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ACE I/D AGT M235T AND MTHFR C677T MARKERS FREQUENCY DISTRIBUTION AND RISK OF CVD DISEASE IN YADAV POPULATION OF BIHAR

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ABSTRACT

Studies have shown that ACE AGT and MTHFR genetic markers are associated with cardio vascular disease CVD. In the present study, an attempt has been made to find the frequencies of ACE I/D, AGT M235T and MTHFR C677T alleles in Yadav population of Bihar. This study is based on 98 healthy unrelated individuals of Yadav population from three villages of the Patna District. 5ml of intravenous blood samples were collected from unrelated subjects. The genomic DNA was obtained from peripheral leukocytes (500µl of whole blood), using salting out procedure (inorganic method) given by Miller *et al.* (1988). The high frequency of D-allele (ACE gene) instead of I allele in the study population demands further investigation. The genetic data were analysed by using the HWE software Version 1.10 utility program for analysis of genetic linkage. The difference in distribution of ACE, AGT and MTHFR genotype and allele were analyzed with χ^2 tests. The allele frequencies of three studied loci are approximately in line with other populations in India as well as world. ACE gene was found to be polymorphic in Yadav population in which ID genotype showed the higher frequency (76.32%) than the DD (13.15%) and II (10.53%) genotypes. It is known that D allele is more prone to hypertension, myocardial infarction, heart failure, vascular disease, stroke, renal failure and various organ disorders (Danser *et al.*, 1995; Pontremoli *et al.*, 2000). In this study the frequency of deletion (D) allele is higher than the frequency of insertion (I) allele indicating that the Yadav population is at risk to these disorders.

INTRODUCTION

Genetic marker is a particular gene or DNA base sequence associated with identifiable chromosome. Increasingly, specific genetic markers are being associated with particular genes or traits. Genetic marker can be used to determine the risk of developing a disease attributed to a gene or genes associated with the marker. Genetic markers are basic and powerful tools of modern genetics. Because of speed and cost effectiveness, genetic markers continue to be used as effective method of

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screening organism and producing initial maps of the genetic material of organism, even when many more precise tools are available. Various studies have compared coronary artery disease (CAD) or (CVD) patients with controls in order to determine which polymorphisms are associated with higher risk of disease. Particularly the human ACE gene contains a number of variable polymorphic regions that can be used in genetic analysis of populations (Rieder *et al.*, 1999), the I/D (insertion/ deletion) polymorphism present in intron 16, has been extensively investigated. The D allele in ACE gene, D allele (Danser *et al.*, 1995; Pontremoli *et al.*, 2000) and in AGT gene, T (Burt *et al.*, 1995; Fukamizu *et al.*, 1990; Kannel, 2000; Mosterd *et al.*, 2002) allele is associated with hypertension, myocardial infarction, heart failure. vascular disease, stroke, renal failure and various organ disorders. MTHFR (Methylene tetrahydrofolatereductase) plays a significant role in methionine metabolism. MTHFR deficiency is inherited as an autosomal recessive trait. There are several mutations that have been identified in MTHFR gene in which C677T is one of important SNPs that has been reported to change the enzyme activity. It replaces the nucleotide cytosine with the nucleotide thymine at important position 677 in exon 4 of the gene which further leads to the substitution of alanine to valine (Frosst *et al.*, 1995).

MTHFR gene plays an important role in cardio vascular disease (CVD) and the disease runs in families. Researchers have identified more than 250 genes responsible for CVD which play a very significant role in development of cardiovascular disease. In the present study an attempt has been made to find the frequencies of ACE I/D, AGT M235T and MTHFR C677T alleles in Yadav population of Bihar.

MATERIALS AND METHODS

This study included 98 healthy unrelated individuals of Yadav population from three villages of the Patna district, in which 50% of population was female and 50% was male. Two types of data were collected for this study: genetic data and demographic data. For the genetic data, 5ml of intravenous blood samples were collected from unrelated individuals belonging to the Yadav community through the help of medical practitioner, after proper informed written consent. The demographic data were collected from 98 families with 55 ever married women ranging in age from 17 to 70 years. The demographic schedule included information related to household composition, educational status of the family members, reproductive profile such as fertility pattern of the ever married women, offspring mortality, and number of child births. In addition, anthropometric measurements were also taken. The interview with the respondents was conducted in the presence of one or the other family members so that correct information could be obtained and verified.

