Ethnic Differences in Distributions of GSTM1 *and* GSTT1 *Homozygous "Null" Genotypes in India*

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Abstract We estimated the frequencies of *GSTM1* and *GSTT1* "null" homozygotes in 10 different ethnic populations of India by a genotyping method based on polymerase chain reaction. These populations, inhabiting diverse geographical locations and occupying various positions in the sociocultural hierarchy, were represented by a sample of 299 unrelated individuals. Frequencies of *GSTM1* and *GSTT1* "null" homozygotes varied from 20% to 79% and 3% to 39%, respectively, across the study populations. Maximum frequencies of *GSTM1* and *GSTT1* "null" homozygotes (79% and 39%, respectively) have been observed in the same population (Jamatia). Frequencies of homozygous "null" genotypes at the *GSTM1* and *GSTT1* loci show a significant positive correlation in these populations, which is contrary to expectations. A possible implication is that the two enzymes are working in tandem, instead of working in a complementary way.

One recognized source of variation in dose-response relationship for exposure-related diseases is inherited genetic polymorphism in disease-associated genes. Knowledge of variations in frequencies of these genes in different populations may help to explain differential responses of different populations to toxic chemicals (Nebert 1997). Most of these gene products are involved either in metabolic oxidation (phase I) or detoxification (phase II) of toxic materials. One group of phase-II enzymes, relevant to this study, consists of GSTs, which have been identified in a variety of human tissues. These enzymes are under the control of at least five gene families (namely, *GSTM, GSTT, GSTP, GSTA,* and microsomal *GST*). GSTs are found in virtually all eukaryotes and may have evolved to provide protection to the organism against toxic substances present in food and the environment (Nebert 1997). Human *GSTM1* and *GSTT1* are two such genes whose products can modify endogenous/exogenous toxic substrates to less reactive species. The mechanism involves binding of glutathione to the insoluble

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electrophilic substrates and rendering them into soluble form. As a result, these modified toxic materials are excreted from the body (Perera 1997).

One polymorphism that has been reported in both *GSTM1* and *GSTT1* genes is a deletion of a segment of DNA that results in the absence of protein synthesis (Seidegard et al. 1988). As a result of deletion mutation/s at either or both of the loci and, consequently, less detoxification of xenobiotic toxic substances, an individual may become susceptible to diseases produced by toxic substances present in the environment. *GSTM1* "null" or "deletion" genotype is present in about 50% of Caucasians, 33% of African Americans and 45% of Japanese (Nelson et al. 1995). In a study on three Indian ethnic populations, we reported that between 20% and 28% of the population carries *GSTM1* "null" genotype (Roy et al. 1998). This "null" genotype has been shown to increase susceptibility of an individual to smoking-related bladder, breast, and lung cancers (Deakin et al. 1996; Bell et al. 1993). It has been reported that *GSTT1* "null" genotype increases the risk of myelodysplastic syndromes in individuals (Chen et al. 1996a). Nelson et al. (1995) also reported that *GSTT1* "null" genotype is present in 64% of Chinese, 60% of Koreans, 28% of Caucasians, 22% of African Americans, and 10% of Mexican Americans. A report on an Indian community from Singapore by Lee et al. (1995) described higher frequencies of homozygous "null" mutations at *GSTM1* and *GSTT1* loci in Chinese populations compared to Malay and Indian populations. Chen et al. (1996) also compared frequencies of *GSTM1* and *GSTT1* "null" genotypes in American whites and blacks. Recently, Nair et al. (1999) reported high frequencies of homozygous "null" genotypes at both *GSTM1* and *GSTT1* loci in oral leukoplakia patients from an Indian cosmopolitan population. Here we report frequencies of *GSTM1* and *GSTT1* homozygous "null" genotypes in 10 ethnically and geographically diverse populations of India. These data may be useful for explaining ethnic variations in the prevalence of diseases resulting from exposures to toxic substances.

