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Allele frequencies of PON1 Q192R polymorphism in four populations of India

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ABSTRACT

The allelic distribution at Paraoxonase 1 (PON1) Q192R polymorphism determines differential sensitivity towards certain organophosphate pesticides. The alleles Q (Glutamine) and R (Arginine) at amino acid position 192 are responsible for the lower and higher activity of the enzyme towards paraoxon respectively, making knowledge of this distribution in different populations vital. This study reports the genotype and allele frequencies of the Gln192Arg polymorphism of PON1 in four populations of India, comprising two caste and two tribal groups hitherto unexamined for this polymorphism. The R allele frequencies in Jat, Meo, Santhal and Zeliangrong populations were found to be 0.47, 0.45, 0.54 and 0.51 respectively. The gene diversity analyses show a high genetic differentiation at this locus indicative of the role of populations' history and other evolutionary forces. A comparison with allele frequencies among 106 populations from different continents showed a concordance with their geographic distribution which will have repercussions in policies targeting pesticide usage.

1. Introduction

Human serum Paraoxonase 1 (HuPON1, EC 3.1.8.1) is a 355 amino-acid long; 43-45KDa sized protein (Hassett et al., 1991) coded by a gene located in chromosome 7q21.3 (Humbert et al., 1993). The enzyme is coded by nine exons (Clendenning et al., 1996). *PON1* belongs to the multigene family comprising two more paraoxonases, *PON2* and *PON3* (Primo-Parmo et al., 1996). The three genes are located adjacent to each other on chromosome 7q21-22. The PON1 enzyme was first reported as an organophosphatase in animal tissue and named because of its ability to hydrolyze paraoxon, an organophosphate (OP) metabolite. In humans, PON1 is associated with high density lipoprotein (HDL) molecules (Serrato and Marian, 1995). The enzyme shows variable specificity towards substrates including esters, lactones and phospholipids, lactonase being the native activity. The serum levels and hydrolytic activity of PON1 determine the susceptibility of an individual towards OP toxicity. The enzyme exerts a protective effect against OP pesticides like parathion, diazinon, and chlorpyrifos and nerve agents like sarin and soman by hydrolyzing their toxic oxon metabolites. The variability of PON1 activity and levels is expected to affect the susceptibility towards these pesticides and their derivatives.

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Studies on population distribution of plasma PON1 activities showed the presence of high, intermediate and low levels of activity (Geldmacher-von Mallinckrodt et al., 1983; Playfer et al., 1976; Brophy et al., 2001; Ginsberg et al., 2009). Genetic variation in enzyme levels and activity lead to studies that unraveled the presence of genetic polymorphisms. The most significant and widely studied among them are coding region polymorphisms Q192R (dbSNP: rs662A>G), L55M (dbSNP: rs854560A>T) and promoter polymorphism −108C/T (dbSNP: rs705379). The variation in enzyme activity was attributed to the presence of Gln192Arg or Q192R polymorphism (Humbert et al., 1993; Adkins et al., 1993). *In vitro* analysis proved that the R192 variant hydrolyzes paraoxon more rapidly than Q192 which, in turn, is an efficient metabolizer of diazoxon, soman and sarin (Furlong et al., 2005). The genotype and allele frequency distribution for Q192R polymorphism has been studied among world populations and in India in lieu of their implications in organophosphate toxicity and cardiovascular health. This is the first attempt to determine Q192R polymorphism in healthy individuals sampled from two caste and two tribal populations from India. The paper outlines the genetic relationship of the study populations with those reported in previous studies at this locus. The polymorphic distribution is explored in other world populations from data reported in literature. The work was aimed to decipher the extent of genetic differentiation at this locus by assessing the allele frequency distribution.

