermediate metabolizers (IMs; *CYP2C19\*1/\*2* ( $n = 21$ ) and *CYP2C19\*1/\*3* ( $n = 5$ )), and poor metabolizers (PMs; *CYP2C19\*2/\*2* ( $n = 4$ ), and *CYP2C19\*2/\*3* ( $n = 3$ )).<sup>12,25,26</sup>

### Effects of polymorphic *CYP2B6*, *CYP3A5*, and *CYP2C19* genotypes on sibutramine pharmacokinetics

We evaluated the effect of the *CYP2B6* genotypes, including the \*4 and \*6 alleles, on the pharmacokinetics of sibutramine. The pharmacokinetic parameters of sibutramine and its M1 and M2 metabolites were not significantly different among the *CYP2B6* genotype groups (**Table 2**). Plasma concentrations of sibutramine, M1, and M2 also showed similar profiles, regardless of the *CYP2B6* genotype (**Figure 1a,c,e**).

With respect to the *CYP3A5* genotype, the pharmacokinetic parameters of sibutramine and its M1 metabolite were not significantly different between *CYP3A5* genotype groups. The mean peak plasma concentration ( $C_{\max}$ ) of the M2 metabolite in subjects with *CYP3A5\*3/\*3* was 17.3% higher than that in subjects with *CYP3A5\*1* ( $P = 0.021$ ), but other pharmacokinetic parameters (except  $C_{\max}$  of the M2 metabolite) were similar between these two groups (**Table 2**). Plasma concentrations of sibutramine, M1, and M2 also showed similar profiles regardless of *CYP3A5* genotype (**Figure 1b,d,f**).

The *CYP2C19* PMs showed higher plasma levels of sibutramine and M1 metabolite than did the EMs and IMs, respectively, with the EMs and IMs exhibiting similar plasma concentration profiles (**Figure 2a,b**). The *CYP2C19* EMs exhibited higher plasma levels of the M2 metabolite than the IMs did, and the PMs had the lowest plasma levels (**Figure 2c**). When we assessed the effect of *CYP2C19* genotypes on the pharmacokinetics of sibutramine, the total area under the plasma concentration–time curve ( $AUC_{\text{all}}$ ) of sibutramine in the IMs and PMs was 18.5 and 252.2% higher, respectively, than that in the EMs ( $P < 0.001$ ) (**Table 3**). In addition, the  $C_{\max}$  of sibutramine of the IMs and PMs was 13.8 and 179.8% higher, respectively, than the corresponding value in the EMs ( $P < 0.001$ ). However, the apparent oral clearance of sibutramine was lower in IMs and PMs—by 52.3 and 16.9%, respectively—than the apparent oral clearance in the EMs (EMs vs. IMs,  $P = 0.083$ ; EMs vs. PMs,  $P = 0.019$ ) (**Table 3**).

In the *CYP2C19* IMs and PMs, the  $C_{\max}$  of M1 was 9.1 and 85.5% higher, respectively, than the corresponding value in the EMs (EMs vs. PMs,  $P = 0.001$ ; EMs vs. IMs,  $P = 0.002$ ). The  $AUC_{\text{all}}$  of the M1 metabolite in the IMs and PMs was 22.5 and 148.0% higher, respectively, than that found in the EMs ( $P < 0.001$ ). However, the  $C_{\max}$  values of the M2 metabolite in the IMs and PMs were 10.4 and 29.2% lower, respectively, than that

**Table 1** Genotype and allele frequencies of *CYP2B6*, *CYP3A5*, and *CYP2C19* genetic polymorphisms in 46 Korean subjects and comparison with previously published data in Japanese populations

Gene	Genotypes	Number	Frequency		Frequency		P value <sup>b</sup>	
			Present (%)	Allele	Present (%)	Japanese <sup>a</sup> (%)		
<i>CYP2B6</i>	*1/*1	27	58.7	*1	78.3	68.5	0.281	
	*1(*4)/*4	9	19.6	*4	12.0	9.3		
	*4/*4	1	2.2	*6	9.8	16.4		
	*1/*6	9	19.6					
<i>CYP3A5</i>	*1/*1(*3)	1	2.2	*1	17.4	25.6	0.209	
	*1/*3	14	30.4	*3	82.6	74.0		
	*3/*3	31	67.4					
<i>CYP2C19</i>	HomoEMs	13	28.3	*1	56.5	53.9	0.878	
	HeteroEMs	*1/*2	21	45.6	*2	34.8		35.0
		*1/*3	5	10.9	*3	8.7		11.1
	PMs	*2/*2	4	87.0	*17	0		
		*2/*3	3	6.5				

EM, extensive metabolizers; PM, poor metabolizers.

