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CYP 2D6*4 polymorphism and interindividual response variation to metoprolol in stage 1 hypertensive patients: no association in a rural Indian population?

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Background/aim: Genetic polymorphism of CYP2D6 shows diverse pharmacokinetic and pharmacodynamic variation. Therefore, the present work was designed to study the variation in therapeutic responses to metoprolol (MP) in stage 1 hypertensive patients and also aims to verify the association of CYP2D6*4 polymorphism and response variation in an Indian population for the first time.

Materials and methods: Clinically, a total of 119 hypertensive patients and 116 healthy individuals as controls were included. Patients were treated with MP extended release 25 mg tablets once daily for 2 weeks. Reduction in systolic blood pressure, diastolic blood pressure, and pulse rate were recorded before and after the treatment. For genotyping, genotypes of 89 hypertensive patients and 71 healthy controls were investigated for CYP2D6*4 polymorphism.

Results: Based on reduction in systolic blood pressure, 26% of the patients did not respond to the MP treatment. Of the patients that responded, 28% responded very slowly, 35% (19 males, 23 females) responded moderately, and 12% (8 males, 6 females) showed a good response to MP. For genotype analysis, we pooled 89 hypertensive patients and 71 controls. No association was found between CYP2D6*4 polymorphism and MP response.

Conclusion: We found no relationship between MP response and CYP2D6*4 genotype in an Indian population in our study.

Key words: Metoprolol, CYP2D6*4, polymorphism, hypertension

1. Introduction

Hypertension is one of the leading causes of cardiovascular morbidity and mortality. Worldwide, it accounts for more than 5% of total deaths (1). This disorder is directly associated with stroke, heart disease, renal failure, and vascular diseases (2). In India, about one-fifth of urban adults (≥20 years old) were reported to be hypertensive. The prevalence of hypertension in India was found to be lower in rural populations (10%) compared to urban populations (25%) (3). Management of hypertension includes administration of antihypertensive drugs such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, beta-blockers, diuretics, Ca^{2+} channel blockers, and peripheral vasodilators (4). Beta-blockers are efficient in reducing the complications of hypertension (1). Metoprolol (beta-blocker) is a cardioselective beta-1 adrenergic receptor antagonist primarily metabolized by CYP2D6. CYP2D6 is located on the human chromosome 22q13.1 (5). It is a member of the cytochrome P450 family with a mixed function oxidase activity that oxidizes/metabolizes xenobiotics and other compounds (6). CYP2D6 is highly polymorphic, with 80 different alleles and allelic variants identified (7,8). Genetic variation of CYP2D6 has great clinical significance in the metabolism of beta-adrenergic blocking agents, antiarrhythmics, psycholeptics, antidepressants, and narcotic analgesics (9). A linear relationship was also observed between the variants of the CYP2D6 gene and the metabolic clearance of metoprolol (10). Individuals with CYP2D6 gene variants are classified functionally into 3 different phenotypes, based on their ability to metabolize drugs: ultrarapid, extensive, and poor metabolizers, with low, normal, and high plasma concentrations of the drug, respectively. Furthermore, increased plasma metoprolol concentration was observed in poor metabolizers (11). Variation in the pharmacokinetics and pharmacodynamics of metoprolol was observed in women as compared with men (12). Women had significantly higher plasma

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metoprolol concentrations than men, independent of CYP2D6 genotype (13). Metoprolol is a beta-blocker and about 70%–80% of the drug is metabolized by the cytochrome P450 2D6 enzyme (CYP2D6). The present study evaluated the interindividual response variation to metoprolol therapy in stage 1 hypertension along with other factors such as age and sex. Despite the vast ethnic variation of the Indian population, no pharmacogenomic study has been carried out on metoprolol in this population. The main aim of the study was to evaluate the association of CYP2D6*4 polymorphism with the therapeutic efficacy of metoprolol as influenced by age, sex, and body mass index (BMI).

