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BamH1 POLYMORPHISM OF HUMAN CYTOCHROME P_{450} GENE, CYP2D6, IN QUIESCENT AND RELAPSE PATIENTS OF PULMONARY TUBERCULOSIS

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BamH I polymorphism of the human cytochrome P_{450} gene, CYP2D6, which encodes drug metabolizing enzyme, was studied to find out whether variant genotypes of this gene are associated with the susceptibility or resistance to bacteriological relapse of pulmonary tuberculosis after stopping treatment with short-course chemotherapy of 6-8 months duration. The study was carried out in control subjects (n=158), patients with pulmonary tuberculosis (n=154), patients with bacteriological relapse (n=50) and quiescent patients (n=50). No difference in the frequency of variant genotypes of *BarnH* I polymorphism of CYP2D6 gene was observed between pulmonary TB patients and control subjects. A trend towards an increased frequency of 22 genotype (homozygous for infrequent allele 2) was observed in bacteriological relapse patients than quiescent patients (odds ratio, OR: 2). Similar increase was observed in male relapse patients than male quiescent patients (OR: 2.2). The present study suggests that 22 genotype of *BamH* I polymorphism of CYP2D6 of human cytochrome P_{450} gene either alone or in combination with closely linked genes may be associated with bacteriological relapse especially in male patients of pulmonary tuberculosis.

Keywords: *BarnH* I, CYP2D6 gene, polymorphism, pulmonary TB.

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INTRODUCTION

Pulmonary tuberculosis is a granulomatous lung disease caused by *Mycobacterium tuberculosis.* Treatment with short course chemotherapy with Rifampicin containing drug regimens has proved to be highly effective (1, 2). Inspite of successful treatment with these drugs, a proportion of patients relapsed, which requires retreatment (1-3). Our earlier studies on human leucocyte antigens (HLA) in quiescent and bacteriological relapse patients of pulmonary tuberculosis revealed the association of HLA-A1, -B17 antigens, HLA haplotypes A1-B17, A1-DR7, HLA-TNFœ haplotypes B17-TNFœ-238/A, B17-TNFœ-308/2 and B17-TNFß/2 with bacteriological relapse (4,5). Studies on pharmacogenetics revealed several genetic polymorphisms that are involved in drug-metabolizing enzymes, transporters, receptors and other drug targets and shown to be linked to inter individual differences in the efficacy or toxicity of many medicines (6,7). The human

Cytochrome P_{450} (CYP) family members are responsible for the metabolism of endogenous substrates, dietary compounds and environmental toxins. Cytochrome P_{450} is involved in the metabolism of commonly used medicines. The polymorphic human cytochrome P_{450} gene, CYP2D6, encodes the drug metabolizing enzyme debrisoquine hydroxylase, a metabolizer for debrisoquine and more than 30 other drugs (8, 9). To understand whether polymorphic variants of drug metabolizing enzyme genes such as Cytochrome P_{450} gene play any role on bacteriological relapse of pulmonary tuberculosis, the present study was carried out. We studied the *BamH*I polymorphism of CYP2D6 gene (human cytochrome P_{450} gene) to find out whether its variant genotypes are associated with the susceptibility or resistance to bacteriological relapse of pulmonary TB after completion of 6-8 months of short course chemotherapy with various anti-TB drugs.

MATERIALS AND METHODS

The study subjects were (i) normal healthy subjects serving as controls (n=158), (ii) pulmonary TB patients (n=154) who were sputum positive for *M. tuberculosis* by smear and culture; (iii) patients who had a relapse indicated by a sputum culture positive for *M. tuberculosis* (n=50); (iv) cured, symptom free, quiescent patients (n=50).