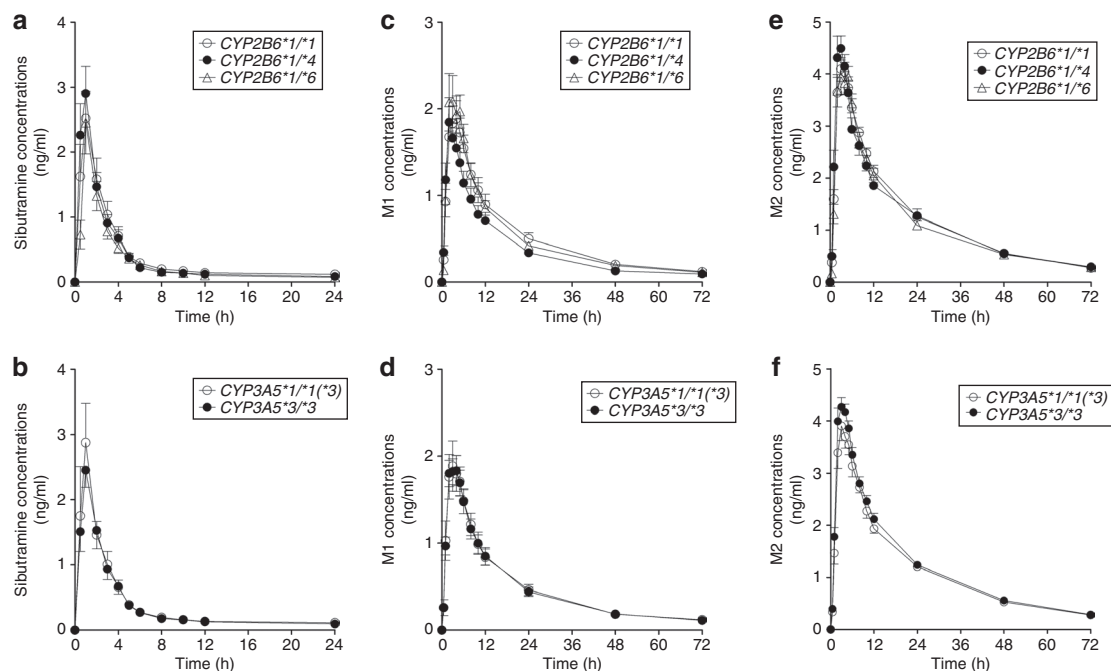
<sup>a</sup>Data obtained from Hiratsuka *et al.*<sup>23</sup> for *CYP2B6* and *CYP3A5* and from Kimura *et al.*<sup>24</sup> for *CYP2C19*. <sup>b</sup>Fisher's exact test.

**Table 2 Comparison of pharmacokinetic variables of sibutramine and M1 and M2 metabolites after a single 15-mg oral dose of sibutramine according to the CYP2B6 and CYP3A5 genotypes**

