

PHARMACOGENETICS

Influence of *CYP2D6**10 on the pharmacokinetics of metoprolol in healthy Korean volunteers

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SUMMARY

Background and objective: Genetic polymorphism of *CYP2D6* leads to differences in pharmacokinetics of *CYP2D6* substrates. The *CYP2D6**10 allele is clinically important in Koreans because of its high frequency in Asians. We investigated whether the pharmacokinetics of metoprolol was altered by the presence of the *CYP2D6**10 allele in Korean subjects.

Methods: One hundred and seven volunteers were recruited and grouped as *CYP2D6**1/*1, *CYP2D6**1/*10 and *CYP2D6**10/*10 according to their genotypes. Metoprolol tartrate 100 mg (Betacloc®) was administered orally once to each subject in these three groups ($n = 6, 7$ and 5 , respectively). The pharmacokinetic parameters of metoprolol and its metabolite, α -hydroxymetoprolol, and the metabolic ratio for the three groups were estimated and compared.

Results and discussion: The area under the plasma concentration–time curve ($AUC_{0 \rightarrow \infty}$), the maximum plasma concentration (C_{max}) and the elimination half-life ($T_{1/2}$) of metoprolol and α -hydroxymetoprolol for the *CYP2D6**10/*10 group were all significantly different from those

of the *CYP2D6**1/*1 group ($P < 0.05$). The $AUC_{0 \rightarrow \infty}$ of metoprolol were 443.7 ± 168.1 , 995.6 ± 321.4 and 2545.3 ± 632.0 ng·h/mL, and the $AUC_{0 \rightarrow \infty}$ of α -hydroxymetoprolol were 1232.0 ± 311.2 , 1344.0 ± 288.1 and 877.4 ± 103.4 ng·h/mL for groups *CYP2D6**1/*1, *1/*10 and *10/*10, respectively. The corresponding $T_{1/2}$ values of metoprolol were 2.7 ± 0.5 , 3.2 ± 1.3 and 5.0 ± 1.1 h, while those of α -hydroxymetoprolol were 5.4 ± 1.5 , 6.0 ± 1.4 and 10.5 ± 4.2 h, respectively. The metabolic ratios of the three groups were significantly different ($P < 0.05$).

Conclusion: The *CYP2D6**10 allele altered the pharmacokinetics of metoprolol in Korean subjects and is likely to affect other drugs metabolized by the *CYP2D6* enzyme, similarly.

Keywords: *CYP2D6*, Korean, metoprolol, pharmacokinetics

INTRODUCTION

Cytochrome P450 (CYP) enzymes play an important role in the metabolism of drugs (1, 2). Differences in the activity of these enzymes are associated with interindividual variability in the efficacy and toxicity of many drugs (3, 4). Approximately 80 CYP enzymes have been characterized in humans, each of which shows distinct catalytic specificity and regulation (5).

CYP2D6 is involved in the metabolism of numerous drugs, including some β -blockers, tricyclic antidepressants, neuroleptics and antiarrhythmics (2, 6). The gene that encodes *CYP2D6*

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has more than 90 variants (<http://www.imm.ki.se/CYPalleles/cyp2d6.htm>). Such polymorphism leads to a variety of enzymatic activities and different phenotypes. Individuals classically described as poor, intermediate, extensive or ultra-rapid metabolizers (PM, IM, EM or UM) show differences in the pharmacokinetics and pharmacodynamics of the different substrates (6–8). The frequency of CYP2D6 PM in Caucasians is about 7% (9). Although the proportion of PM individuals among Asians, including Koreans, is no more than 1%, genetic polymorphism of CYP2D6 plays an important role in these populations because of the high frequency of the *CYP2D6*10* allele (>50%), which is associated with low CYP2D6 activity (9–14).

Metoprolol, a lipophilic and β_1 -selective antagonist, has been used effectively in the treatment of coronary artery disease, hypertension and chronic heart failure (15–18). It is metabolized into at least four metabolites, α -hydroxymetoprolol, *O*-demethyl metoprolol, *N*-dealkyl metoprolol and metoprolol acid (19). CYP2D6 is responsible for about 70–80% of metoprolol's metabolism (20), and α -hydroxylation is exclusively mediated by CYP2D6 (21–23).