The genomic DNA was obtained from peripheral leukocytes (500µl of whole blood), using salting out procedure (inorganic method) as given by Miller *et al.* (1988). After the extraction of DNA samples, further analysis was conducted by PCR amplification of the desired DNA fragments. Genotyping was based on the

polymerase chain reaction technique, with further restriction analysis when required.

The ACE I/D was determined by PCR and was performed with sense primer 5′ CTG GAG ACC ACT CCC ATC ATT TCT 3′ and antisense primer 5′ GAT GTG GCC ATC ACA TTC GTC AGA 3′ to amplify D and I allele, which will resulted in the amplification of 490bp and190-bp products, respectively. The AGT and MTHFR were determined by PCR performed with forward primer 5′ GAT GCG CAC AAG GTC CTG TC 3′ and 5′ TGA AGG AGA AGG TGT CTG CGG GA 3′ respectively and reverse primer 5′ CAG GGT GCT GTC CAC ACT GGA CCC C 3, 5′ AGG ACG GTG CGG TGA GAG TG 3, respectively the product size of AGT gene is 330bp to 279bp and the product size of MTHFR gene is 175bp to 198bp. For the MTHFR gene *Hinf*I enzyme was used for the digestion. However, due to paucity of funds, not all 98 samples could be analysed for DNA markers. The respective sample sizes for individual markers are as shown in Table 1.

The genetic data were analysed by using the HWE software Version 1.10 utility program for analysis of genetic linkage. The difference in distribution of ACE, AGT and MTHFR genotype and allele were analyzed with χ^2 tests. The Hardy- Weinberg equilibrium was examined by the Marker chain method with a programme for population genetic data analysis. A p value ≤ 0.05 was considered statically significant.

RESULTS AND DISCUSSION

ACE gene was found to be polymorphic in Yadav population in which ID genotype showed the higher frequency (76.32%) than the DD (13.15%) and II (10.53%) genotypes. In the present study the population does not follow the Hardy-Weinberg equilibrium with respect to ACE polymorphism. This can be because of endogamous nature of the population and second the sample size is small. As mentioned above, D allele is more prone to hypertension, myocardial infarction, heart failure, vascular disease, stroke, renal failure and various organ disorders (Danser *et al.*, 1995; Pontremoli *et al.*, 2000). In this study the frequency of deletion (D) allele is higher than the frequency of insertion (I) allele indicating that the Yadav population is at risk to these disorders.

As we know the ACE gene is located on chromosome 17q23 i.e., this gene is located on q arm of 17 chromosome in 23rd locus. In this gene I allele is considered as ancestral or wild allele while D allele is considered as mutant allele. The Human ACE gene contains a number of variable polymorphic regions that can be of potential use in genetic analysis of populations (Rieder *et al.*, 1999). The insertion/deletion polymorphism is present in intron 16, and in the present study this particular region has been investigated. There are several studies available from north east India, North West India, southern India, and west India. The I allele frequency in India ranges from 0.38 (Kashmeri) to 0.69 (Brokpas). In present study frequency of I allele (0.48) in Yadav population falls in this range (Fig. 1).

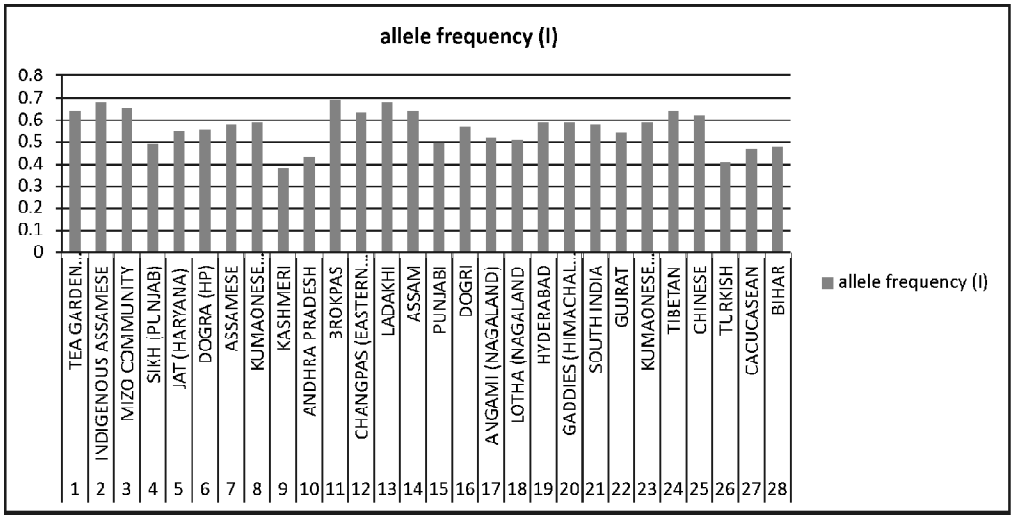

Figure 1: Insertion(I) allele frequency distribution of ACE gene in some population groups