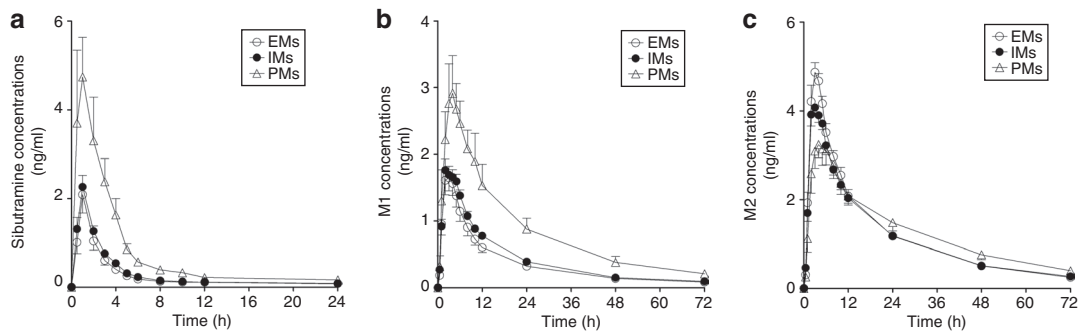
	CYP2B6			P value	CYP3A5		P value
	*1/*1 (n = 27)	*1(*4)/*4 (n = 10)	*1/*6 (n = 9)		*1/*1(*3) (n = 15)	*3/*3 (n = 31)	
<b>M1 metabolite</b>							
Half-life (h)	20.7 ± 5.85	21.0 ± 4.91	20.4 ± 5.89	0.974	20.3 ± 5.20	21.0 ± 5.78	0.693
t <sub>max</sub> (h)	3.37 ± 1.39	2.8 ± 1.31	3.44 ± 1.23	0.475	3.46 ± 1.50	3.16 ± 1.26	0.475
C <sub>max</sub> (ng/ml)	2.18 ± 1.17	2.01 ± 0.73	2.42 ± 0.83	0.688	2.16 ± 1.11	2.21 ± 1.00	0.868
AUC <sub>all</sub> (ng-h/ml)	35.5 ± 22.8	27.5 ± 9.60	34.5 ± 11.4	0.522	33.85 ± 15.93	33.47 ± 20.33	0.950
AUC <sub>inf</sub> (ng-h/ml)	39.1 ± 25.5	30.0 ± 10.6	37.9 ± 12.4	0.512	37.48 ± 18.50	36.66 ± 22.34	0.903
<b>M2 metabolite</b>							
Half-life (h)	22.6 ± 4.70	21.4 ± 4.85	25.4 ± 4.16	0.173	22.9 ± 4.57	22.8 ± 4.87	0.950
t <sub>max</sub> (h)	3.55 ± 1.21	3.2 ± 1.13	3.66 ± 1.11	0.647	3.86 ± 1.12	3.32 ± 1.16	0.141
C <sub>max</sub> (ng/ml)	4.50 ± 1.12	4.89 ± 0.82	4.45 ± 0.65	0.529	4.10 ± 0.86	4.81 ± 0.96	0.021
AUC <sub>all</sub> (ng-h/ml)	86.7 ± 18.4	85.2 ± 19.9	81.9 ± 14.9	0.788	81.78 ± 13.54	87.25 ± 19.52	0.335
AUC <sub>inf</sub> (ng-h/ml)	96.1 ± 19.0	94.8 ± 23.7	92.8 ± 17.2	0.911	91.57 ± 16.09	96.95 ± 20.86	0.384
<b>Sibutramine</b>							
Half-life (h)	9.69 ± 8.84	9.51 ± 10.1	7.27 ± 4.30	0.755	8.64 ± 9.09	9.44 ± 8.11	0.765
t <sub>max</sub> (h)	1.09 ± 0.48	0.75 ± 0.26	1.22 ± 0.44	0.051	1.1 ± 0.50	1.01 ± 0.43	0.566
C <sub>max</sub> (ng/ml)	2.95 ± 2.72	3.28 ± 1.45	2.53 ± 1.39	0.777	3.26 ± 2.80	2.78 ± 1.98	0.505
AUC <sub>all</sub> (ng-h/ml)	9.81 ± 9.19	9.18 ± 4.30	7.54 ± 3.93	0.742	9.59 ± 7.47	9.05 ± 7.61	0.824
AUC <sub>inf</sub> (ng-h/ml)	10.4 ± 9.71	9.88 ± 4.84	7.90 ± 4.06	0.719	10.1 ± 7.90	9.63 ± 8.09	0.827
CL/F (l/h)	1873.4 ± 1753.9	1301.5 ± 859.1	2384.1 ± 3422.0	0.519	2067.2 ± 2745.3	1743.4 ± 1625.9	0.618

Data are expressed as mean values ± SD.

AUC<sub>inf</sub>, AUC from zero to infinity; AUC<sub>all</sub>, total area under the plasma concentration–time curve; CL/F, apparent oral clearance; C<sub>max</sub>, peak plasma concentration; t<sub>max</sub>, time to C<sub>max</sub>.



**Figure 1** Mean (±SEM) plasma concentrations of (a,b) sibutramine, (c,d) M1, and (e,f) M2 metabolites after a single 15-mg oral dose of sibutramine according to CYP2B6 (\*1/\*1 (n = 27)), \*1(\*4)/\*4 (n = 10), and \*1/\*6 (n = 9) and CYP3A5 (\*1/\*1(\*3) (n = 15) and \*3/\*3 (n = 31)) genotypes.



**Figure 2** Mean ( $\pm$ SEM) plasma concentrations of (a) sibutramine, (b) M1, and (c) M2 metabolites after a single 15-mg oral dose of sibutramine according to *CYP2C19* genotype (EMs (\*1/\*1,  $n = 13$ )), IMs (\*1/\*2 or \*1/\*3,  $n = 26$ ), PMs (\*2/\*2 or \*2/\*3,  $n = 7$ ). EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

**Table 3** Comparison of pharmacokinetic variables of sibutramine and M1 and M2 metabolites after a single 15-mg oral dose of sibutramine according to the *CYP2C19* genotype.