Materials and Methods

Populations Studied. We studied 299 unrelated individuals belonging to 10 endogamous population groups of India. Of these populations, three are tribal groups speaking Austro-Asiatic (two groups) or Tibeto-Burman (one group) languages; six are caste groups at different levels of the social hierarchy (upper, middle, and lower) speaking Indo-European languages; and one is an Islamic religious group of Indo-European-speaking Muslims. With the exception of the tribal Lodha, all of the populations are numerically large (>100,000). The Lodhas number between 25,000 and 30,000. There is no custom of preferential matings between relatives among the tribal or caste groups included in this study. However, the Muslims practice cousin marriages. The populations are primarily endogamous; the extent of interpopulation marriages per generation is negligibly small. It may, however, be mentioned that, although insignificant, there are occasional marriages across caste barriers; sometimes females of lower caste groups marry

Table 1. Names of Study Populations, Sampling Locations, and Ethnolinguistic Details

Population	*Location of Sampling*	*Ethnicity and Language*
Bagdi	East India, West Bengal	Hindu low caste; Indo-European, Bengali
Mahishya	East India, West Bengal	Hindu middle caste; Indo-European, Bengali
Brahmin–West Bengal	East India, West Bengal	Hindu upper caste; Indo-European, Bengali
Lodha	East India, West Bengal	Tribe; Austro-Asiatic, Lodha
Santal	East India, West Bengal	Tribe; Austro-Asiatic, Santali
Brahmin–Uttar Pradesh	North India, Uttar Pradesh	Hindu upper caste; Indo-European, Hindi
Chamar	North India, Uttar Pradesh	Hindu low caste; Indo-European, Hindi
Muslim	North India, Uttar Pradesh	Islamic religious group; Indo-European, Hindi
Rajput	North India, Uttar Pradesh	Hindu middle caste; Indo-European, Hindi
Jamatia	Northeast India, Tripura	Tribe; Tibeto-Burman, Kokborok

men of higher caste groups. The samples were drawn from three geographical locations of the states of West Bengal, Tripura, and Uttar Pradesh. Further details are given in Table 1 and Figure 1.

PCR Analysis. Blood samples (5–10 mL venous blood in EDTA) were drawn from individuals who had given informed consent. DNA was isolated using a standard protocol (Miller et al. 1988) and suspended in Tris-EDTA buffer. *GSTM1* homozygous "null" genotypes in DNA samples were detected by the absence of a polymerase chain reaction (PCR) product (630 base pairs [bp]) on a 1.5% agarose gel but in the presence of an internal control band (Comstock et al. 1990; Roy et al. 1998). Similarly, *GSTT1* homozygous "null" genotypes were detected by the absence of a 480-bp PCR product (Pemble et al. 1994) but in the presence of an internal control PCR product band.

Statistical Analyses. Binomial tests of proportions were performed to compare appropriate subsets of data. The coefficient of correlation between population frequencies of homozygous "null" genotypes at *GSTM1* and *GSTT1* loci was calculated and the regression line was estimated by the least-squares method.

To assess genomic relationships among populations, dendograms were constructed by the neighbor-joining (NJ) method (Felsenstein 1993).

Results

***GSTM1* "Null" Mutation.** The frequency of the homozygous "null" genotype shows considerable variation among the 10 populations, from 20% among the Brahmins of Uttar Pradesh to 79% among the Jamatia tribals of Tripura (Table 2).

Figure 1. Geographical map of India and locations of study populations.

We have noted interesting patterns of variation. First, the Tibeto-Burman-speaking tribals (Jamatias) show a high frequency of the homozygous "null" genotype (79%), while the Austro-Asiatic-speaking tribals (Lodha and Santal) show a much lower frequency (26%). Second, while there is little variation (37% to 48%) among caste groups belonging to different social ranks (Table 1 and Table 2) residing in eastern India, the extent of variation among comparable caste populations of northern India is much higher (20% to 78%). Third, caste populations belonging to the same social rank but residing in different geographical locations, such as the Brahmins of Uttar Pradesh and the Brahmins of West Bengal, show very different frequencies (20% and 40%, respectively).

The Tibeto-Burman-speaking Jamatias show statistically significant differ-

Table 2. Frequencies of Homozygous "Null" Individuals in Populations

Populations (n)	*GSTM1 "Null" (%) ± S.D.*	*GSTT1 "Null" (%) ± S.D.*
Bagdi (30)	37 ± 9	3 ± 3
Mahishya (33)	48 ± 9	13 ± 6
Brahmin–West Bengal (20)	40 ± 11	11 ± 7
Lodha (31)	26 ± 8	6 ± 4
Santal (23)	26 ± 9	9 ± 6
Brahmin–Uttar Pradesh (25)	20 ± 8	19 ± 8
Chamar (23)	78 ± 9	25 ± 9
Muslim (28)	36 ± 9	14 ± 7
Rajput (48)	44 ± 7	12 ± 5
Jamatia (38)	79 ± 7	39 ± 8

ences in the frequency of the homozygous "null" genotype at the 1% level with caste groups of both northern and eastern regions, as also with the other tribal groups. Similarly, Jamatias show significant differences (at the 1% level) with pooled Indo-European speakers. The Austro-Asiatic-speaking tribal groups (Santal and Lodha) show statistically significant differences at the 5% level with the pooled Indo-European speakers and caste groups of eastern India. The difference in proportions of the *GSTM1* "null" homozygotes is not significant between the northern and eastern Indian caste groups.