It has been reported that *CYP2D6*10* allele is responsible for the pharmacokinetic variability of metoprolol seen in Chinese and Japanese populations (24, 25). Sohn *et al.* (10) studied the differences in the metabolic ratio of metoprolol between EM and PM in Koreans. However, the influence of *CYP2D6* genotype, not phenotype, on the pharmacokinetics of metoprolol in Koreans remains unclear. Therefore, the purpose of this study was to investigate the relationship between the *CYP2D6*10* allele, common among Koreans, and the pharmacokinetics of metoprolol in Korean volunteers.

MATERIALS AND METHODS

Subjects and study protocol

This research was approved by the Institutional Review Board of the Inha University Hospital at Incheon. Written informed consent was received from each subject before participating in this study. One hundred and seven healthy Korean subjects were genotyped for *CYP2D6*10*, and then classified into three groups (*CYP2D6*1/*1*, **1/*10* and

**10/*10*). Eighteen volunteers (14 males, four females and aged 21–27 years) were selected for studying the pharmacokinetics of metoprolol. Their mean ($\pm$ SD) age, body weight and height were 23.2 ± 1.5 years, 66.9 ± 11.5 kg and 168.7 ± 6.8 cm, respectively. There were no significant differences in sex, age, body weight or height among the three groups.

After fasting overnight, a single oral dose of 100 mg of metoprolol tartrate (Betaloc[®] tablet; AstraZeneca Korea, Seoul, Korea) was administered to each subject. Blood samples were taken from a peripheral vein at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after drug administration. Plasma samples, separated from blood, were stored at -80°C before analysis.

Genotyping

Genomic DNA was isolated from peripheral blood by phenol–chloroform extraction to determine the *CYP2D6*10* alleles (26). *CYP2D6*10* allele, which contains the C100T mutation in exon 1, was screened by allele specific polymerase chain reaction (PCR) analysis. Alleles not containing the C100T mutation were classified as *CYP2D6*1*. The first PCR was carried out using primers 9, 10 and 10B listed in Table 1 (12). Secondary PCR was done using primer 9, wild-specific primer (primer 11) and a mutant-specific primer (primer 12). Identification of genotype was performed through electrophoresis. Genomic DNA from subjects participating in the pharmacokinetic study was reassayed using the long PCR amplification reported by Hersberger *et al.* (27) to identify any subjects with the *CYP2D6*5*.

Determination of metoprolol and α -hydroxymetoprolol

Concentrations of metoprolol and α -hydroxymetoprolol in plasma were analysed by high-performance liquid chromatography (HPLC) (Nanospace SI-2 system; Shiseido Co., Tokyo, Japan) (28, 29). To 1 mL of plasma, 50 μL of the internal standard (dextrophan tartrate, 10 $\mu\text{g}/\text{mL}$) and 300 μL of 5% trichloroacetic acid were added, and then centrifuged at 3000 rpm for 10 min. The samples were alkalized with 1 mL of 1 mol/L sodium hydroxide and extracted with 5 mL of *tert*-butylmethyl

Table 1. Primers for the amplifying the fragments of *CYP2D6**10 allele

Primer name	Position	Sequences
Primer 9	-196 ~ -179	5'-ACCAGGCCCTCCACCGG-3'
Primer 10	321 ~ 302	5'-TCTGGTAGGGGAGCCTCAGC-3'
Primer 10B	L320 ~ 302	5'-GTGGTGGGGCATCCTCAGG-3'
Primer 11	201 ~ 188	5'-AGGGGGCCTGGTGG-3'
Primer 12	201 ~ 188	5'-AGGGGGCCTGGTGA-3'

ether. Subsequently, the organic phase was extracted with 1 mL of 1 mol/L hydrochloric acid. The aqueous phase was alkalized again and back-extracted with 4 mL of *tert*-butylmethyl ether. The organic solvent was evaporated and the remnants dissolved in 150 µL of mobile phase (87.5% pH 3.0 phosphate buffer and 12.5% acetonitrile). Validation of the method was performed using three seeded controls of low, medium and high concentrations as well as the lower limit of quantification (LLOQ), following the principles of Shah *et al.* (30). HPLC was carried out isocratically with a Capcell Pak UG120 column (4.6 × 150 mm, 5 µm; Shiseido Co., Tokyo, Japan) and 10 µL aliquots of each sample were delivered by an autosampler. The flow rate of the mobile phase was 1 mL/min. Fluorometric detection was performed at 225 nm (excitation) and 320 nm (emission).

