International Journal of Scientific Reports
Pandey S et al. Int J Sci Rep. 2016 Jan;2(1):13-17
http://www.sci-rep.com

pISSN 2454-2156 | eISSN 2454-2164

DOI: http://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20160088

Research Article

CYP1A1 gene polymorphism among central Indian population and its genetic distribution

Snehlata Pandey[1]*, Arvind Tripathi[1], Jitendra Tripathi[1], Sanjeev Dubey[2]

[1]Center of Biotechnology studies, School of Environmental Biology, APSU, Rewa, MP-486001, India
[2]Department of Botany, Government Model Science College, Rewa, MP-486001, India

Received: 07 October 2015
Accepted: 17 December 2015

***Correspondence:**
Dr. Snehlata Pandey
E-mail: snehlatapandey23@gmail.com

ABSTRACT

Background: The human gene, CYP1A1, encodes a member of the cytochrome P450 superfamily of enzymes. Cytochrome P450 (CYP1A1) enzymes are involved in the oxidative metabolism of endogenous compounds such as steroids, fatty acids, leukotrienes, prostaglandins, in the metabolism of foreign chemicals such as drugs, carcinogens, and other environmental pollutants and in polycyclic hydrocarbon metabolism. This was the first study done in the region of vindhyan Madhya Pradesh.
Methods: The study population included total 300 samples from random population patients and normal healthy individuals (between 192 urban's and 108 tribals). Physical data was collected during the sample collection.Genetic polymorphism study was carried out by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and the observed genotype frequencies, allele frequencies and P- value for CYP1A1 gene polymorphism. All the statistical analyses were performed using spss software.
Results: The genotype distribution pattern was not found different between tribal's and urban population ($\chi 2$ =1.434 and P Value=0.4882) and overall allele frequency was also not statistically significant between two respective groups. Smoking and drinking activity data was generated through questionnaire organized during Sampling and the findings suggest that smoking have high effect over CYP1A1 gene polymorphism susceptibility.
Conclusions: The genotype distribution pattern was not found different between tribal's and urban population ($\chi 2$=1.434 and P Value=0.4882) and overall allele frequency was also not statistically significant between two respective groups.

Keywords: CYP1A1 gene, Polymorphism, Genetic distribution

INTRODUCTION

A gene is called to be polymorphic if more than one allele occupies that gene's locus within a population. Cytochrome P450 enzymes are involved in the oxidative metabolism of endogenous compounds such as steroids, fatty acids, leukotrienes, and prostaglandins and in the metabolism of foreign chemicals such as drugs, carcinogens, and other environmental pollutants.[1]

The cytochrome P-450 1A1 gene (CYP1A1) is part of a super gene family located at 15q22-q24 human chromosome.[2] The human CYP1 family consists of two functional genes: CYPlAl, involved in polycyclic hydrocarbon metabolism and CYP1A2, involved in aryl amine metabolism. Both genes are up-regulated (induced) by certain foreign chemicals such as benzo[a]pyrene, 3-methylcholanthrene, 0-naphthoflavone, and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. As the inducibility of CYPlAl increases, so does the metabolism of poly-cylic hydrocarbon procarcinogens to reactive carcinogenic intermediates; enhanced metabolism often leads to a

higher risk of malignancies. CYP1A1 may function as a carcinogen-detoxication enzyme, whereas the paradoxical activation of natural dietary compounds with chemo preventative activity provides further insight into the cancer-protecting role of this enzyme.[3,4]

CYPs exhibit considerable catalytic diversity among the enzymes of phase I biotransformation. This enzyme super family, existing in over 50 forms, is the most important enzyme system involved in the biotransformation of many endogenous and exogenous substances including drugs. CYPs are variably distributed in different tissues. Most can be found in the membrane of the endoplasmic reticulum in the liver, although CYPs are found in almost all tissues and organs (intestine, lung, kidney, brain, lymphocytes, and placenta). Physiological substrates of these enzymes include steroids, fatty acids, prostaglandins, leukotrienes, and biogene amines, while xenobiotic substrates include drugs, herbal toxins and toxic chemicals from the environment. CYPs predominantly catalyse oxidative reactions, insertion of an atom from molecular oxygen into a substrate, i.e. a typical activating (or Phase I) reaction, serving as monooxygenases, oxidases and peroxidases, although they can act in reduction reactions too. Several reports have suggested that the metabolic activation of PAHs by cytochrome P450 (CYPs), including CYP1A1 is a necessary step for PAH-induced atherosclerosis. CYP1A1 is the main metabolizing enzyme of PAHs that generates a highly electrophilic diol-epoxide metabolite capable of creating DNA adducts.[5,6]