In ACE I/D polymorphism D allele are considered as mutant allele. The D allele frequency in India ranges from 0.31 (Brokpas) to 0.61 (Kashmiri). In the present study frequency of D allele of Yadav population is 0.51 which is within the range of other Indian populations (Figure 2).

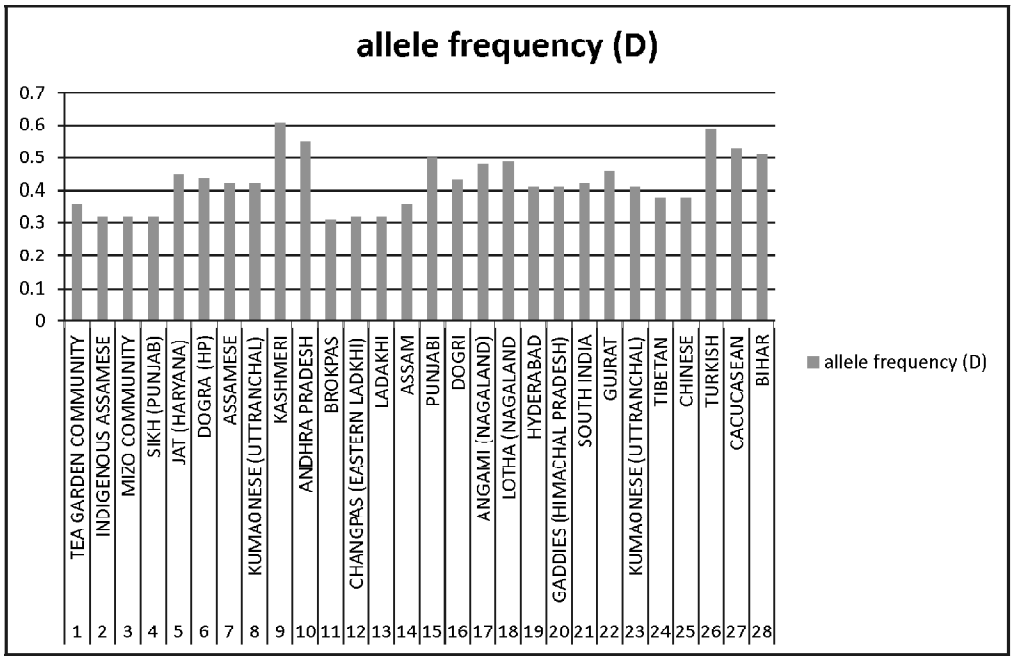

Figure 2: Deletion (D) allele frequency distribution in ACE gene in some population groups

In comparative study of D and I allele of present population with other Indian populations we can see that mutant allele D has lower frequency than the ancestor allele I except among Kashmir and Andhra Pradesh. The Yadav population in India and Turkish Caucasian population have higher D allele frequency than I allele; it means that these populations are at risk of having cardiovascular and other RAS pathway related diseases and need to be further investigated.

Angiotensinogen is also known as AGT and Serpin A8, is member of serpin family. The official name of AGT gene is "Angiotensinogene (Serpin Peptidase Inhibitor, Clade A, member 8)". Angiotensinogen is an essential component of the renin-angiotensin system (RAS) and potent regulator of blood pressure. The first study on AGT M235T gene was conducted by Ward in 1993 on Caucasian population. AGT M235T gene was found to be polymorphic in present population, in which MT genotype showed the highest frequency (57.14) followed by TT (34.14) and MM (8.53). In this study AGT M235T gene also does not obey the Hardy - Weinberg equilibrium. It may be that some external force is working in this population or it might be possible that due to less sample size it is not in equilibrium.