The intra- and inter-day coefficients of variation for metoprolol between 5 and 250 ng/mL ranged from 3.5% to 12.6%, and for α -hydroxymetoprolol, they ranged from 2.9% and 20.3%. The accuracy of the assay between 5 and 250 ng/mL ranged from 90.8% to 115.0% (intra-day run) and from 95.9% to 117.6% (inter-day run). The LLOQ for this assay was determined as 5 ng/mL for both metoprolol and α -hydroxymetoprolol. The mean recovery rates ranged from 95.9% to 98.2% for metoprolol and 88.5% to 92.8% for α -hydroxymetoprolol between 10 and 100 ng/mL, respectively.

Pharmacokinetic analysis

WinNonlin, version 4.1 (Pharsight Co., Mountain View, CA, USA) was used for the analysis of pharmacokinetic parameters, such as maximum plasma concentration ($C_{\max}$), time to reach the maximum plasma concentration ($T_{\max}$), area under the plasma concentration-time curve ($AUC_{0 \rightarrow \infty}$) and elimination half-life ($T_{1/2}$). These parameters were calculated by non-compartmental analysis.

The metabolic ratio was calculated as the plasma concentration ratio of metoprolol to α -hydroxymetoprolol at each blood sampling time.

Statistical analysis

The statistical significance of differences in pharmacokinetic parameters among the three groups was tested using one-way ANOVA, followed by Duncan's method and Dunn's method for comparison of parameters between any two groups. $P < 0.05$ was considered to be statistically significant.

RESULTS

One hundred and seven volunteers were classified by their genotypes using allele-specific PCR amplification for the C100T mutation in the *CYP2D6* gene. Of these, 33 were homozygous for *CYP2D6**1, 27 for *CYP2D6**10, while the remaining 47 were heterozygous for these alleles. Of these volunteers, 18 participated in the pharmacokinetic study and were classified into three groups: *CYP2D6**1/*1 (six subjects), *CYP2D6**1/*10 (seven subjects) and *CYP2D6**10/*10 (five subjects).

The plasma concentration-time curves of metoprolol and α -hydroxymetoprolol are shown in Figs 1 and 2. There is no mean plasma concentration at 24 h for the *CYP2D6**1/*1 group in Fig. 1 because the plasma concentrations of metoprolol of all samples were under the LLOQ. The $AUC_{0 \rightarrow \infty}$ and the $C_{\max}$ of metoprolol in the *CYP2D6**10/*10 group were significantly greater than those of the *CYP2D6**1/*1 or *CYP2D6**1/*10 group, whereas the $AUC_{0 \rightarrow \infty}$ and the $C_{\max}$ of α -hydroxymetoprolol in this group were significantly lower than in the *CYP2D6**1/*1 or *CYP2D6**1/*10 groups ($P < 0.05$). However, there was no significant difference between the three groups in the $T_{\max}$ of metoprolol and α -hydroxymetoprolol. The $T_{1/2}$ of metoprolol and α -hydroxymetoprolol in the *CYP2D6**10/*10

group was significantly longer than those of the *CYP2D6**1/*1 or *CYP2D6**1/*10 groups ($P < 0.05$).

The metabolic ratios of metoprolol to α -hydroxymetoprolol was markedly higher in the *CYP2D6**10/*10 group than in the *CYP2D6**1/*1 and *CYP2D6**1/*10 groups (Fig. 3). In addition,

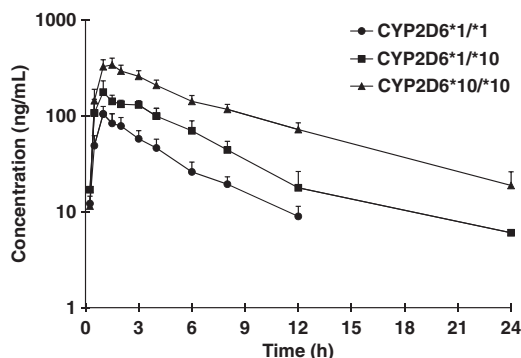


Fig. 1. Time profiles of mean plasma concentration of metoprolol after an oral administration of metoprolol tartrate 100 mg (*CYP2D6**1/*1, $n = 6$; *CYP2D6**1/*10, $n = 7$; *CYP2D6**10/*10, $n = 5$).