METHODS

Study population

The study population consisted of 300 unrelated subjects comprising between 192 urban's and 108 tribals between the age of 20- 40yr from the Sanjay Gandhi hospital and advance X-ray pathology Rewa, and District hospital Satna and Sidhi. All the participants were asked to fill a detailed questionnaire at the time of recruitment, seeking information regarding individual's age, sex, ethnicity, dietary habits, physical activity, and life style, personal and family medical history.

Blood collection and plasma/serum separation

Approx. 5ml. of Venous blood samples of each individual were collected in storage vials containing EDTA as anticoagulant (50µl. EDTA for 1ml. blood).Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min at 4°C.

Deoxyribonucleic acid (DNA) isolation

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller.[7]

Detection of CYP1A1 polymorphism via PCR-RFLP

The human cytochrome P-450 1A1 (CYP1A1) gene located on chromosome 15 at q^{22}-q^{24} encodes monooxygenases, a phase I enzyme involved in the activation and metabolism of environmental pro-carcinogens. The CYP1A1 allele consists of a T -to-C substitution in the 3' non-coding region, which creates on msp I restriction enzyme cleavage site. Primer was designed to amplify 340 bp sequences to study this polymorphism.

Genotyping

Genotyping was carried out by polymerase chain reaction (PCR) and restriction enzyme digestion. The *CYP1A1* polymorphism was detected using the following primers: CYP1A1 - F-5′-TTCCACCCGTTGCAGGATAGCC-3′ and CYP1A1 - R-5′-CTGTCTCCCTCTGGTTACAGGAAG -3′.The PCR was carried out in a final volume of 25 µl, containing 100 ng of genomic DNA(4-5 µl), 2.5 µl of 10X *Taq* polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concent ration 1X; Genetix Biotech Asia Pvt. Ltd.,India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of secific forward and reverse primers and 1 µl of unit of 1U/µl Red Taq DNA polymerase (Bangalore genei).

Thermal profile used for the amplification of desired segment of gene was as follows: Initial denaturation at 950C for 5 min and 30 cycles of denaturation at 94°C for 45 Sec, annealing at 59.40C for 45 sec and extension at 720C for 1 min, followed by final extension at 720C for 10 min. PCR products were separated on 2% agarose gel (2%w/v, Sigma) using a 100 bp molecular weight (MW) marker to confirm the PCR product size of 340 bp.

For restriction digestion of the C -to-T substitution in the 3' non-coding region, which creates an msp I restriction enzyme cleavage site, the reaction mix included 0.2 µl of 10,000U/ml Msp I restriction enzyme (final concentration 2.5 U), 2.5 µl of 10X GENAI Buffer c (final concentration 1X; 50 mM potassium acetate, 20 mM Tris acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9(‘ 10.0 µl of PCR product and 10 µl of sterile water. Reaction was incubated for 16 hrs at 37^0C for complete digestion. 10 µl of digested PCR product was loaded on 2% Agarose gel. Electrophoresis was done at 80 V in 1 X Tris-borate EDTA buffer (89mM Tris pH 7.6, 89 mM Boric acid, 2 mM EDTA pH 8.0) buffer.) A 100 bp gene DNA ladder (genetix india) was run concurrently as molecular weight marker. The gel was than stained with ethidium bromide (10mg/ml). The products were visualized using an ultraviolet Transilluminator. The gel picture was captured using a digital camera and gel documentation software (Vilber Lourmate, Cedex I, France).