<i>CYP2C19</i> genotype	EMs	IMs	PMs	A vs. B	<i>P</i> value*	B vs. C
	A ( $n = 13$ )	B ( $n = 26$ )	C ( $n = 7$ )			
<i>M1</i> metabolite						
Half-life (h)	19.47 $\pm$ 7.38	20.94 $\pm$ 4.84	22.74 $\pm$ 3.99	1.000	0.653	1.000
$t_{\max}$ (h)	3.15 $\pm$ 1.28	3.16 $\pm$ 1.38	3.86 $\pm$ 1.35	0.814	0.680	1.000
$C_{\max}$ (ng/ml)	1.86 $\pm$ 0.81	2.03 $\pm$ 0.72	3.45 $\pm$ 1.50	1.000	0.001	0.002
AUC <sub>all</sub> (ng·h/ml)	24.84 $\pm$ 11.96	30.44 $\pm$ 9.80	61.60 $\pm$ 29.15	0.801	<0.001	<0.001
AUC <sub>inf</sub> (ng·h/ml)	27.27 $\pm$ 12.71	33.18 $\pm$ 10.52	68.82 $\pm$ 32.60	0.855	<0.001	<0.001
<i>M2</i> metabolite						
Half-life (h)	21.03 $\pm$ 3.73	23.05 $\pm$ 4.94	25.97 $\pm$ 4.42	0.593	0.077	0.421
$t_{\max}$ (h)	3.31 $\pm$ 1.18	3.34 $\pm$ 1.13	4.43 $\pm$ 0.98	1.000	0.118	0.087
$C_{\max}$ (ng/ml)	5.11 $\pm$ 0.72	4.58 $\pm$ 0.97	3.62 $\pm$ 0.79	0.259	0.003	0.044
AUC <sub>all</sub> (ng·h/ml)	86.80 $\pm$ 15.54	83.00 $\pm$ 16.89	92.18 $\pm$ 25.13	1.000	1.000	0.706
AUC <sub>inf</sub> (ng·h/ml)	94.65 $\pm$ 17.45	92.27 $\pm$ 17.67	107.11 $\pm$ 26.78	1.000	0.517	0.227
<i>Sibutramine</i>						
Half-life (h)	8.64 $\pm$ 9.34	7.13 $\pm$ 5.54	17.80 $\pm$ 10.76	1.000	0.042	0.006
$t_{\max}$ (h)	1.08 $\pm$ 0.28	1.00 $\pm$ 0.49	1.14 $\pm$ 0.63	1.000	1.000	1.000
$C_{\max}$ (ng/ml)	2.18 $\pm$ 1.45	2.48 $\pm$ 1.40	6.10 $\pm$ 3.50	1.000	<0.001	<0.001
AUC <sub>all</sub> (ng·h/ml)	6.20 $\pm$ 4.60	7.35 $\pm$ 3.61	21.84 $\pm$ 10.16	1.000	<0.001	<0.001
AUC <sub>inf</sub> (ng·h/ml)	6.79 $\pm$ 5.33	7.70 $\pm$ 3.45	23.32 $\pm$ 10.74	1.000	<0.001	<0.001
CL/F (l/h)	3061.2 $\pm$ 3359.7	1601.1 $\pm$ 824.3	517.4 $\pm$ 236.9	0.083	0.019	0.552

Data are expressed as mean  $\pm$  SD. *CYP2C19* genotypes are as follows: EMs (\*1/\*1), IMs (\*1/\*2, \*1/\*3), and PMs (\*2/\*2, \*2/\*3). \**P* values for the comparison between genotype groups are calculated using one-way analysis of variance followed by Bonferroni test or Kruskal–Wallis test with a *posteriori* testing with the Dunn test (for  $t_{\max}$ ).

AUC<sub>inf</sub>, AUC from zero to infinity; AUC<sub>all</sub>, total area under the plasma concentration–time curve; CL/F, apparent oral clearance;  $C_{\max}$ , peak plasma concentration; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer;  $t_{\max}$ , time to  $C_{\max}$ .

found in the EMs (EMs vs. IMs,  $P = 0.003$ ; IMs vs. PMs,  $P = 0.044$ ). Other pharmacokinetic parameters of the M2 metabolite did not differ between *CYP2C19* genotype groups (Table 3).

In a stepwise multiple linear regression analysis with *CYP2B6*, *CYP3A5*, and *CYP2C19* genotypes as independent variables, only the *CYP2C19* genotype was found to be an independent predictor of the pharmacokinetic parameters of sibutramine and M1 metabolite. In addition to *CYP2C19*, *CYP3A5* was also an independent predictor of the  $C_{\max}$  of the M2 metabolite but not of the area under the curve (AUC) (Table 4).

#### Effects of polymorphic *CYP2B6*, *CYP3A5*, and *CYP2C19* genotypes on the metabolic conversion of sibutramine into M1 and M2 metabolites

In order to compare the metabolic conversion of sibutramine into M1 and M2 metabolites with respect to CYP genotype, we calculated the ratios of  $C_{\max}$  and AUC for the genotypes. The *CYP2C19* genotype was found to play a significant role in the metabolic conversion of sibutramine into M1 and M2 metabolites (Table 5). However, the *CYP2B6* and *CYP3A5* genotypes showed no association with the conversion (see Supplementary Table S1 online).