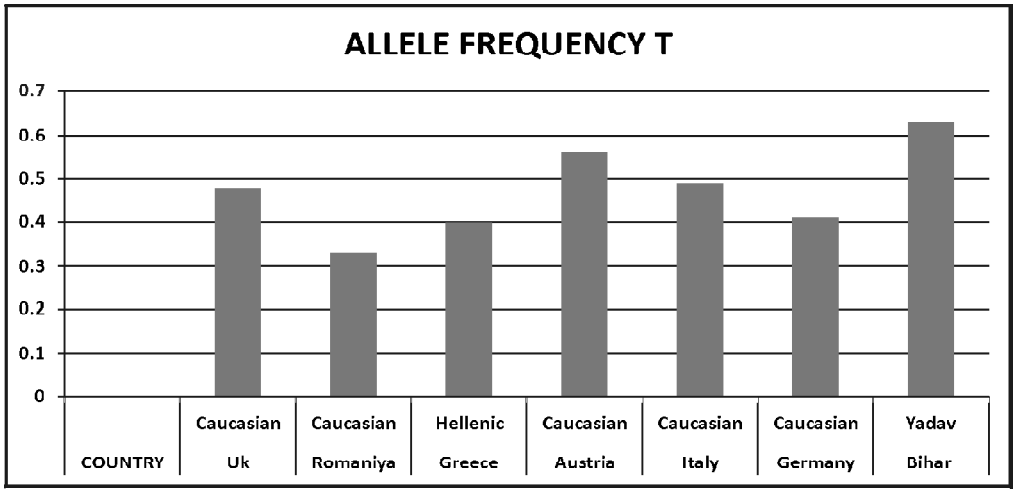

Figure 3: Comparison of T Allele of Yadav population of Bihar with some European populations

If we compare the T allele in Yadav population of Bihar with European populations, it shows the highest frequency (0.63) as compared to the European population (Figure 3). In American populations frequency of T allele ranges from 0.15 (USA Mixed studied by Wang *et al.*, 2006) to 0.79 (USA African American studied by Jenkins *et al.*, 2008). In Asian and Indian population groups the frequency of T allele ranges from 0.20 (mixed Indian; Srivastava *et al.*, 2012) to 0.85 (Figure 5). According to Schmidt *et al.* (1995) a higher frequency of T allele is found to be a risk for family history disease and early onset of hypertension.

Methylenetetrahydrofolatereductase (MTHFR) plays a wide range of roles in different cellular functions such as 5,10-methyltetrahydrofolate to 5–

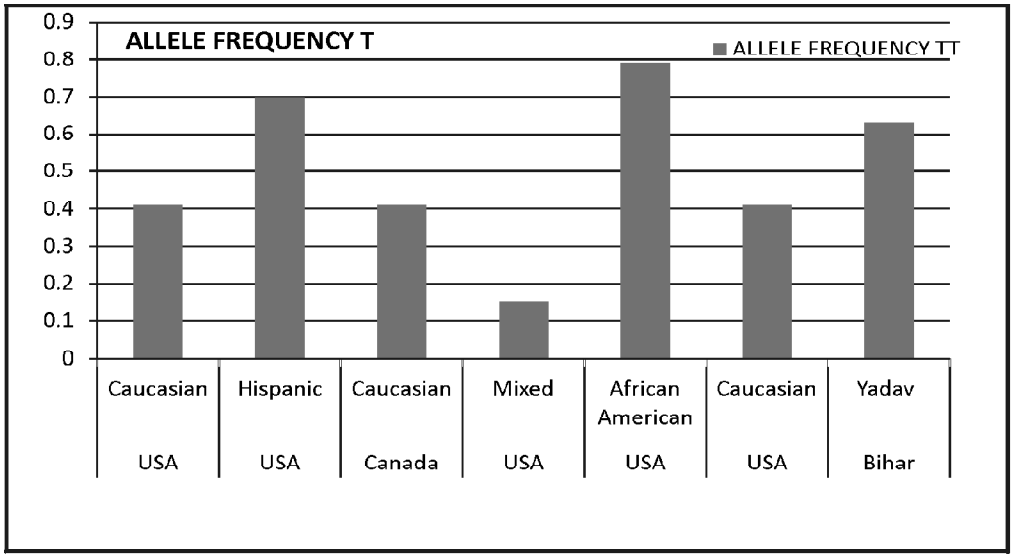

Figure 4: Comparison of T Allele of Yadav population of Bihar with some populations of American Continent

