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Frequency of homozygous null mutation at the glutathione-s-transferase M1 locus in some populations of Orissa, India

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With 1 figure and 1 table in the text

Summary: GSTM1, one of the mu type glutathione-s-transferase isozymes in human, has been observed to be inherited dominantly, and the polymorphism of this gene can be detected by polymerase chain reaction (PCR). In this report we have determined the frequency of a homozygous deletion mutation of the GSTM1 gene in some populations of the Sundergarh District in Orissa, India. About 24 % of unrelated individuals (n = 72) were detected to carry this deletion mutation in homozygous form. Possible implications of this homozygous deletion are discussed.

Zusammenfassung: GSTM1, eine der mu-Typen des Glutathion-S-Transferase-Isozyms beim Menschen, konnte als dominant vererblich nachgewiesen werden. Der Polymorphismus dieses Gens läßt sich mit Hilfe der Polymerasekettenreaktion (PCR) nachweisen. In dieser Untersuchung wird über die Häufigkeit einer mutativ bedingten homozygoten Deletion des GSTM1-Gens in verschiedenen Populationen aus dem Sundergarh-Distrikt in Orissa, Indien, berichtet. Unter 72 nicht miteinander verwandten Individuen wurde das Vorliegen dieser Deletion in homozygoter Form in einer Häufigkeit von ca. 24 % beobachtet. Die möglichen Implikationen dieser homozygoten Deletion werden diskutiert.

Introduction

Most cancers are caused by exposure to environmental carcinogens, not by inheritance of specific susceptibility genes such as the retinoblastoma gene (Marx 1991). Exposure to carcinogens is effected by intake of various kinds of food items, life-style factors (smoking, tobacco chewing, drinking etc.) and various enzyme participate in the process of detoxification of carcinogens. Genetic polymorphisms have been described for a number of enzymes involved in the metabolic activation or detoxication of carcinogens. These metabolic enzymes can be divided into two categories: the 'phase I' enzymes which almost exclusively belong to the cytochrome P-450 group and are mostly involved in the activation process, and the 'phase II' enzymes such as glutathione-s-transferase (GST) which are generally involved in the detoxification process. Polymorphism in detoxification enzymes may provide a genetic basis for inter-individual variability in the susceptibility to toxic materials. Several reports have demonstrated that the GSTM1 gene, one of

the GST mu class genes on chromosome 1p13 (Pearson et al. 1993), is polymorphic and a deletion mutation in this gene has been identified in individuals of different ethnic backgrounds, using Southern blotting (Zhong et al. 1991) and PCR (Comstock et al. 1990) techniques. Individuals homozygous for deletion mutation do not produce the enzyme and as a result have very low or undetectable enzymatic activity in lymphocytes (Seidegard et al. 1988). GSTM1 null genotype is present in about 40–50 % of Caucasians (Zhong et al. 1991), 33 % of African-Americans (Bell et al. 1993) and 45 % of Japanese (Kihara et al. 1993) population. Recently Soni et al. (1995) reported that 46 % of randomly selected individuals of Indian origin, of unspecified ethnicity, exhibited low/undetectable GST mu enzyme activity in lymphocytes. These individuals may be assumed to be of GSTM1 null genotype. In this study we have directly determined the frequency of GSTM1 null genotype in 72 unrelated individuals with known ethnic backgrounds.

Materials and methods

About 10 ml of blood was collected by venipuncture from each of 72 individuals living in Sundergarh District, Orissa. Data on ethnic background of each individual were collected. Most individuals belong to middle caste group, Agharia, and others belong to various backward communities (e. g., Gaud, Tanti) or tribes (e. g. Munda).

Genomic DNA was isolated from lymphocytes by salt (NaCl) precipitation method as described in Miller et al. (1988). The DNA sequences between exons 3–5 were amplified according to the procedure described in Sekine et al. (1995). The pair of primers for detecting homozygous deletion in GSTM1 gene were custom synthesized according to the nucleotide sequences described by Seidegard et al. (1988). Two additional primers were used in the same reaction tube to co-amplify the 916 bp sequences in exon 1 of delta-amino levulinate dehydratase (ALAD) gene (Wetmur et al. 1991). The ALAD primers produce an internal control amplification product during PCR of GSTM1 gene. The sequences of the primers used are described as follows:

GSTM1 primers: sense primer, 5'-CCTGATTATGACAGAAGCCAG-3'
antisense primer, 5'-CTGGATTGTAGCAGATCATGC-3'
ALAD primers: sense primer, 5'-AGACAGACATTAGCTCAGTA-3'
antisense primer, 5'-GGCAAAGACCACGTCCATTC-3'