**Table 4** Assessment of the effects of *CYP2B6*, *CYP3A5*, and *CYP2C19* genotypes on pharmacokinetic parameters of sibutramine and M1 and M2 metabolites, using a multiple linear regression model

	Sibutramine				M1 metabolite				M2 metabolite			
	$C_{max}$		AUC		$C_{max}$		AUC		$C_{max}$		AUC	
	$\beta$	<i>P</i> value	$\beta$	<i>P</i> value	$\beta$	<i>P</i> value	$\beta$	<i>P</i> value	$\beta$	<i>P</i> value	$\beta$	<i>P</i> value
<i>CYP2B6</i> *4	-0.069	0.606	0.018	0.887	0.107	0.436	0.185	0.14	-0.139	0.280	0.066	0.682
<i>CYP2B6</i> *6	0.016	0.908	0.023	0.855	-0.183	0.186	-0.114	0.373	0.174	0.178	0.127	0.436
<i>CYP3A5</i>	-0.068	0.613	0.006	0.961	0.055	0.689	-0.029	0.820	0.309	0.019	0.172	0.278
<i>CYP2C19</i>	0.481	0.001	0.580	<0.001	0.435	0.002	0.559	<0.001	-0.446	0.001	-0.050	0.747

AUC, area under the curve;  $C_{max}$ , peak plasma concentration;  $\beta$ , standardized regression coefficient.

**Table 5** Comparison of metabolic conversion of  $C_{max}$  and AUC of sibutramine and M1 and M2 metabolites according to the *CYP2C19* genotype

		EMs	IMs	PMs	<i>P</i> value*		
		A (n = 13)	B (n = 26)	C (n = 7)	A vs. B	A vs. C	B vs. C
$C_{max}$	M1/SIB	1.12 ± 0.56	1.00 ± 0.51	0.63 ± 0.21	1.000	0.116	0.241
	M2/SIB	4.19 ± 4.18	2.57 ± 1.66	0.76 ± 0.41	0.206	0.019	0.309
	(M1 + M2)/SIB	5.31 ± 4.50	3.57 ± 2.09	1.39 ± 0.51	0.246	0.017	0.243
	M2/M1	3.64 ± 2.81	2.53 ± 1.02	1.26 ± 0.69	0.191	0.014	0.250
AUC	M1/SIB	5.24 ± 2.65	4.55 ± 1.30	3.05 ± 1.05	0.754	0.032	0.153
	M2/SIB	25.94 ± 24.06	13.80 ± 6.55	5.12 ± 2.70	0.037	0.007	0.433
	(M1 + M2)/SIB	31.18 ± 25.46	18.34 ± 7.44	8.16 ± 3.21	0.040	0.005	0.330
	M2/M1	5.17 ± 5.20	3.00 ± 1.06	1.72 ± 0.90	0.096	0.043	0.907

Data are expressed as mean values ± SD. \**P* values for the comparison between the groups of the *CYP2C19* genotype are calculated using one-way analysis of variance followed by Bonferroni test. *CYP2C19* genotype groups are as follows: EMs (\*1/\*1), IMs (\*1/\*2, \*1/\*3), and PMs (\*2/\*2, \*2/\*3).

AUC, area under the curve;  $C_{max}$ , peak plasma concentration; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; SIB, sibutramine.

## DISCUSSION

The results of this study demonstrate that the *CYP2C19* genotype is a major determinant of the pharmacokinetic variability of sibutramine in humans and that the *CYP2B6* and *CYP3A5* genotypes play a minor role. The AUC and  $C_{max}$  values of sibutramine itself in *CYP2C19* PMs were 252.2 and 179.8% higher, respectively, than those in the EMs, and this difference is statistically significant. To exclude possible masking effects of the *CYP2C19* genotype on other genotypes tested, we analyzed the data by stratifying the *CYP2B6* and *CYP3A5* genotypes on the basis of the *CYP2C19* genotype and stepwise multiple linear regression analysis in the population studied. We confirmed that only *CYP2C19*—not *CYP2B6* or *CYP3A5*—is a major determinant of the pharmacokinetics of sibutramine in the population studied.

Sibutramine is a prodrug and must be metabolized into the M1 and M2 metabolites to gain pharmacological activity.<sup>4,5</sup> Thus far, only *CYP3A4* has been shown to be involved in the metabolism of sibutramine into M1 and M2 metabolites.<sup>7</sup> However, Bae *et al.* recently showed that *CYP2B6* and, to a lesser extent, *CYP3A4/5* are involved in the metabolism of sibutramine *in vitro*.<sup>8</sup> On the basis of this evidence, we first explored the relationships between the *CYP2B6* and *CYP3A5* genotypes and the pharmacokinetics

of sibutramine. The *CYP2B6* polymorphism has been associated with the pharmacokinetics of various *CYP2B6* substrates, including bupropion,<sup>27</sup> thiotepa,<sup>28</sup> and efavirenz.<sup>29</sup> In addition, the *CYP3A5* polymorphism has been shown to affect the pharmacokinetics of various *CYP3A* substrates, including alprazolam, simvastatin, and many others.<sup>10,30,31</sup> However, despite the evidence that both *CYP2B6* and *CYP3A5* are involved in the metabolism of sibutramine and that their polymorphisms modulate the pharmacokinetics of the respective *CYP2B6* and *CYP3A* substrates, our results suggest that neither the *CYP2B6* nor the *CYP3A5* genotype is associated with the variability of sibutramine disposition and its pharmacokinetics in the population studied. On the contrary, our results strongly indicate that *CYP2C19*, formerly considered to play a minor role in the metabolism of sibutramine *in vitro*,<sup>8</sup> substantially influences plasma levels of sibutramine and significantly changes its pharmacokinetic characteristics.