Statistical analysis

Statistical analysis was done by comparing the distribution of genotype frequencies and allele frequencies for all polymorphisms in all groups of study subjects. The proportions of different genotypes for a gene in a population are known as genotype frequencies. The proportion of a genotype in a sample will be the ratio of the number of individuals having that genotype of the total number of individuals in the sample. The proportions of different alleles for a gene present in a population are known as allele frequencies. The proportion of an allele in a sample will be the ratio of number of occurrence of the investigated allele in the population to the total number of alleles. Data was analyzed using Microsoft Excel 2002, Microsoft Corporation and graphPad Instat program (GraphPad Software, San Diego California USA, Copyright 1992-1998 GraphPad Software Inc.

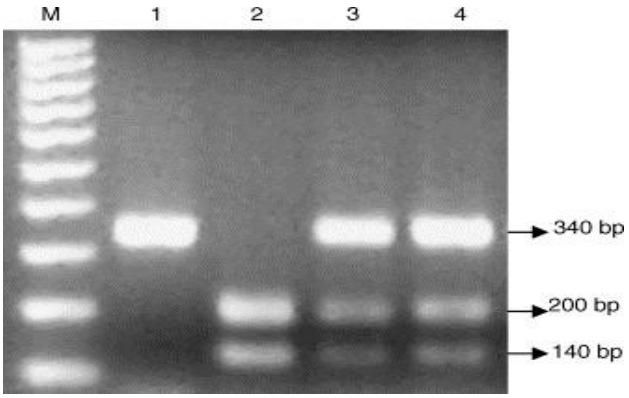
RESULTS

Hardy – Weinberg equilibrium

The genotype frequencies of each study group were tested to be in accordance with hardy – Weinberg equilibrium using chi square test for independence. All the calculated values were compared with tabulated values and found that all the frequencies were in Hardy – Weinberg equilibrium. The standard tabulated values was 3.8 at 1 degree of freedom and P = 0.05 level of significance.

Table 1: Values of Chi square test for Hardy-Weinberg equilibrium for CYP1A1 gene in study population.

Gene polymorphism	Study Groups (χ^2 Values)	
	Urban's	Tribal's
CYP1A1	1.08683	0.877182148



(Wild type=1, Heterozygous=2, Mutant=3, 4, Ladder=100bp ladder, M)

Figure 1: Detection of CYP1A1 polymorphism.

Detection of CYP1A1 polymorphism

The 340 bp sequence of Exon 7 on chromosome 15 at q^{22}-q^{24} was amplified by PCR and after that PCR product were subjected to BSDR I digestion. The wild type allele was undigested (denoted as CC genotype), and mutant allele had given 200 and 140 bp fragment (denoted as TT genotype) and other was Heterozygous (denoted as CT genotype) as they had given the three bands of 340, 200 and 140 bp.

The genotype frequency (in %) of CC, CT and TT Genotypes scored 37.5%, 55.21% and 07.29% respectively in Urban's. In Tribal population genotype frequencies were found CC=46.30%, CT=44.44 and TT=07.41%.

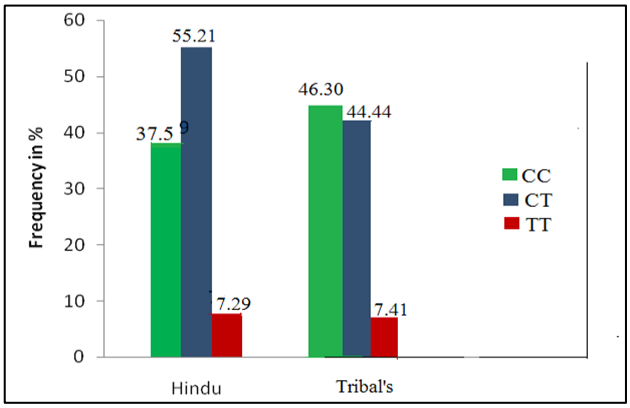


Figure 2: CYP1A1 genotype frequency (In %) in study groups (Hindu & Tribal).

