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Research Article

Frequency of HLA B*15:02 Variant Allele In A Healthy South Indian Population

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ABSTRACT

Purpose: To determine the frequency of HLA B*15:02 variant allele in a healthy South Indian population and compare it with other population frequencies.

Methods: Five millilitres of venous blood samples were collected from 203 unrelated healthy volunteers of South Indian origin between the age group of 18 and 60 years and of either gender. DNA extraction was performed using the standard Phenol-Chloroform method. Genotyping of tagging SNPs of HLA B*15:02 variant allele was done using Real-Time PCR. The tagging SNPs of HLA B*15:02 variant allele includes G > C (rs3909184) and G > A (rs2844682).

Results: The variation was found to occur at frequencies of GG (66%), GC (14%), GA (16%), CC (2%) and AA (2%). The genotype frequencies were found to be in Hardy Weinberg equilibrium. A statistically significant difference was seen in the frequency distribution of genotypes between the study population and the people belonging to ethnicities of Japanese, Hispanics, African and Non-Caucasian Americans.

Conclusion: The frequency of occurrence of HLA B*1502 genetic variation has been established in south Indian healthy population. The results of this study will serve as the benchmark for future studies related to antiepileptic drugs and HLA B*15:02 in south Indian population.

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INTRODUCTION

Epilepsy is one of the most prevalent neurological diseases. Antiepileptic drugs inherently can lead to serious adverse drug reactions like Steven-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN).[1] Major histocompatibility complex class I (MHC) gene includes three Human Leucocyte Antigen (HLA) genes. These peptides play the important role in differentiating between self and foreign proteins.[2] Among the three HLA genes, HLA B has been implicated to have the highest number of polymorphisms.[2] It has been found that variations in HLA B alleles give rise to the development of hypersensitivity reactions to drugs.[3] HLA-B*15:02 has been implicated in the development of phenytoin and carbamazepine-induced SJS/TEN.[2, 3]

It has been stated that there is an increased risk of SJS/TEN in patients with HLA-B*15:02 variant allele in Han Chinese and Asian population.[2] Mehta *et al* have shown the association between HLA-B*15:02 variant allele and carbamazepine-induced SJS in 6 of 8 Indian patients who fulfilled the criteria for carbamazepine-induced SJS.[4]

In order to prevent the development of SJS/TEN, FDA recommended genetic screening for HLA B*15:02 and HLA A*31:01 before initiating antiepileptic drugs for patients who belong to ethnicities where these variations occur at higher frequencies.[1] In order to translate the recommendation of FDA into clinical practice and to determine the relevance of this genetic variation in our population, it is essential to establish the frequencies of these variant alleles. In our study we have evaluated the frequency distribution of HLA B*15:02 genetic variation in a south Indian healthy population.

METHODOLOGY:

The study was conducted in a tertiary care teaching hospital in south India after obtaining approval from Institute Ethics Committee (Human Studies). The study was done in compliance with good clinical practice according to the principles of the Declaration of Helsinki. Written informed consent was obtained from the study participants prior to their participation in the study. Two Hundred and three unrelated healthy volunteers of south Indian origin between the age group of 18 and 60 years of either gender were included in the study.

Five milliliters of venous blood were collected from the cubital fossa for DNA extraction and genotyping. The cellular layer including the buffy coat was separated using ultracentrifugation at 2500 rotations per minute (RPM) and stored at -200° C, until extraction. DNA extraction was performed using the standard Phenol-Chloroform method. Genotyping of the polymorphisms was carried out with Allelic Discrimination assays, with the kits obtained from Applied BioSystems (ABI) USA. The assay kits were based on Taqman Technology, and Real-Time PCR platform used was ABI 7300. The allelic call was read with the help of sequence manipulation suite (SMS) version 1.4.

*HLA B*1502* gene has triallelic sites. Hence variations can be either i) G > C (rs3909184) or ii) G > A (rs2844682). Frequency distribution of variant alleles was expressed as descriptive statistics. Comparison of the frequency distribution with other world populations and Hardy Weinberg equilibrium was analyzed using Chi-square test. A p-value of less than 0.05 was considered to be significant. All statistical tests were done using GraphPad InStat v.3.6 California, USA.