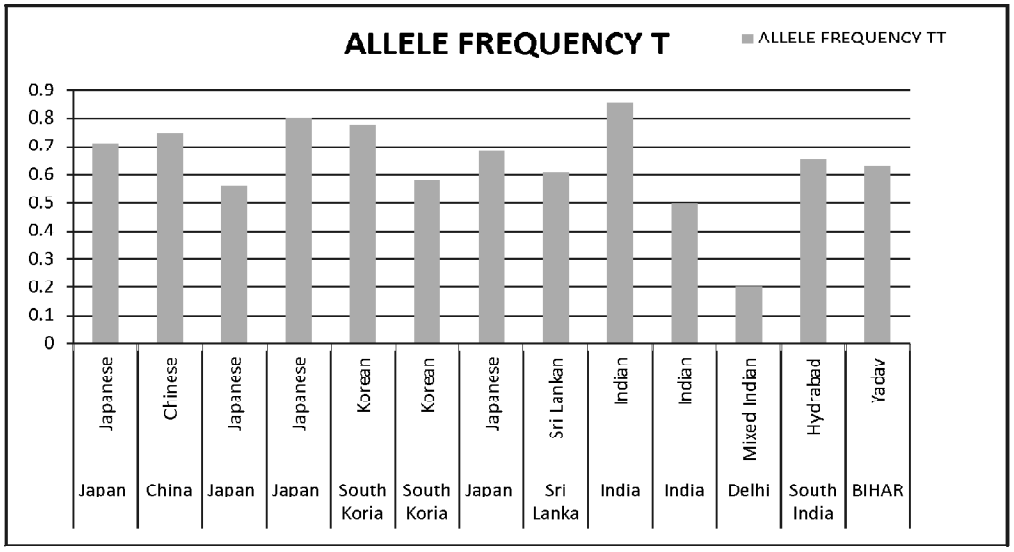

Figure 5: Comparison of T Allele of Yadav population of Bihar with Indian some Indian and other Asian populations

methyltetrahydrofolate DNA methylation, synthesis and repair, etc. The MTHFR gene provides instructions for making an enzyme called methylenetetrahydrofolatereductase. This enzyme plays a role in processing amino acids, the building blocks of proteins.

In present study, the MTHFR gene is also found to be polymorphic among Yadavs of Bihar. The CC homozygote genotype has the highest frequency (79.4%) followed

by heterozygote CT (17.65%) and TT (3%). Of the various SNPs of MTHFR, C677T is reported as an important SNP leading to change in the enzyme activity. It replaces the nucleotide cytosine with the nucleotide thymine at important position 677 in exon 4 of the gene which further leads to the substitution of alanine with valine (Frosst *et al.*, 1995).

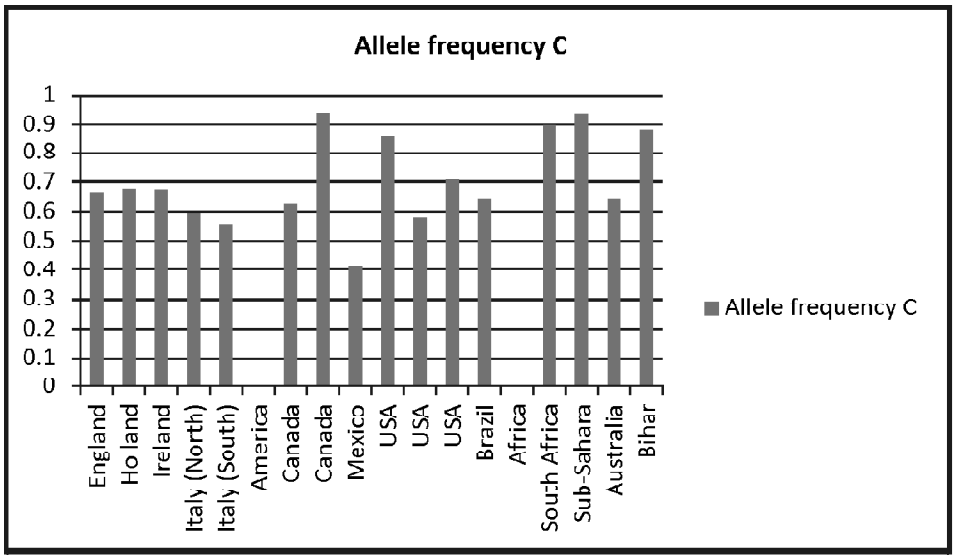

Figure 6: Frequency of C allele in MTHFR C677T SNP of Yadav population of Bihar in comparison with European, American, African, and Australians population groups