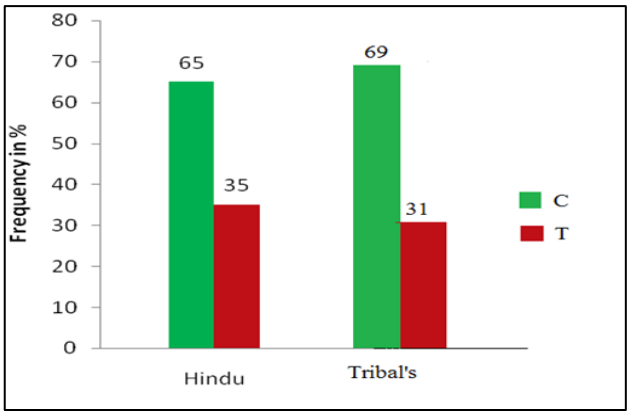


Figure 3: CYP1a1 allele frequency in (in %) study groups (Hindu & Tribal).

The association of genotype distribution pattern in all two groups (Urban's and Tribal's) was studied and as recorded, there is no statically significant differences exist as the χ^2 and P values were χ^2=1.434 (P = 0.4882) between Urban's-Tribal's.

The distribution of C and T alleles in various groups were found C = 65% and T = 35% in Urban's, T = 31% in

Tribal population. The association between Allelic distribution in all three groups were studied by Fisher's exact test and values obtained were, P = 1.0000 between Urban's – Tribal. All the values were non-significant which showed that the distribution pattern C and T allele in all two groups were statically not different.

Table 2: Frequency distribution (in %) and association of genotype and alleles of CYP1A1 genes in study groups.

CYP1A1 Gene	Study groups			
	Urban's No. 192	%	Tribals No. 108	%
CC	72	37.5	50	*46.30*
CT	106	55.21	48	44.44
TT	14	07.29	08	07.41
	χ^2 Values		P- Value	
Urban's-Tribal's	1.434		0.4882 (NS)	
Alleles	Urban's		Tribal	
	Frequency	%	Frequency	%
C	65		69	
T	35		31	
P- Values (Fisher's exact test)				
Urban's- Tribal's			0.6521 (NS)	

DISCUSSION

Indian populations are known for their unique cultural and linguistic diversity.[8] Broadly, Indian population can be categorized as the castes, tribes and religious communities. Tribes represent ~8.2% of the total population of India. There are currently about 530 tribal groups in India. Madhya Pradesh (MP) is the second largest Indian state by area, is located in the central part and is homeland of several caste and tribal groups. It is bordered by the states of Uttar Pradesh in the north, Chhattisgarh in the east, Maharashtra in south, Gujarat in the west and Rajasthan in the northwest. Except for the valleys of the Narmada and the Tapti, Madhya Pradesh consists of a plateau, straddled by the river Narmada and interspersed with the mountains of the Vindhya and the Satpura ranges. It is one of the largest states of India inhabited by the bulk of tribal populations of the country constituting 20.3% of the total tribal populations. There are 46 Scheduled Tribes (ST), among which Gond, Bhil, Baiga, Sahariya, Oraon, Korku and Kol are the most prominent.[9]

Over the past few years, genetic studies using haploid and diploid genetic markers have provided a substantial understanding of the human origins and dispersal patterns in South Asia [10]. In present investigation we planned to perform a comparative study of CYP1a1 alpha gene polymorphism and genotypic distribution in tribal population of Madhya Pradesh with normal urban population.

CYP1A1 gene, encodes a member of the cytochrome P450 super family of enzymes. The cytochrome P450 proteins are monooxygenases which catalyse many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Cytochromes P450 are a group of heme-thiolate monooxygenases. In liver microsomes, this enzyme is involved in an NADPH-dependent electron transport pathway. It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. The aim of our study to analyse CYP1A1 gene polymorphism among random population in our central Indian population. A large number of studies have been conducted to investigate the associations between variants and its association with disease.[11]

The genotype frequencies of CYP1A1 BSDR I recorded in our study population were significantly different with Caucasian, African- American and Turkish population as it was reported as CC=79.5%, CT=19.2% and TT=1.3% in Caucasian, CC=70.7%, CT=27.6% and TT=1.6% in African- American and CC=75%, CT=19.1%, TT=5.6% in Turkish population, which showed that these population are distinct from our population ,Turkish Journal of Cancer 2005. The very low degree of differences found those reported in African population as it was CC=58.1%, CT=36% and TT=5.9%, which indicated a possible link between Asian, African and our population and same origin of these races.[12]