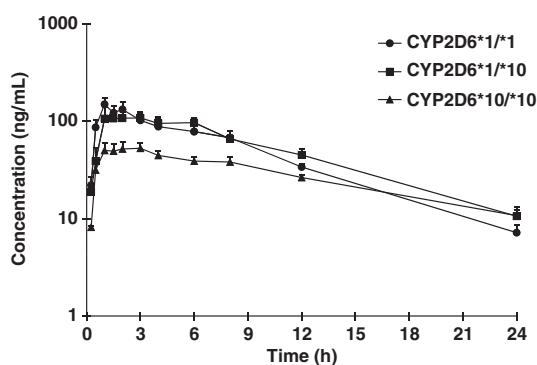


Fig. 2. Time profiles of mean plasma concentration of α -hydroxymetoprolol after an oral administration of metoprolol tartrate 100 mg (*CYP2D6**1/*1, $n = 6$; *CYP2D6**1/*10, $n = 7$; *CYP2D6**10/*10, $n = 5$).

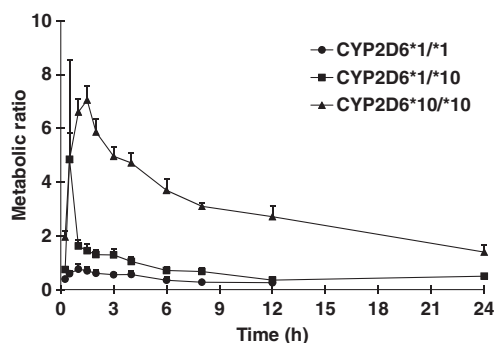


Fig. 3. Metabolic ratios of metoprolol to α -hydroxymetoprolol.

metabolic ratios determined at each blood sampling time (0.5, 1, 1.5, 2, 3, 4, 6 and 8 h) were significantly different between groups ($P < 0.05$). In summary, the metabolism of metoprolol to α -hydroxymetoprolol in the *CYP2D6**10/*10 group was remarkably different from those of the other two groups (Table 2).

DISCUSSION

The *CYP2D6**10 allele altered the pharmacokinetics of metoprolol in Korean volunteers. Although *CYP2D6* is only a small fraction of the total *CYP450* content in the liver, many drugs acting on the central nervous and circulatory systems are metabolized by it (31). There are also clear inter-ethnic differences in the frequency of the different allelic forms of the *CYP2D6* gene. For example, the frequency of *CYP2D6**4 allele is 1% in Asians, but 12–21% in Caucasians. The *CYP2D6**3, *4, *5 and *6 alleles are known to be associated with PM (7, 32–34). The *CYP2D6**9, *10, *17 and *41 alleles are termed IM alleles, coding for less active enzymes or low level of enzyme expression (7, 32). The respective allele frequencies of *CYP2D6**3, *4 and *5 in Caucasians are 2.3%, 21.5% and 4%, but they are 0%, 0.4% and 5.7% in Asians (2). For the *CYP2D6**10 allele, the frequency in Asians is about 50%, whereas it is only 1–2% in Caucasians (8). One important mutation, the C100T substitution in exon 1 of *CYP2D6**10 allele shows clear ethnic differences in frequency. This causes an amino acid substitution (Pro34Ser) leading to an unstable form of the enzyme with low metabolic activity (12). The common *CYP2D6**10 allele among Asians, including Koreans, could help explain their lower ability than Caucasians to metabolize *CYP2D6* substrates (35). Therefore, this allele could be clinically important for Koreans, compared with other alleles, such as *CYP2D6**3, *4, *5 or *6, which are rare in this population. The different prevalence of the various alleles lead to differences in the pharmacokinetics of various *CYP2D6* substrates between Asians and Caucasians (36). The metabolism of risperidone was reported to be dependent on *CYP2D6* among Korean patients with schizophrenia (37). It was further reported that the *CYP2D6**10 allele played an important role in the regulation of enzyme activity. Fukuda *et al.* reported that *CYP2D6**10 affected the pharmacokinetics

Table 2. Pharmacokinetic parameters of metoprolol and α -hydroxymetoprolol in the three groups by CYP2D6 genotype