Amplifications of GSTM1 and ALAD nucleotide sequences were performed for screening each sample in a volume of 50 µl of reaction mixture containing 200 µM dNTPs, 1 pM of each primer, 400–800 ng of genomic DNA, 2.5 U of Taq polymerase (Cetus inc. USA) using a thermal cycler P-2400 (Perkin-Elmer, USA). Thirty cycles of amplification were carried out at 94 °C for 1 min (denaturation), 58 °C for 1.5 min (annealing) as well as 72 °C for 1 min (extention) with an initial denaturation step at 95 °C for 4 min. Amplified products were electrophoresed on a 1.5 % agarose gel in TBE buffer, stained with ethidium bromide and photographed. The primers for GSTM1 gene were chosen in such a way that amplification will occur in case of GSTM1 positive homozygous (+/+) and heterozygous (+/–) individuals but not in homozygous null or negative (–/–) individuals. Therefore, samples for which PCR products for GSTM1 gene were absent on the gel, were considered as homozygous GSTM1 null (–/–) individuals.

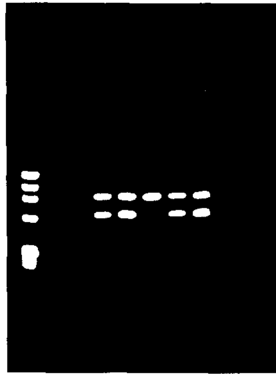

Fig. 1. Ethidium bromide stained PCR products of GSTM1 (630 bp) and ALAD genes (916 bp: internal control) obtained by amplifying DNA samples with oligonucleotide primers. Results of analysis of five DNA samples are shown. Lane 1 (leftmost lane) is a sizing marker (ØX174 DNA digested with HaeIII). Lanes 2, 3, 5 and 6 correspond to the presence and Lane 4 corresponds to the absence of amplified products of GSTM1 gene from five individuals.

Results and discussion

The amplified products from GSTM1 gene for (+/+) or (+/–) individuals showed a single band of 630 bp following agarose gel electrophoresis (Fig. 1). In addition to the target region of GSTM1 gene, internal control gene (ALAD) product was also perfectly amplified in all samples.

Sample sizes, genotypes and allele frequencies are presented in Table 1 by ethnicity. To avoid vagaries of small sample sizes, data on some ethnic groups were pooled and classified as "mixed" in Table 1. The frequency of the GSTM1 null allele is seen to vary between 45 and 52 % (pooled estimate = 48 %). The allele frequency differences between ethnic groups are not statistically significant at the 5 % level. The proportion of individuals of the null genotype (–/–) in the ethnic

Table 1. Sample sizes, genotypes and allele frequencies of GSTM1 null mutation in various ethnic groups of Orissa.

Ethnic Group	No. of individuals	% Individuals with null (–/–) mutation	Frequency ± s.d. of GSTM1 (–) allele
Agharia (Middle caste)	25	7 (28.0)	0.52 ± 0.08
Goud and Tanti (Lower caste)	27	6 (22.2)	0.47 ± 0.08
"Mixed" ethnicity	20	4 (20.0)	0.45 ± 0.10
Total	72	17 (23.8)	0.48 ± 0.05

groups studied is considerably smaller (24 %) than reported from other populations (33–50 %).

The physiological consequences of deletion mutation in GSTM1 gene in individuals still remain ill-defined. The central role of GST in detoxification implies that sensitivity to chemical toxins and carcinogens may be affected by this mutation. GSTM1 null polymorphism has attracted much attention owing to its possible association with increased susceptibility to certain malignancies, such as lung cancer (Seidegard et al. 1986, Zhong et al. 1991), astrocytoma (Strange et al. 1992), stomach cancer (Harada et al. 1992, Strange et al. 1991), pituitary adenoma (Fryer et al. 1993), bladder cancer (Katoh et al. 1995). Thus, the frequency of the GSTM1 null (–/–) genotype in a defined population may be a positive correlate of cancer prevalence in the population. Further, the association may be site specific and vary by ethnicity. Stronger association between GSTM1 null genotype and lung cancer was observed among the Japanese (Nakachi et al. 1993, Kihara et al. 1994) than among the Caucasian (London et al. 1995 and references therein) population. Such ethnic differences in associations may have important public health consequences and, therefore, it is important to study the associations in diverse ethnic groups. Population data, as presented in this study, of normal individuals are essential for such association study.

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