2. Materials and methods

2.1. Subjects

The institutional human ethics committee approved the study (1299/PD/Ethics/Dean/GCH/Chennai) and the study was conducted according to good clinical practice guidelines. Newly diagnosed hypertensive patients without antihypertensive drug therapy were considered as the subject group. All the patients included in the study were attending the hypertensive clinic at the Government General Hospital, Madras Medical College, Chennai. Normotensive individuals attending the general medical outpatient unit were considered as the controls. The inclusion criteria for the study group were ambulatory patients of either sex, aged 30–65, with systolic blood pressure ranging from 140 to 160 mmHg and diastolic blood pressure ranging from 90 to 100 mmHg. The control group consisted of 116 healthy individuals matched to age and sex with the study subjects, with systolic blood pressure ranging from 90 to 130 mmHg and diastolic blood pressure ranging from 60 to 85 mmHg without clinically significant renal, hepatic, gastroenterology, or metabolic disorders. The exclusion criteria included a history of bronchospasm or asthma, thyrotoxicosis and peripheral vascular diseases, diabetes mellitus, pregnancy or breastfeeding, sitting diastolic blood pressure greater than 110 mmHg, malignant hypertension, secondary hypertension, cardiac surgery and other cardiac intervention or stroke in the past 3 months, congestive heart failure, and clinically significant hepatic, renal, neurological, gastrointestinal, metabolic, hematological, or psychiatric problems. In addition to the above, the following patients were also excluded: patients under treatment with Ca^{2+} channel blockers, ACE inhibitors and digoxin, lithium, catecholamine-depleting drugs (reserpine, MOA inhibitors), quinidine, methadone, fluoxetine, fluvoxamine, paroxetine, sertraline, or propafenone; known smokers and alcoholics; and those with known hypersensitivity to metoprolol or beta-blockers. Moreover, patients with SGOT/SGPT values of >1.5 times the upper limit of the normal range (10–40 unit/mL), alkaline phosphatase (13–39 IU/L) or total serum bilirubin of >1.2 times the upper limit of normal, or creatinine of >1.2 mg/dL were excluded. Informed consent was obtained from the patients and the control group after explaining the purpose of study.

Based on the inclusion criteria, 166 patients were selected out of 525 hypertensive patients screened. A metoprolol extended release tablet (25 mg/day) was administered orally to 166 patients for 2 weeks. One hundred and nineteen hypertensive patients (51 males and 68 females) completed the study, as the remaining 47 patients failed to turn up. Blood samples were collected from 119 patients and subjected for DNA extraction. DNA extraction was completed successfully for 89 samples and they were analyzed for genotypic variation; the remaining 30 samples were not successful. Blood samples were collected from 116 healthy individuals, of which 71 were subjected to genotypic analysis; the remaining samples were not successful during DNA extraction.

2.2. Clinical investigations

Detailed medical history, physical examination, and baseline laboratory investigations were documented. Sitting blood pressure, pulse rate, respiratory rate, and body temperature were measured before and after medication. ECG and chest X-rays were taken to rule out cardiac and respiratory complications. In both patient and control groups, the following tests were conducted: renal function test (sugar, urea, and creatinine), liver function test (SGOT, SGPT, and bilirubin), lipid profiles (total cholesterol, HDL, and triglycerides), and serum electrolytes.

2.3. Genomic DNA extraction and analysis

The genotypes of 89 hypertensive patients and 71 controls were analyzed for CYP2D6*4. Five milliliters of venous blood was collected from the subject and control groups. Genomic DNA was extracted from peripheral blood cells and dissolved in 1X TE buffer or Milli-Q water and quantified spectrophotometrically. Genotyping was carried out using the PCR-RFLP method. CYP2D6*4 (355 bp) was detected by PCR amplification using GCCTTCGCCAACCACTCCG and AAATCCTGCTCTTCCGAGGC as the forward and reverse primers, respectively. PCR was performed in a 10-µL volume containing 50–100 ng of DNA, 5 pmol of each primer, 10 mM dNTPs, 10X PCR reaction mixture, 50 mM $MgCl_2$, and 0.25 U of Taq polymerase. Following initial denaturation for 5 min at 94 °C, PCR was performed for 35 cycles of denaturation for 45 s at 94 °C, annealing for 30 s at 59 °C, and an extension for 30 s at 72 °C, and further extended for 3 min at 72 °C. Amplification was checked on 2% agarose gel. The amplified PCR product was digested with *Mva*I (37 °C) and the digested PCR products were separated by 3% agarose gel electrophoresis. The restriction site was generated by a substitution of G→A at nucleotide

position 1934 (7). The RFLP assay distinguished the wild type allele (250 bp and 105 bp) from the mutant allele (355 bp).

2.4 Statistical analysis

The data generated from the study were analyzed by ANOVA and multiple regression analysis with SPSS. CYP2D6*4 allele frequency and odds ratio were calculated by chi-square test.