Parameters	CYP2D6*1/*1 (n = 6)	CYP2D6*1/*10 (n = 7)	CYP2D6*10/*10 (n = 5)	P value
Metoprolol				
AUC _{0→∞} (ng·h/mL)	443.7 ± 168.1	995.6 ± 321.4	2545.3 ± 632.0 ^c	<0.001*
C _{max} (ng/mL)	114.4 ± 37.4	218.4 ± 70.8 ^a	349.4 ± 117.8 ^{b,c}	<0.001*
T _{max} (h)	1.2 ± 0.4	1.6 ± 1.0	1.4 ± 0.4	0.601
T _{1/2} (h)	2.7 ± 0.5	3.2 ± 1.3	5.0 ± 1.1 ^{b,c}	0.006*
α-Hydroxy metoprolol				
AUC _{0→∞} (ng·h/mL)	1232.0 ± 311.2	1344.0 ± 288.1	877.4 ± 103.4 ^{b,c}	0.024*
C _{max} (ng/mL)	159.8 ± 52.6	139.6 ± 38.3	57.1 ± 18.5 ^{b,c}	0.002*
T _{max} (h)	1.4 ± 0.5	2.3 ± 1.2	2.0 ± 0.7	0.247
T _{1/2} (h)	5.4 ± 1.5	6.0 ± 1.4	10.5 ± 4.2 ^c	0.032*

Each value represents the mean ± SD.

*Statistically significant difference among genotype groups, $P < 0.05$.

^aSignificant difference between CYP2D6*1/*1 and CYP*1/*10 groups, $P < 0.05$.

^bSignificant difference between CYP2D6*1/*10 and CYP*10/*10 groups, $P < 0.05$.

^cSignificant difference between CYP2D6*1/*1 and CYP*10/*10 groups, $P < 0.05$.

of venlafaxine in a Japanese population (38). Reduced trough level and total clearance of haloperidol was significantly correlated with the number of active CYP2D6 genes in a German population as well (39). Taken together, these data show that the genetics of metabolic enzymes should be taken into account when designing pharmacokinetic studies.

In this study, we also looked for the CYP2D6*5 allele and confirmed that no one had this allele, which is associated with the absence of drug-metabolizing activity (40).

Our results are consistent with those of Kirchheiner in that the C_{max}, AUC and T_{1/2} were altered significantly according to the CYP2D6 genotype (41). Sohn *et al.* (10) compared the metabolic ratios of metoprolol between the different phenotype groups (EM and PM) among Asians. They reported that Chinese EM had a lower capacity to metabolize metoprolol to α -hydroxymetoprolol than Koreans and Japanese. Our results are in agreement with other studies on the effect of CYP2D6*10 allele on the pharmacokinetics of metoprolol in Japanese and Chinese (24, 25). Taguchi *et al.* (25) reported that the oral clearance value in Japanese patients homozygous for the CYP2D6*10 allele decreased by 60.4% when compared with those of patients with the CYP2D6*1/*1 or *1/*2 genotypes. Huang *et al.* (24) found significant differences in AUC, C_{max} and T_{1/2}

among homozygous C188, heterozygous C/T188 and homozygous T188 subjects for either *R*- or *S*-metoprolol in a Chinese population. However, the mean plasma concentrations of metoprolol in Malaysian cardiovascular patients with CYP2D6*1/*1 genotype were not significantly lower than those of the CYP2D6*10/*10 group according to Ismail *et al.* (42). It has been reported that females in the fertile age range have a slightly higher CYP2D6 activity than males, although the evidence is conflicting (43). We investigated the difference in the pharmacokinetics of metoprolol between three females and four males with the CYP2D6*1/*10, because four women (one in the CYP2D6*1/*1, three in the CYP2D6*1/*10) participated. However, there were no significant differences in the mean pharmacokinetic parameters of the two groups.

This study was designed to analyse the plasma concentration of racemic metoprolol. We chose a simpler method than measuring the plasma concentrations of the enantiomers, because the plasma concentrations of *S*-metoprolol, a pharmacologically active form, were similar to those of *R*-metoprolol reported by Huang *et al.* (24).

There were no distinct interethnic differences in the observed effect of CYP2D6*10 allele between Japanese and Chinese subjects on the pharmacokinetics of metoprolol. The lack of effect reported for Malay subjects may be attributed to the differences in the experimental conditions. Our results

must be interpreted within the following limitations. The results were obtained from a healthy volunteer group, not from a patient group. Thus, the therapeutic effects of metoprolol need to be evaluated in patients with heart failure, and additional studies on the correlation between pharmacodynamics and genotypes in Korean should be performed before any application of this information to clinical practice.