We evaluated the differences in sibutramine pharmacokinetics between *CYP2C19* EMs and PMs. PMs showed higher plasma levels and a lower disposition rate as compared with EMs. Although there were differences between EMs and IMs in the pharmacokinetics of sibutramine and its metabolites, none of these differences was statistically significant. Similarly,

many pharmacogenetics studies on *CYP2C19* are performed by classifying subjects as EMs (with IMs also categorized as EMs) or PMs.<sup>26,32,33</sup> Therefore, a comparison of sibutramine pharmacokinetics between EMs and IMs might provide only limited information. On the other hand, the values related to the extent of metabolic conversion—as assessed by the ratios of AUC and  $C_{\max}$ —were highest in EMs, followed by IMs and PMs. This finding strongly suggests that *CYP2C19* enzymatic function determined by the polymorphic *CYP2C19* is a major determinant of interindividual variability in sibutramine pharmacokinetics. Our results pharmacogenetically show that the *CYP2C19* genotype, but not the *CYP2B6* or the *CYP3A5* genotype, plays a crucial role in the disposition of sibutramine in humans. We cannot clearly explain the discordance between *in vitro* and *in vivo* findings. Previous reports have suggested that the roles of CYP enzymes other than *CYP2B6* and *CYP3A4/5*—including *CYP2C19*—should be considered in the disposition of sibutramine. Although *in vitro* studies have shown that multiple CYP enzymes are involved in the metabolism of sibutramine, the relative contribution of each CYP enzyme to the metabolism of sibutramine has not been assessed.<sup>8</sup> Therefore, there is a limitation to predicting the *in vivo* capacity of each CYP on the basis of *in vitro* results.<sup>34–36</sup> On the other hand, metabolic pathways other than the known pathway from sibutramine to M1 and M2 metabolites should be considered. Previous reports on obese patients who were given sibutramine demonstrated that both ketoconazole and erythromycin moderately increased steady-state plasma concentrations of sibutramine as well as its active metabolites.<sup>37,38</sup> Therefore, if these inhibitors only block the metabolic conversion of sibutramine into M1 and M2 metabolites, when plasma levels of sibutramine increase, the levels of M1 and M2 metabolites should decrease. It is interesting to note that in our study, despite the apparently significant role of *CYP2C19* in the metabolic conversion of sibutramine into M1 and M2 metabolites, *CYP2C19* PMs showed higher AUC and  $C_{\max}$  levels of sibutramine and of the M1 metabolite. If *CYP2C19* were the only enzyme involved in the sibutramine-to-M1 metabolic pathway, plasma levels of M1 metabolite would have exhibited the opposite result, i.e., decreased M1 metabolite levels. With respect to the M2 metabolites,  $C_{\max}$  levels in PMs were lower than those in EMs, but other pharmacokinetic parameters were similar, regardless of *CYP2C19* genotype. Therefore, it is likely that, besides the pathway from sibutramine to M1 and M2 metabolites, there are other dominant metabolic pathways of sibutramine that are associated with *CYP2C19*. In addition, drugs causing interactions with sibutramine, e.g., ketoconazole and erythromycin, are well-known inhibitors of *CYP3A4/5* but not of *CYP2C19*.<sup>8,33,34</sup> Therefore, it is possible to assume that *CYP3A4/5* is involved in the disposition of sibutramine. However, because we could not confirm this with the results of the present study, further evaluation should be undertaken in order to identify these pathways.

Because the anti-obesity effects of sibutramine are caused by the action of M1 and M2 metabolites but not of the parent sibutramine,<sup>6</sup> and given that *CYP2C19* PMs exhibited a moderate increase in  $C_{\max}$  (1.9-fold) and AUC (2.5-fold) levels as

compared with those of EMs in terms of the M1 metabolite, it is possible to postulate *a priori* that the *CYP2C19* genotype is a factor that modulates the therapeutic effects of sibutramine.

Although sibutramine is frequently prescribed for weight reduction,<sup>39</sup> it has several undesirable side effects.<sup>40</sup> The associate director of the US Food and Drug Administration's Office of Drug Safety has indicated that sibutramine is one of five drugs currently on the market that may be endangering patients.<sup>41</sup> It was also reported that sibutramine is associated with QT prolongation and adverse cardiovascular effects, such as tachycardia, hypertension, and arrhythmia.<sup>40,42,43</sup> Recently it was revealed that sibutramine itself blocks hERG channels in a concentration-, voltage-, state-, and time-dependent manner.<sup>44</sup> The hERG channel is the most important contributor to drug-induced QT prolongation and cardiac arrhythmia.<sup>21,22</sup> Therefore, despite our evidence that *CYP2C19* PMs may achieve a more therapeutic effect by the elevation of plasma metabolite levels, concurrent elevation of sibutramine levels may cause or exacerbate side effects related to sibutramine itself.