The same trend observed in allelic distribution pattern of CYP1A1 BSDR I polymorphism as it was observed C=65%, T=35% in Hindu, C=64% and C=69%, T=31% in Tribal. Which can be related with the others finding in Asian population (C=77% and T=23%), but surprisingly allelic frequency did not match with the African population (C=97% and T=03%) which could be due to differences in mating pattern or other unknown factors. A significant difference observed between results from our population and Caucasian population (C = 95% and T = 05%).[12] The differences of allelic distribution between our population and African population can open the new phenomena in field of population genetics. The higher frequency of T allele in our population keep them at the more risk for carcinogenesis than the other ethnic groups of world population.

CONCLUSION

To study distribution of CYP1A1 allele distribution in Vindhyan region 108 samples from tribal's from Rewa, Sidhi, Umaria, Shahdol and 192 numbers of samples were collected from urban population of Rewa. The genotype distribution pattern was not found different between tribal's and urban population (χ2=1.434 and P Value=0.4882) and overall allele frequency was also not statistically significant between two respective groups.

Although scope of our study is limited because many of other genes are their which are also associated with risk

of many disease but in future a genome wide association study with large sample size is recommended to investigate the population and risk of disorders. Molecular Biology has opened new ways to discover new medicines which can battle with much life threatening disease. This study contains small sample size but we recommend a large population based functional genomics study.

ACKNOWLEDGEMENTS

We thank all the subjects who participated in this study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. UK Essays. Genetic Polymorphism Governing the Cyp2d6 Cytochrome P450 Enzyme Subfamily in Drug Metabolism. [Accessed 7 October 2015]. http://www.ukessays.com/dissertation/examples/sciences/genetic-polymorphism-governing-the-cyp2d6-cytrochrome-p450-enzyme-subfamily-in-drug-metabolism.php?cref=1.
2. Hildebrand CE, Gonzalez FJ, McBrid OW, Nebert DW. Assignment of the human 2, 4, 7, 8-tetrachlorodibenzo-p-dioxin-inducible cytochrome P1-450 gene to chromosome 15. Nucleic Acids Res. 1985;13(6):2009-16.
3. Gonzalez FJ, Gelboin HV. Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins**.** Drug Metab Rev 1994;26:165-83.
4. Nebert DW, McKinnon RA, Puga A. Human drugmetabolizing enzyme polymorphisms: effects on risk of toxicity and cancer. DNA Cell Biol. 1996;15:273-80.
5. Qiang MA, Lu AY. CYP1A induction and human risk assessment: an evolving tale of in vitro and in vivo studies. Drug Metab. Dispos. 2007;35:1009-16.
6. Jacquet M, Lambert V, Baudoux E, Muller M, Kremers P, Gielen J. Correlation between P450 CYPlAl inducibility, MspI genotype and lung cancer incidence. Eur J Cancer. 1996;32:1701-6.
7. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.
8. Chaubey G. The demographic history of India: A perspective based on genetic evidence. 2010:9-81. Universitatis Tartuensis, Estonia. Ph.D Thesis. (http://hdl.handle.net/10062/15240).
9. Census of India [http://www.censusindia.net/].
10. Thangaraj K, Naidu BP, Crivellaro F, Tamang R, Upadhyay S, Sharma VK. The influence of natural barriers in shaping the genetic structure of Maharashtra populations. 2010;PLoS One 5 e15283.
11. Anti-Cytochrome P450 1A1 antibody [6G5] ab126828. Product Datasheet. available at: http://www.abcam.com/Cytochrome-P450-1A1-antibody-6G5-ab126828.tml
12. Garte S, Gaspari L, Alexandrie S, Ambrosone C, Autrup H, Autrup J. Metabolic Gene Polymorphism Frequencies in Control Populations. Cancer Epidemiol Biomarkers. 2001;10:1239.

Cite this article as: Pandey S, Tripathi A, Tripathi J, Dubey S. CYP1A1 gene polymorphism among central Indian population and its genetic distribution. Int J Sci Rep 2016;2(1):13-7.