The study patients were selected from an earlier chemotherapy study conducted by our centre. Patients attending Tuberculosis Research Centre (TRC), Chennai, with respiratory symptoms and radiographic abnormalities suggestive of pulmonary TB and who had been cured were studied. They were sputum positive for *M. tuberculosis* by smear and culture initially. Among the PTB patients, 108 were males and 46 were females, aged 40.9±1.0 and 36.9±5.4 years respectively. All these patients had received supervised short course chemotherapy for 6 to 8 months duration and had been followed up for five years after treatment. During follow-up, around 10 percent of the treated patients had a bacteriological relapse of the disease. The relapse patients were treated with other drug regimens and cured.

Patients under follow up were classified as having bacteriological relapse or being quiescent. The main criteria for relapse were clinical deterioration, persistent radiograph deterioration (chest X-ray) and sputum cultures positive for *M. tuberculosis.* The relapse patients were culture proven cases. Patients who had a bacteriological relapse (n=50) and an equal number of patients with quiescent disease (n=50), matched for treatment regimen and duration of follow up were selected. The relapse and quiescent patients were age matched. Among the relapse patients, 42 were males and 8 were females aged 41±1.9 years and 40±4.9 years respectively. Among the quiescent patients 36 were males and 14 were females, aged 40±1.6 years and 40±2.5 years respectively. The relapse patients and the quiescent patients were recruited from the same environment. They belonged to the same socioeconomic status and the same ethnic origin.

The controls comprised of spouses of the patients (n=76) (family contacts) who were living together with the patients before, during and after treatment for a period of 10-15 years and staff of TRC (n=72) working for more than 3 years. Of the controls, 79 were males and 79 were females aged 40±1.1 and 36.8±1.0 years respectively. All the family contacts and other control subjects were clinically normal at the time of blood collection. The patients and the spouses were not

consanguineous. The other control subjects were not related to any of the patients. The patients and the controls were randomly selected and belonged to the same ethnic group.

Genomic DNA was extracted from the peripheral blood white cells using a salting out procedure (10). For *BamHI* polymorphism, the exon 2 and parts of the flanking introns of the CYP2D6 gene was amplified using the following primers as described earlier (8, 11). Primers for intron 1 (base pairs 585 to 605): 5'-GAT GAG TTA GTC CTG AGT GCC – 3' and for intron 2 (base pairs 1162 to 1182); 5'-TCC CAC GGA AAT CTG TCT CTG – 3'.

The polymerase chain reaction (PCR) conditions were: an initial denaturation step at 94°C for 4 mins followed by 35 cycles of denaturation at 94°C for 30 secs, annealing 58°C for 30 secs and extension 72°C for 60 secs and finally 2 minutes extension at 72°C, using 10X PCR amplification buffer (Gibco BRL, Grand Island, NY, USA), 1mM $MgSO_4$, 0.6 mM of dNTPs, 5pmoles of each primer, (Gibco BRL, Grand Island, NY, USA) 100 ng of DNA and 1unit of Taql polymerase (Gibco BRL, Grand Island, NY, USA) in a 25 µl reaction mix in a programmable thermocycler (MJ Research, Inc., Watertown, MA, USA).

The amplified 598 base pair product was checked on a 1% agarose gel stained with ethidium bromide along with F X174 *Hae*III digest as a marker. 5 µl of the PCR product was restriction digested with 3-5 units of *BamH*I enzyme at 37°C for 3 hours and run on 1.5% gel for 45 minutes at 80V along with F X174 *Hae*III marker. Absence of *BamH*I restriction site (598 bp) was assigned as common allele and the presence of restriction site resulting in 349 and 249 bp fragments was assigned as infrequent allele 2. Genotypes were assigned accordingly: homozygotes for common allele 11 and homozygotes for infrequent allele 22. Presence of 598, 349 & 249 bp fragments was assigned as heterozygotes 12.

The frequencies of the genotypes in the patient group and controls were analysed using x^2 test with Yate's correction (x^2y) using Statcalc program (Epi Info, Version-5; USD; Stone Mountain, GA) to find out the statistical significance, Odds ratio (OR) and Confidence Interval (CI).

RESULTS

No difference in the genotype frequency of *BarnH*I polymorphic variants of CYP2D6 gene was observed between the control group and pulmonary tuberculosis group.