3. Results

The mean age, BMI, cholesterol level, systolic and diastolic blood pressure, and reduction of systolic and diastolic blood pressure in males and females on metoprolol treatment are represented in Tables 1 and 2. As observed in control subjects, females had significantly higher BMIs than males. A significant age difference was found in BMI and diastolic blood pressure.

The hypertensive patients were classified into 4 different groups (nonresponders, very slow, moderate, and good responders) based on the reduction in systolic blood pressure and diastolic blood pressure following metoprolol treatment as represented in Tables 3 and 4, respectively. About 26% of the patients did not respond to metoprolol treatment. Of the patients that responded, 27% responded very slowly, 35% responded moderately, and 12% of the patients showed a good response to metoprolol.

Gene frequencies of CYP2D6*4 for the patients and control subjects are given in Table 5. The frequency of the '+' allele was about 15.5% in control subjects and about 20.8% in hypertensive patients. Odds ratios for the association of the CYP2D6*4 allele with the responders and nonresponders to metoprolol treatment are given in Table 6. Nonresponders are those patients whose blood pressure did not reduce or reduced by less than 4 mmHg following an administration of 25 mg/day metoprolol for 15 days. Responders are those patients whose blood pressure reduced by ≥4 mmHg. The RFLP assay results are depicted in the Figure.

Table 1. Mean age, BMI, and cholesterol level of hypertensive patients.

Parameter	Males	Females
N	51	68
Age (years)	49 ± 9.2	50.8 ± 9.7
BMI (kg/m^2)	25.7 ± 3.5	27.7 ± 4.2
Cholesterol (mg/dL)	185.2 ± 32.1	179.4 ± 44.4

BMI: Body mass index. Values are expressed as mean ± standard deviation.

Table 2. Influence of metoprolol on SBP, DBP, and HR in hypertension patients.

Parameters	Males BT	Males AT	Difference	Females BT	Females AT	Difference
SBP (mmHg)	148.3 ± 8.3	135.4 ± 15	12.8 ± 11.8	149.3 ± 8.5	137.7 ± 10.3	11.8 ± 9.7
DBP (mmHg)	95.5 ± 4.7	87.4 ± 8.7	8.3 ± 7.5	95.1 ± 4.8	88.0 ± 8.6	6.9 ± 7.4
HR(Pulse/min)	83.6 ± 7.2	78.0 ± 8.0	5.4 ± 6.5	84.8 ± 8.9	80.4 ± 7.7	4.5 ± 7.8

SBP: Systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; BT: before treatment; AT: after treatment. Values are expressed as mean ± standard deviation.

Table 3. Classification of hypertensive patients based on the reduction in systolic blood pressure after metoprolol treatment.

Variants		Nonresponders	Responders			Total
			Very slow responders	Moderate responders	Good responders	
Males	n	13	11	19	8	51
	%					42.86
Females	n	18	21	23	6	68
	%					57.14
Total	n					119

Nonresponders: ≤4 mmHg; very slow responders: >4 and ≤10 mmHg; moderate responders: >10 and ≤20 mmHg; good responders: >20 mmHg.

Table 4. Classification of hypertensive patients based on the reduction in diastolic blood pressure after metoprolol treatment.

Variants		Nonresponders	Responders			Total
			Very slow responders	Moderate responders	Good responders	
Males	n	14	25	11	1	51
	%					42.86
Females	n	25	28	15	0	68
	%					57.14
Total	n					119

Nonresponders: ≤4 mmHg; very slow responders: >4 and ≤10 mmHg; moderate responders: >10 and ≤20 mmHg; good responders: >20 mmHg.

Table 5. Gene frequency of CYP2D6*4 allele among responders, nonresponders, and controls.

Groups	AA	GA	GG	P (± SE)
Responders (N = 65)	2 (3.1%)	20 (30.8%)	43 (66.2%)	0.185 (0.018)
Nonresponders (N = 24)	1 (4.2%)	11 (45.8%)	12 (50%)	0.479 (0.051)
Controls (N = 71)	1 (1.4%)	20 (28.2%)	50 (70.4%)	0.1549 (0.016)

N: Sample size. P < 0.05 is significant; SE: standard error. AA, GA, GG: genotypes. Nonresponders are those patients whose blood pressure did not reduce or reduced by 4 mmHg or less following administration of 25 mg/day metoprolol for 15 days. Responders are those patients whose blood pressure reduced by ≥4 mmHg.