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REFERENCES

- Gonzalez FJ (1988) The molecular biology of cytochrome P450s. *Pharmacological Reviews*, **40**, 243–288.
- Bertilsson L, Dahl ML (1996) Polymorphic drug oxidation: relevance to the treatment of psychiatric disorders. *CNS Drugs*, **5**, 200–223.
- Dorne JLCM, Walton K, Slob W, Renwick AG (2002) Human variability in polymorphic CYP2D6 metabolism: is the kinetic default uncertainty factor adequate? *Food and Chemical Toxicology*, **40**, 1633–1656.
- Nagata K, Yamazoe Y (2002) Genetic polymorphism of human cytochrome P450 involved in drug metabolism. *Drug Metabolism and Pharmacokinetics*, **17**, 167–189.
- Nelson DR, Koymans L, Kamataki T *et al.* (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, **6**, 1–42.
- Ingelman-Sundberg M (2005) Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *The Pharmacogenomics Journal*, **5**, 6–13.
- Zanger UM, Raimundo S, Eichelbaum M (2004) Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **369**, 23–37.
- Ingelman-Sundberg M (2001) Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy. *Journal of Internal Medicine*, **250**, 186–200.
- Bertilsson L, Lou YQ, Du YL *et al.* (1992) Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquine and S-mephenytoin. *Clinical Pharmacology and Therapeutics*, **51**, 388–397.
- Sohn DR, Shin SG, Park CW, Kusaka M, Chiba K, Ishizaki T (1991) Metoprolol oxidation polymorphism in an Korean population comparison with native Japanese and Chinese populations. *British Journal of Clinical Pharmacology*, **32**, 504–507.
- Bertilsson L, Dahl ML, Dalén P, Al-Shurbaji A (2002) Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. *British Journal of Clinical Pharmacology*, **53**, 111–122.
- Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjöqvist F, Ingelman-Sundberg M (1994) Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. *Molecular Pharmacology*, **46**, 452–459.
- Roh HK, Dahl ML, Johansson I, Ingelman-Sundberg M, Cha YN, Bertilsson L (1996) Debrisoquine and S-mephenytoin hydroxylation phenotypes and genotypes in a Korean population. *Pharmacogenetics*, **6**, 441–447.
- Yue QY, Zhong ZH, Tybring G, Dalén P, Dahl ML, Bertilsson L, Sjöqvist F (1998) Pharmacokinetics of nortriptyline and its 10-hydroxymetabolite in Chinese subjects of different CYP2D6 genotypes. *Clinical Pharmacology and Therapeutics*, **64**, 384–390.
- Olsson G, Rehnqvist N, Sjögren A, Erhardt L, Lundman T (1985) Long-term treatment with metoprolol after myocardial infarction: effect on 3 year mortality and morbidity. *Journal of the American College of Cardiology*, **5**, 1428–1437.
- Wikstrand J, Warnold I, Tuomilehto J *et al.* (1991) Metoprolol versus thiazide diuretics in hypertension. Morbidity results from the MAPHY study. *Hypertension*, **17**, 574–588.
- MERIT-HF Study Group (1999) Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomized intervention trial in congestive heart failure (MERIT-HF). *Lancet*, **353**, 2001–2007.
- Benfield P, Clissold SP, Brogden RN (1986) Metoprolol: an updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in hypertension, ischaemic heart disease and related cardiovascular disorders. *Drugs*, **31**, 376–429.
- Li F, Cooper SF, Côté M (1995) Determination of the enantiomers of metoprolol and its major acidic metabolite in human urine by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B*, **668**, 67–75.
- Murthy SS, Shetty HU, Nelson WL, Jackson PR, Lennard MS (1990) Enantioselective and diastereoselective aspects of the oxidative metabolism of metoprolol. *Biochemical Pharmacology*, **40**, 1637–1644.