Since, tuberculosis is more prevalent in males, the data were analysed further for male and female controls and patients. Interestingly, a significantly increased genotype frequency of 22 (homozygous for infrequent allele) of CYP2D6 gene was observed in female control subjects than male controls (P=0.03; Odds Ratio (OR) 2.3; 95% Confidence interval = 1.07-5.23) whereas, no such increase in the 22 genotype was observed in female pulmonary TB patients than male patients (Table 1).

A trend towards an increased frequency of 22 genotype (homozygous for infrequent allele) was observed in bacteriological relapse patients than quiescent patients (Odds ratio = 2.0). The data were further analysed for male and female relapse and quiescent cases. An increased frequency of the genotype 22 was observed in male relapse patients than male quiescent patients (Odds ratio=2.2). However, these increases were not significant (Table 2).

Table 1 **Genotype frequency of *BamH*I polymorphism of CYP2D6 gene among pulmonary TB patients and healthy controls**

Variant genotypes	Genotype Frequency (%)					
	Total		Controls		Pulmonary-TB	
	Controls n=158	PTB n=154	Males n=79	Females n=79	Males n=108	Females n=46
11	29.1 (46)	25.3 (39)	31.6 (25)	26.6 (21)	23.2 (25)	30.4 (14)
12	43.7 (69)	49.4 (76)	49.4 (39)	38.0 (30)	50.0 (54)	47.8 (22)
22	27.2 (43)	25.3 (39)	19* (15)	35.4* (28)	26.8 (29)	21.8 (10)

n = subjects studied: numbers in parentheses represent the subjects positive for variant genotypes:
PTB : pulmonary tuberculosis;
*Female vs male X^2yP=0.03: Odds ratio = 2.34; 95% confidence interval = 1.07-5.23

Table 2 **Genotype frequency of *BamH*I polymorphism of CYP2D6 gene among quiescent and relapse patients of pulmonary TB**

Variant genotypes	Genotype Frequency (%)					
	Total		Quiescent		Relapse	
	Quiescent n=50	Relapse n=50	Males n=36	Females n=14	Males n=42	Females n=8
11	30 (15)	22 (11)	27.8 (10)	35.7 (5)	23.8 (10)	12.5 (1)
12	52 (26)	48 (24)	55.5 (20)	42.9 (6)	45.2 (19)	62.5 (5)
22	18* (9)	30* (15)	16.7@ (6)	21.4 (3)	31.0@ (13)	25 (2)

n = subjects studied; numbers in parentheses represent the subjects positive for variant genotypes
*P=0.24 (not significant); odds ratio = 1.95: 95% confidence interval = 0.69-5.69
@P=0.23 (not significant): odds ratio of n = 2.24; 95% confidence interval = 0.67-8.13

DISCUSSION

The frequency of variant genotypes (11, 12, 22) of *BamH*I polymorphism of CYP2D6 gene was similar among controls and pulmonary TB patients indicating a lack of association between the gene and susceptibility to pulmonary TB.

The frequency of 22 genotype was higher among healthy females than healthy males suggesting that this genotype in combination with closely linked genes along with other host factors such as hormones may influence drug metabolism in males and females. It is known that sex steroid hormones are synthesized from a series of enzymatic reactions involving several of the cytochrome P_{450} enzymes (12). The drug metabolism enzyme gene (CYP2D6) is one of the cytochrome P_{450} gene family (13). The observed difference in the male and female subjects may probably be due to gene-gene interaction.

The frequency of genotype 22 was higher among relapse patients compared to quiescent patients particularly among males. It is likely that closely linked CYP2D6 genes in combination with other genes and host factors may influence the metabolism of anti-tuberculous drugs during treatment. Due to altered drug metabolism, some of the bacteria may undergo dormancy and later be reactivated after completion of treatment. Further research may explore whether other alleles of CYP2D6 gene and other genes of Cytochrome P_{450} family are associated with bacteriological relapse of pulmonary TB.

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