Table 6. Odds ratio of CYP2D6*4 allele with the hypertensive patients, controls, responders, and nonresponders.

Groups	Chi-square	P-value	Odds ratio	95% CI	
HT patients vs. controls (N = 89)	1.472	0.225	1.431	0.804	2.546
Nonresponders vs. controls (N = 24)	3.207	0.073	2.026	0.937	4.390
Responders vs. controls (N = 65)	0.426	0.514	1.235	0.659	2.316

N: Sample size. CI: Confidence interval, P < 0.05 is significant. Nonresponders are those patients whose blood pressure did not reduce or reduced by 4 mmHg or less following administration of 25 mg/day metoprolol for 15 days. Responders are those patients whose blood pressure reduced by ≥4 mmHg.

4. Discussion

Metoprolol has large pharmacokinetic and pharmacodynamic variation due to the inherited variations in the drug-metabolizing enzyme (CYP2D6) and adrenergic beta-1 receptor polymorphism (14). Even though there is large ethnic variation in the Indian population, to date limited pharmacogenetic data are available for metoprolol. This pilot study aimed to discover the variation in response to metoprolol therapy in stage 1 hypertension.

Large interpatient variability in therapeutic response to drugs, including cardiovascular drugs, has been reported by many scientists (15). In a study by the US Veterans Administration, there were substantial variations in blood pressure responses to antihypertensive therapy, but to date there is no accurate means of identifying the individuals who respond quite well to drugs (14,16).

In the case of males, individuals in the 40–45 age group and the 55–60 age group were slow responders. Individuals in the 35–40 age group were good responders. In the case of females, individuals in the 35–40 and 45–50 age groups were poor responders compared to other age groups. A gradual increase in response was observed above 45 years. The frequencies of the CYP2D6*4 allele

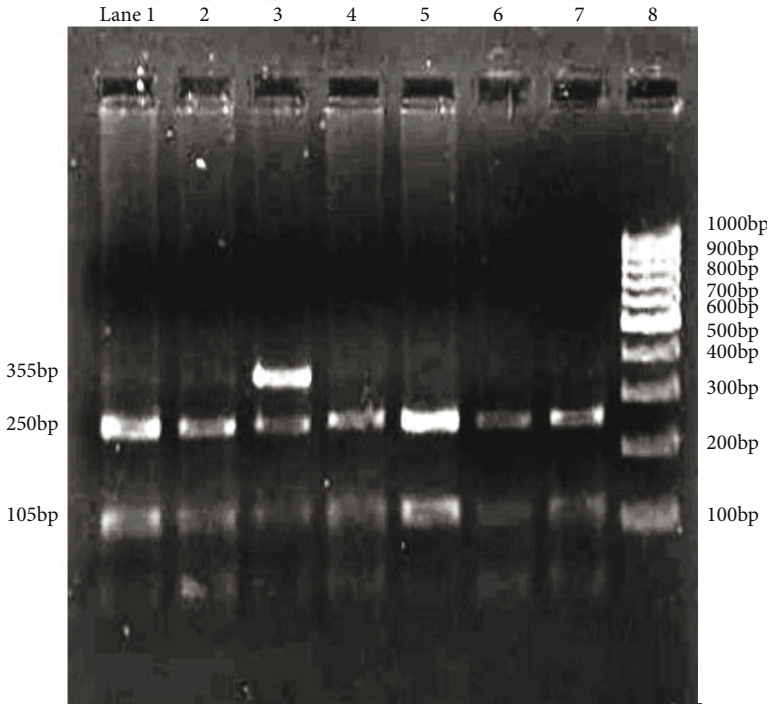

Figure. Agarose gel electrophoresis (1.5%), CYP2D6*4 allele primer digested with *Mva*I restriction enzyme. Lanes 1 and 5, 250 and 105 bp; lane 3, 355, 250, and 105 bp; and lane 8, 1000 bp ladder.

in control subjects and hypertensive patients were 15.5% and 20.8%, respectively. The CYP2D6*4 allele was not associated with hypertension (P = 0.23). No association was found between the presence of CYP2D6*4 allele and nonresponders to metoprolol. Responders to metoprolol also showed no significant association with the CYP2D6*4 allele.