2. Materials and methods

The project was approved by the Institutional Ethical Committee, Department of Anthropology, University of Delhi. Blood samples were collected from unrelated individuals belonging to four different populations from the western, eastern and north-eastern parts of the Indian sub-continent after explaining them about the project and taking their consent. The blood samples were stored at 4 °C till genomic DNA extraction. A total of 348 individuals were sampled comprising 100 Jats from Bhiwani, Haryana, 83 Meos from Nuh, Haryana, 95 Santhals from Baripada, Odisha and 70 individuals of the Zeliangrong tribe of Tamenglong district, Manipur. Genomic DNA was extracted from each blood sample by following the salting-out procedure described by Miller et al. (1988). The DNA was quantified using Nanodrop1000 and diluted to a concentration of 40 ng/μl for polymerase chain reaction (PCR). For *PON1* Q192R polymorphism genotyping, primers given by Humbert et al. (1993) were used for PCR. The PCR product was incubated with *AlwI* (Fermentas Ltd.) at 37 °C for 3 h for digestion. The digested products were visualized on a 3% agarose gel in 0.5× Tris-borate EDTA buffer using a Gel Documentation system (Syngene, UK).

Allele frequencies were determined by gene-counting method. A χ^2-goodness-of-fit test was performed to determine if the genotype distribution was in agreement with Hardy–Weinberg equilibrium. Heterozygosities were computed as $h = 1 - \sum_{i=1}^{j} x_i^2$ where h is the heterozygosity per locus, x_i is the frequency of the ith allele of a locus in the population and j is the number of alleles (Nei, 1973). Average heterozygosities were calculated by gene identities. The results were tabulated (Table A.1). An extensive literature search was done to identify populations sampled to genotype for Q192R in India and other parts of the world. Allele frequency data was also compiled from the Allele Frequency Database (Rajeevan et al., 2012). Samples composed of related individuals were excluded. Those studies were selected which reported samples to be healthy, randomly selected individuals of defined ethnicity. The areas from which samples were collected were noted for finding their respective latitude and longitude. The final data set comprised 10,658 individuals representing 110 populations from all over the world (Table A.1 and Appendix) (Antikainen et al. (1996), Chen et al. (2003), Gamboa et al. (2006), Lahiry et al. (2007), Tripi et al. (2006)).

The allele frequency distribution was analyzed at major geographical levels. Gene diversity indices H_T, H_S and G_{ST} were calculated following Nei's (1973) method (Table A.2). The genetic distance, DA (Nei, 1972) was calculated and the multidimensional matrix generated was used to deduce the Neighbor-Joining (Saitou and Nei, 1987) dendrogram using the DISPAN software.

The patterns of latitudinal and longitudinal clines were examined in different parts of the world which can be used for evaluating the role of genetic variation at this locus with respect to the risk of organophosphate poisoning. In order to achieve this, the datasets in Table A.1 and Appendix were used for a regression analysis that was performed using R version 3.0.1. (R Core Team, 2013).

3. Results

3.1. Allele and genotype frequency distribution in India

The allele and genotype frequency distribution and the heterozygosity estimates of different populations included in this study are summarized in Table A.1. All populations except Santhal showed genotype distribution in concordance with Hardy-Weinberg expectations. The QQ genotype was most frequent in Meo (28%) closely followed by Jat (24%) and was only 13% in Santhal. In all the four populations QR was the most frequent genotype: Santhal (64%), Jat (59%), Zeliangrong (56%), Meo (53%). Q was found to be more frequent in Jats (0.53 ± 0.035) and Meo (0.55 ± 0.039) from the north-western state of Haryana. Santhal (0.54 ± 0.036) and Zeliangrong (0.51 ± 0.042) had a higher R allele frequency. Heterozygosity levels among populations included in this study were not very different but differed from estimates reported in other populations from India.

3.2. Allele frequency distribution in other world populations

The estimated allele frequencies and heterozygosities of Q192R polymorphism in various populations across the world have been grouped region-wise and listed in Appendix. The lowest Q allele frequency is reported in Poturujara from Brazilian Amazon where it was absent (Santos et al., 2005). When the Q allele frequency was pooled for different continents, the maximum frequency was observed in Europe (0.7281 ± 0.0787). The gene diversity analysis revealed a high