The distribution of *CYP2C19* EM and PM genotype groups encompassed a wide range of ethnicities.<sup>16</sup> Although nongenetic factors—such as enzyme inhibition and induction, age, and liver function—can also modulate *CYP2C19* activity, plasma levels of *CYP2C19* substrates can be modulated by the *CYP2C19* genotype. Consistently, the polymorphic *CYP2C19* genotype influenced the pharmacokinetics of sibutramine via the functional change of *CYP2C19* in this study.

Our study has several potential limitations. First, it was a single-dose study in healthy male subjects, and it is not known whether comparable or aggravated results would be obtained if the drug were administered repeatedly to patients. The exclusion of female subjects from the study might be a limitation to the assessment of the pharmacokinetics of sibutramine. However, the recruitment of both genders might have had potential confounding effects on the pharmacogenetic evaluation. The literature addressing the effect of gender on *CYP2C19* activity includes findings supporting this. The disposition of S-mephenytoin, a *CYP2C19* substrate, was shown to be higher in female subjects than in male subjects.<sup>45,46</sup> Second, although we demonstrated the substantial role of the *CYP2C19* genotype in sibutramine pharmacokinetics, the number of homozygous mutants for *CYP2B6* and *CYP3A5* enrolled in the study was too small for statistical significance. In order to fully assess the effects of these genes, a larger number of subjects with the mutant allele is needed. In this study, we did not assess the effect of the *CYP3A4* gene. Like the *CYP3A5* gene, the *CYP3A4* gene is very polymorphic. Although >40 single-nucleotide polymorphisms have been identified in the *CYP3A4* gene,<sup>47</sup> the allele frequencies are very low, making it unlikely that genotypic differences in *CYP3A4* account for interindividual variability in sibutramine metabolism.

The clinical implication of this study is that the *CYP2C19* genotype is an important factor affecting the disposition of sibutramine. As mentioned, there is a considerable interethnic difference in the frequency of the *CYP2C19* PM genotype.<sup>16</sup> Given that *CYP2C19* PM subjects had higher plasma levels of

sibutramine, a determination of a patient's genotype may help explain the toxicity of the drug and help clinicians better manage sibutramine therapy.

In summary, the *CYP2C19* genotype, but not *CYP2B6* or *CYP3A5* genotype, affects the plasma levels of sibutramine and its metabolites. This means that the *CYP2C19* genotype is a major determinant in the modulation of the disposition of sibutramine in humans.

## METHODS

**Subjects.** A total of 46 healthy volunteers were recruited from previous pharmacokinetic studies. All the subjects were men, and their mean ( $\pm$ SD) age was  $24.8 \pm 3.4$  years (range, 19–38 years); weight,  $68.4 \pm 7.6$  kg (range, 53–88 kg); and height,  $175.4 \pm 5.2$  cm (range, 165–186 cm).

All 46 subjects were determined by physicians to be healthy on the basis of the results of a detailed physical examination, 12-lead electrocardiography, serum biochemistry, hematology, and urinalysis. Subjects were excluded if they had a history of or evidence of a hepatic, renal, gastrointestinal, or hematologic abnormality, a hepatitis B or C or HIV infection, any other acute or chronic disease, or an allergy to any drug. All the subjects were nonsmokers and were not taking any medication, and all gave written informed consent.

**Study design.** The study protocol was approved by the institutional review board of Anam Hospital, Korea University College of Medicine, Seoul, Korea. All the study subjects were admitted to the clinical trial center during the evening prior to the day of drug administration. After an overnight fast, the subjects were given a single, 15-mg oral dose of sibutramine HCl (Reductil; Abbott Korea, Seoul, Korea) with 240 ml of water in the morning. Blood samples were collected immediately before drug administration (baseline) and then at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24, 48, and 72 h after drug administration. Blood samples were collected in heparinized tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Plasma was separated by centrifugation at 3,000 r.p.m. at 4°C for 15 min and stored at –70°C until assayed.