RESULTS:

Of the 203 healthy volunteers screened, 135 were males and 68 were females. The genotype frequency of *HLA B*1502* gene is shown in Table 1. The genotype frequencies follow Hardy Weinberg Equilibrium (P value 0.79). The alleles of *HLA B*1502* genetic variation were found to be occurring at frequencies of G allele 82%, A allele 14% and C allele 4% . The frequencies of the variant alleles C and A together were found to be pervasive in our study. The allele frequency in our population is comparable to the other Asian populations

namely. Thai, Han Chinese, Malay and Vietnamese. However, a statistically significant difference was observed in the allele frequencies between the study population and that of those reported in Japanese, Hispanics, Africans and Non Caucasian Americans. (Table 2)

*Table 1: Genotype frequencies of HLA B*1502 genetic variation*

S. No:	Gene	Triallelic SNP	Genotype frequencies (%)					P value
			GG	GC	GA	CC	AA	
1.	*HLAB*1502*	rs3909184 G>C rs2844682 G>A	66	14	16	2	2	0.79

P > 0.05 Indicates consistency with Hardy-Weinberg Equilibrium (HWE; Chi square test

Table: 2 Comparison of allele frequency in comparison with other populations

Population	**N**	**Allele frequencies (%)**		**P value**
		X	**Y**	
Present Study	203	82	18	Reference
Thai[3]	42	88.1	11.9	0.322
Han Chinese[5]	113	92	8	0.058 5
Malay[6]	300	84.3	15.7	0.703
Japan[6]	371	99.9	0.1	0.0001
Spain[6]	173	100	0	< 0.0001
Vietnam[6]	170	86.5	13.5	0.562
African[7]	271	100	0	< 0.0001
Non-Caucasian American[7]	371	99.61	0.39	< 0.0001

P value < 0.05 is considered significant. X = wild allele, Y = variant allele; Chi Square test

DISCUSSION:

India is a country with extreme diversity. Indians are genetically distinct in comparison with other races in the world. Based on Ancestral elements the Indian population is distinguished into ancestral North Indians and ancestral South Indians. An Indian genome variation consortium study of genetic markers demonstrated a notable heterogeneity amongst the diverse Indian population. There is no published data available on the allele frequencies of *HLA B*15:02* gene for south Indians.[8]This may be the first study to establish the frequency of HLA B*15:02 variant allele in south Indian population. In our study, we found that the frequency of the variant allele was 18%. The frequency of HLA B*15:02 variant allele in south Indian population was similar to other Asian population viz. Thailand, China, Malaysia, and Vietnam.[3,5,6] Lonjou et al exhibited a result were in 4 of 12 European patients who had developed carbamazepine-induced SJS. All the 4 patients were of Asian ancestry.[9]

Two other studies done in Thai population by Tassaneeyakul et al[10] and Locharernkul et al[11] also revealed similar association between the HLA-B*15:02 variant allele and carbamazepine and phenytoin-induced SJS. Chang et al in his study revealed that 12 of 16 Malay patients had an association between HLA-B*15:02 variant allele and carbamazepine-induced SJS.[6]

The result of our study was not comparable to the Japanese, Spanish, African and Non-Caucasian American populations.[6,7] These

populations did not have the variant allele. The findings from the above-mentioned studies show that the Asian populations have a higher frequency of the HLA-B*15:02 variant allele and in turn are more susceptible to develop SJS/TEN with carbamazepine or phenytoin therapy.

The results suggest that screening for HLA-B*15:02 variant allele in south Indian population can be incorporated in clinical practice, to minimize the adverse effects developed due to carbamazepine and phenytoin as suggested by the FDA.

CONCLUSION:

The frequency of HLA B*1502 variant alleles in south Indian population is 18%. The results of this study will serve as the benchmark for future studies related to antiepileptic drugs and HLA B*15:02 in south Indian population.

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