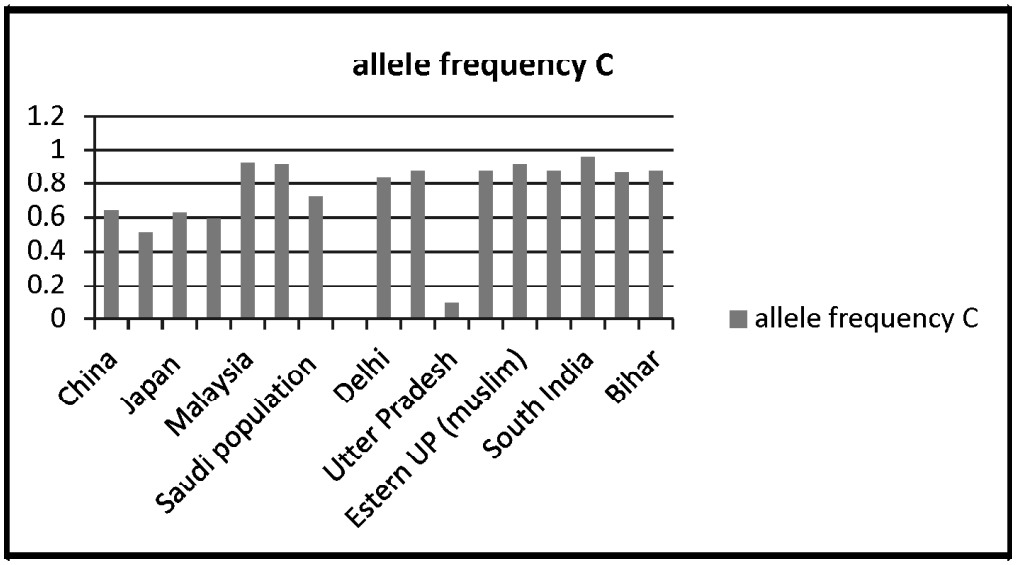

Figure 7: Comparison of C allele of Yadav population in MTHFR C677T SNP with Asian and Indian population groups

In present study, if we compare the frequency of C allele with world and other Asian and Indian populations, frequency of C allele falls closer to the Mexicans (0.41), sub-Saharans and Canadian (0.93) populations. Recently, Indian genome

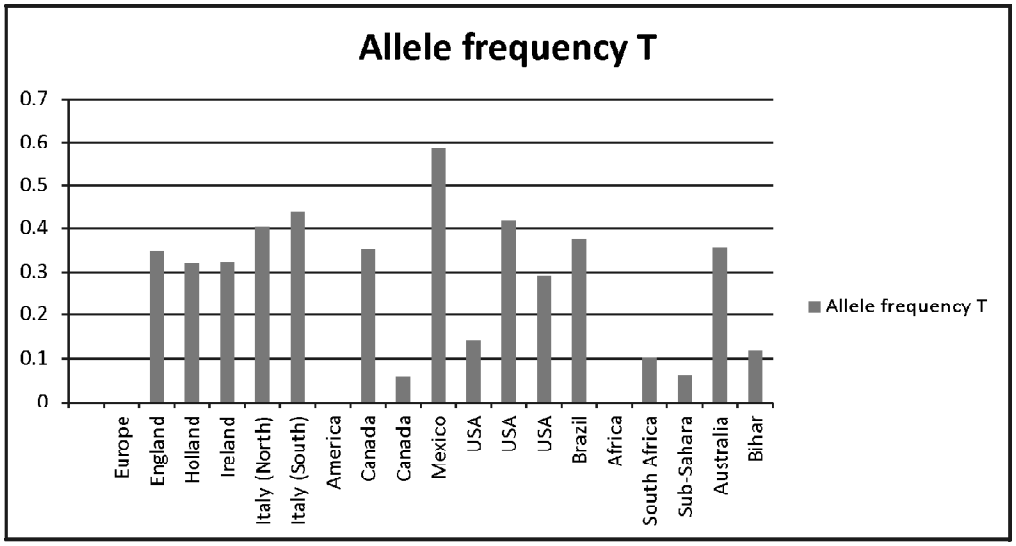

Figure 8: Frequency of T allele in MTHFR C677T SNP of Yadav population of Bihar in comparison with European, American, African, and Australian populations