**Analytical methods.** Plasma concentrations of the metabolites of sibutramine, M1 and M2, were analyzed using a validated high-performance liquid chromatographic–mass spectrometric method (HPLC/MS/MS), as previously described, with a slight modification.<sup>48</sup> In brief, plasma samples (0.4 ml) were spiked with an internal standard (10  $\mu$ l at 1  $\mu$ g/ml propiverine), alkalized with 20  $\mu$ l of 0.1 mol/l sodium carbonate solution, and extracted with 5 ml of methyl-*t*-butyl ether. After vigorous vortex mixing, the organic phase was evaporated to dryness under a flow of nitrogen gas. The dry residue was reconstituted with 100  $\mu$ l of mobile phase, and a 5  $\mu$ l aliquot of this solution was injected into the API 3000 HPLC-MS/MS system (Sciex Division of MDS, Toronto, Ontario, Canada) equipped with an Agilent 100 series high-performance liquid chromatographic system (Agilent, Wilmington, DE). Chromatographic separation of the compounds was accomplished using a Shiseido Capcell Pak C18 column (5  $\mu$ m, 3.0  $\times$  150 mm; Shiseido, Tokyo, Japan) with a mobile phase consisting of 62% acetonitrile, including 0.1% trifluoroacetic acid. The mass spectrometer with an electrospray source was run in the positive mode and  $m/z$  280.4  $\rightarrow$  124.9, 266.4  $\rightarrow$  124.9, 252.3  $\rightarrow$  124.9, and 368.0  $\rightarrow$  182.8 were monitored for sibutramine, the M1 and M2 metabolites, and the internal standard, respectively. Linearity calibration curves in the ranges of 0.05–40 ng/ml for sibutramine, M1, and M2 were established ( $r^2 = 0.99854$  for sibutramine,  $r^2 = 0.99777$  for M1, and  $r^2 = 0.99980$  for M2). For sibutramine, M1, and M2, intraday and interday coefficients of variation were <11.04%.

**Genotyping.** For genetic analysis, a 2-ml EDTA blood sample was drawn from each subject and stored at –20°C until DNA extraction. DNA was extracted using standard methods (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany).

***CYP2B6* genotyping:** All subjects were genotyped for the *CYP2B6*\*4 (18053A>G) (rs2279343) and *CYP2B6*\*6 (15631G>T and 18053A>G) (rs3745274) alleles by pyrosequencing methods, using a Pyrosequencer (Biotage, Uppsala, Sweden) as described previously by Rohrbacher *et al.*<sup>49</sup> The validity of the method was confirmed by sequencing.

***CYP3A5* genotyping:** All subjects were genotyped for the *CYP3A5*\*3 (6986A>G) (rs776746) allele by pyrosequencing methods using a Pyrosequencer (Biotage). The primers used in *CYP3A5*\*3 genotyping were 5'-biotin-CCCACGTATGTACCACCCAGC-3' (forward) and 5'-CACACAGGAGCCACCCAAGG-3' (reverse). The sequence primer was 5'-AGCTCTTTTGTCTTTCA-3'. The PCR conditions for both assays were 1 cycle at 94°C for 5 min, followed by 45 cycles at 94°C for 30 s, at 58°C for 30 s, and at 72°C for 30 s. The validity of the method was confirmed by sequencing.

***CYP2C19* genotyping:** All subjects were genotyped for the *CYP2C19*\*2 (681G>A) (rs4244285), *CYP2C19*\*3 (636G>A) (rs4986893), and *CYP2C19*\*17 (–806C>T) (rs12248560) alleles using pyrosequencing methods as described previously by Kim *et al.*<sup>50</sup> The validity of the method was confirmed by sequencing.

**Pharmacokinetic analysis.** The pharmacokinetic variables of sibutramine, M1, and M2 were estimated by noncompartmental methods using WinNonlin version 5.2 (Pharsight, Cary, NC). The peak concentrations ( $C_{\max}$ ) and their time to reach  $C_{\max}$  ( $t_{\max}$ ) were estimated directly from the observed plasma concentration–time data. The  $AUC_{\text{all}}$  was calculated using the linear trapezoidal rule. The AUC from zero to infinity ( $AUC_{\text{inf}}$ ) was calculated as  $AUC_{\text{inf}} = AUC_{\text{last}} + C_t/K_e$  (where  $C_t$  is the last plasma concentration measured). The elimination rate constant ( $K_e$ ) was determined by linear regression analysis of the log-linear part of the plasma concentration–time curve. The half-life ( $t_{1/2}$ ) of metabolites was calculated using the equation half-life =  $\ln 2/K_e$ . The apparent clearance of orally administered sibutramine (apparent oral clearance) was calculated as follows: apparent oral clearance = dose/ $AUC_{\text{inf}}$ .

**Statistical analysis.** Data are expressed as mean values  $\pm$  SD in the text and tables and, for clarity, as mean values  $\pm$  SEM in the figures. A stepwise forward multiple regression analysis was carried out to investigate the relative effects of *CYP2B6*, *CYP3A5*, and *CYP2C19* genotypes on the pharmacokinetic variables of sibutramine and its metabolites, M1 and M2. Statistical comparisons of all pharmacokinetic variables between genotype groups were carried out using analysis of variance, followed by *a posteriori* testing with the Bonferroni test. Data for  $t_{\max}$  were analyzed using the Mann–Whitney *U* test or the Kruskal–Wallis test, with *a posteriori* testing using the Dunn test. The data were analyzed using the statistical program SPSS 12.0 for Windows (SPSS, Chicago, IL). Differences were considered statistically significant at  $P < 0.05$ .

**Supplementary Material** is linked to the online version of the paper at <http://www.nature.com/cpt>

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## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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