The study by Fredman et al. had 22% nonresponders to metoprolol (17). Similarly, in our study, we observed that one-fourth of the study population did not respond to metoprolol. Females were more likely to be nonresponders than males. Males within the 35–40 age group were good responders to metoprolol; females aged 30–35 and 40–45 also responded well. No sex difference was observed in blood pressure, pulse rate, or cholesterol level for patients. Among nonresponders, the '+' allele frequency was over 2 times higher than that observed in the overall sample of hypertensive patients and over 3 times higher than the value observed in control subjects.

Among the 3 genotypes, frequency of the AA genotype is very small (usually <5%) in comparison with the other 2 genotypes. Since the AA genotype frequency and sample size were very small, the odds ratios were calculated using allele frequencies instead of genotype frequencies. The result of this analysis was compared with various subject samples of hypertensive patients. In all 3 correlations (all hypertensive patients, nonresponders, and responders), the odds ratios were positive or above 1. However, none of them were statistically significant (P < 0.05).

In conclusion, one-fourth of the study patients were nonresponders to metoprolol. Males aged from 30 to 35 years and females aged from 40 to 45 years were good responders. However, we found no relationship between metoprolol response and CYP2D6*4 genotype. This study must be further continued in larger populations to explore other genetic factors to confirm this finding.

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References

1. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. JAMA 2003; 389: 2560–2572.
2. MacMahon SW, Wilcken DEL, Macdonald GJ. The effect of weight reduction on left ventricular mass: a randomized controlled trial in young overweight hypertensive patients. N Engl J Med 1986; 314: 334–339.
3. Mohan V, Deepa M, Farooq S, Datta M, Deepa R. Prevalence, awareness and control of hypertension in Chennai--The Chennai Urban Rural Epidemiology Study (CURES-52). J Assoc Physicians India 2007; 55: 326–332.
4. Khan NA, Hemmelgan B, Padwal R, Larochelle P. The 2007 Canadian Hypertension Education programme recommendations for the management of hypertension. Can J Cardiol 2007; 23: 603–604.
5. Eichelbaum M, Baur MP, Dengler HJ. Chromosomal assignment of human cytochrome P-450 (debrisoquine/spartiene type) to chromosome 22. Br J Clin Pharmacol 1987; 23: 455–458.
6. Mabic S, Castagnoli K, Castagnoli JN. Oxidative metabolic bioactivation of xenobiotics. In: Woolf TE, editor. Handbook of Drug Metabolism. New York, NY, USA: Marcel Dekker; 1999. pp. 49–79.
7. Zanger UM, Fischer J, Raimundo S, Sturen T, Evert BO, Schwab M. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP 2D6. Pharmacogenetics 2001; 11: 573–585.
8. Sachse C, Brockmöller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1977; 60: 284–295.
9. Daly AK. Molecular basis of polymorphic drug metabolism. J Mol Med 1995; 173: 539–553.
10. Kirchheiner J, Heesch C, Bauer S, Meisel C, Seringer A, Goldammer M, Tzvetkov M, Meineke I, Roots I, Brockmüller J. Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetic and pharmacodynamics. Clin Pharmacol Ther 2004; 98: 302–312.
11. Johnson AJ, Cavallari LH. Cardiovascular pharmacogenomics. Exp Physiol 2004; 90: 283–289.
12. Sharma A, Pibarot P, Pilote S, Dumesnil JG, Arsenault M, Bélanger PM, Meibohm B, Hamelin BA. Toward optimal treatment in women: the effect of sex on metoprolol-diphenhydramine interaction. Clin Pharmacol 2010; 50: 214–225.
13. Jochmann N, Stangl K, Garbe E, Baumann G, Stangl V. Female-specific aspects in the pharmacotherapy of chronic cardiovascular diseases. Eur Heart J 2005; 26: 1585–1595.
14. Veterans Administration Co-Operative Study Group on Antihypertensive Agents. Racial differences in response to low dose captopril are abolished by the addition of hydrochlorothiazide. Br J Clinical Pharmacol 1982; 14: 97–101.
15. Namath A, Chen C, Agrawal R. β1 and β2 Adrenergic receptor polymorphisms: their impact on cardiovascular physiology, diseased states and response to therapeutic agents. Seminars in Anesthesia, Perioperative Medicine and Pain 2007; 26: 2–9.
16. Materson BJ. Variability in response to antihypertensive drug treatment. Hypertension 2004; 43: 1166–1167.
17. Fredman RM. Metoprolol for hypertension. Open trial in general practice. Med J Aust 1982; 8: 378–380.