21. Hamelin BA, Bouayad A, Méthot J *et al.* (2000) Significant interaction between the nonprescription antihistamine diphenhydramine and the CYP2D6 substrate metoprolol in healthy men with high or low CYP2D6 activity. *Clinical Pharmacology and Therapeutics*, **67**, 466–477.
22. Lennard MS, Tucker GT, Silas JH, Woods HF (1986) Debrisoquin polymorphism and the metabolism and action of metoprolol, timolol, propranolol and atenolol. *Xenobiotica*, **16**, 435–447.
23. McGourty JC, Silas JH, Lennard MS, Tucker GT, Woods HF (1985) Metoprolol metabolism and debrisoquin oxidation polymorphism-population and family studies. *British Journal of Clinical Pharmacology*, **20**, 555–566.
24. Huang JD, Chuang SK, Cheng CL, Lai ML (1999) Pharmacokinetics of metoprolol enantiomers in Chinese subjects of major CYP2D6 genotypes. *Clinical Pharmacology and Therapeutics*, **65**, 402–407.
25. Taguchi M, Nozawa T, Kameyama T, Inoue H, Takesono C, Mizukami A, Hashimoto Y (2003) Effect of CYP2D6*10 on pharmacokinetic variability of routinely administered metoprolol in middle-aged and elderly Japanese patients. *European Journal of Clinical Pharmacology*, **59**, 385–388.
26. Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory Press.
27. Hersberger M, Mrti-Jaun J, Rentsch K, Hänseler E (2000) Rapid detection of the CYP2D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. *Clinical Chemistry*, **46**, 1072–1077.
28. Rau T, Heide R, Bergmann K, Wuttke H, Werner U, Feifel N, Eschenhagen T (2002) Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment. *Pharmacogenetics*, **12**, 465–472.
29. Mistry B, Leslie J, Eddington NE (1998) A sensitive assay of metoprolol and its major metabolite α -hydroxymetoprolol in human plasma and determination of dextromethorphan and its metabolite dextrorphan in urine with high performance liquid chromatography and fluorometric detection. *Journal of Pharmaceutical and Biomedical Analysis*, **16**, 1041–1049.
30. Shah VP, Midha KK, Dighe S *et al.* (1991) Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. *European Journal of Drug Metabolism and Pharmacokinetics*, **16**, 249–255.
31. Rendic S, Di Carlo FJ (1997) Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metabolism and Reviews*, **29**, 413–580.
32. Zanger UM, Fischer J, Raimundo S, Stüven T, Evert BO, Schwab M, Eichelbaum M (2001) Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics*, **11**, 573–585.
33. Shimada T, Tsumura F, Yamazaki H, Guengerich FP, Inoue K (2001) Characterization of ($\pm$)-bufuralol hydroxylation activities in liver microsomes of Japanese and Caucasian subjects genotyped for CYP2D6. *Pharmacogenetics*, **11**, 143–156.
34. Sachse C, Brockmüller J, Hildebrand M, Müller K, Roots I (1998) Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics*, **8**, 181–185.
35. McElroy S, Sachse C, Brockmüller J *et al.* (2000) CYP2D6 genotyping as an alternative to phenotyping for determination of metabolic status in a clinical trial setting. *AAPS PharmSci*, **2**, E33.
36. Kim K, Johnson JA, Derendorf H (2004) Differences in drug pharmacokinetics between East Asians and Caucasians and the role of genetic polymorphisms. *Journal of Clinical Pharmacology*, **44**, 1083–1105.
37. Roh HK, Kim CE, Chung WG, Park CS, Svensson JO, Bertilsson L (2001) Risperidone metabolism in relation to CYP2D6*10 allele in Korean schizophrenic patients. *European Journal of Clinical Pharmacology*, **57**, 671–675.
38. Fukuda T, Yamamoto I, Nishida Y, Zhou Q, Ohno M, Takada K, Azuma J (1999) Effect of the CYP2D6*10 genotype on venlafaxine pharmacokinetics in healthy adult volunteers. *British Journal of Clinical Pharmacology*, **47**, 450–453.
39. Brockmüller J, Kirchheiner J, Schmider J, Walter S, Sachse C, Müller-Oerlinghausen B, Roots I. (2002) The impact of the CYP2D6 polymorphism on haloperidol pharmacokinetics and on the outcome of haloperidol treatment. *Clinical Pharmacology and Therapeutics*, **72**, 438–452.
40. Mizutani T (2003) PM frequencies of major CYPs in Asians and Caucasians. *Drug Metabolism and Reviews*, **35**, 99–106.
41. Kirchheiner J, Heesch C, Bauer S *et al.* (2004) Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics. *Clinical Pharmacology and Therapeutics*, **76**, 302–312.
42. Ismail R, Teh LK (2006) The relevance of CYP2D6 genetic polymorphism on chronic metoprolol therapy in cardiovascular patients. *Journal of Clinical Pharmacy and Therapeutics*, **31**, 99–109.
43. Hägg S, Spigset O, Dahqvist R (2001) Influence of gender and oral contraceptives on CYP2D6 and CYP2C19 activity in healthy volunteers. *British Journal of Clinical Pharmacology*, **51**, 169–173.