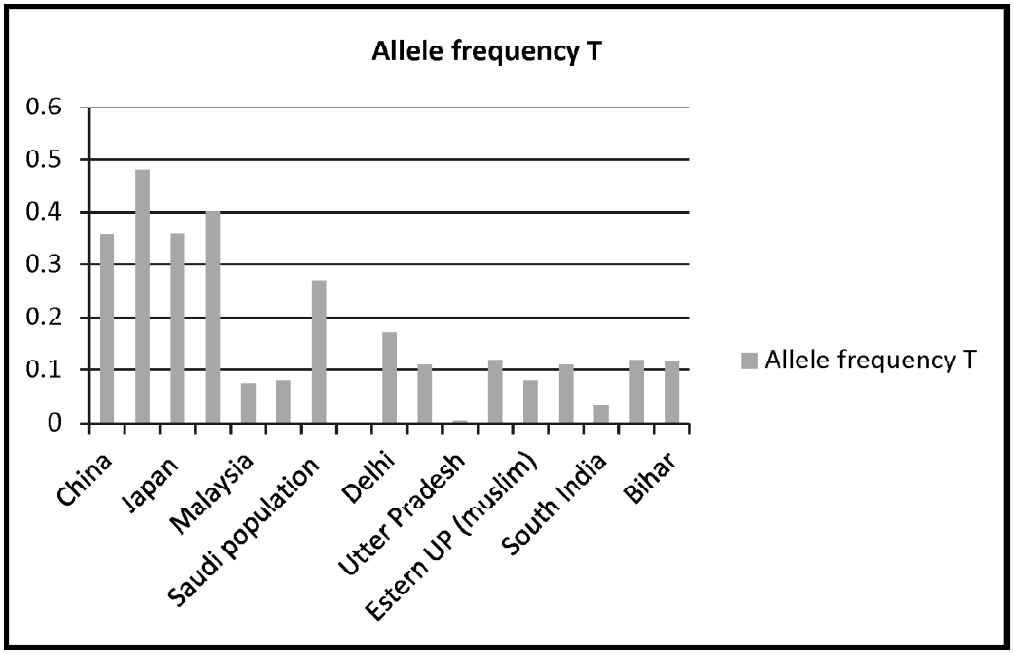

Figure 9: Comparison of T allele of Yadav population in MTHFR C677T SNP with Asian and Indian populations

variation consortium (2008) reported the overall frequency of Mutant T allele among the Indian population to be 14%.

All these three clinical markers are highly associated with CVD disease and various metabolic diseases like renal disease and hypertensions, but in population of Bihar studies on these genes have not been conducted. We need to conduct more extensive studies with larger samples on Yadavs of Bihar and other ethnic groups to know the association of CVDs and these genomic markers.

The allele frequencies of three studied loci are approximately in line with other populations in India as well as world. The high frequency of D-allele (ACE gene) instead of I allele in the population under study demands further investigation. The Hardy-Weinberg disequilibrium observed in the Yadav population of Bihar for polymorphism related to ACE and AGT is possibly due to evolutionary force (especially selection) in the studied populations. Given the high public health importance of screening the studied polymorphism, the large scale studies on different populations are required for developing effective counseling strategies.

Table 1: Genotypic distribution of ACE, AGT and MTHFR gene in Yadavs of Bihar

Gene	*Observed number*	*%*	*Expected number*	*%*	*HWE p-value*
ACE (I/D)					
II	8	10.53	18	23.68	<0.0001
ID	58	76.32	38	50	
DD	10	13.15	20	26.32	
Total	76	100	76	100	
I Allele	0.486				
D Allele	0.512				
AGT (M235T)					
MM	7	8.33	6.9	8.22	0.0374
MT	48	57.14	48.3	57.5	
TT	29	34.53	28.8	34.28	
Total	84	100	84	100	
M Allele	0368				
T Allele	0.630				
MTHFR (C677T)					
CC	54	79.41	52.9	77.79	0.261
CT	12	17.65	14.1	20.88	
TT	2	2.94	0.9	1.33	
Total	68	100	68	100	
C Allele	0.882				
T Allele	0.117				

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