***GSTT1* "Null" Mutation.** The frequencies of the homozygous "null" genotype at this locus also show considerable variation among these populations (Table 2); from 3% among the low-caste Bagdi of West Bengal to 39% among the Jamatias of Tripura. Patterns of variation reveal several interesting features. First, the Tibeto-Burman-speaking Jamatias show the highest frequency (39%) of the homozygous "null" genotype and the Austro-Asiatic-speaking Lodhas and Santals show much lower frequencies (6% and 9%, respectively). Second, there is more variation among caste groups of eastern India (from 3% to 13%) compared to the caste populations of northern India (from 12% to 25%). Third, caste populations belonging to the same social rank, such as Bagdi of West Bengal and Chamar of Uttar Pradesh, show very different frequencies (3% and 25%, respectively).

The pattern of statistical significance of the differences in proportions of *GSTT1* "null" homozygotes among the Indo-European and tribal groups is exactly the same as that for the *GSTM1* "null" proportions, except that the difference between Indo-European and Austro-Asiatic groups is not significant.

Correlation between Population Frequencies. Although the number of populations is small, we noted that population frequencies of *GSTM1* and *GSTT1*

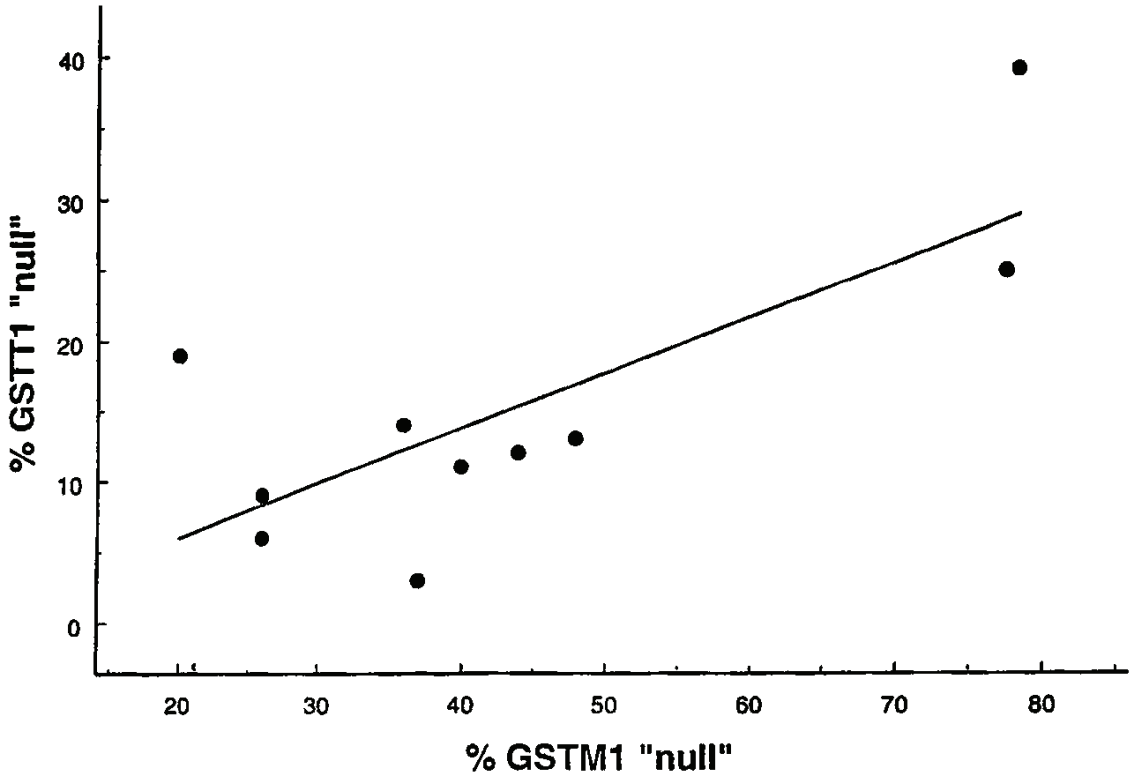

Figure 2. Correlation between frequencies of *GSTM1* and *GSTT1* "null" genotypes in populations.

"null" homozygotes are positively correlated ($r = 0.76$, $p = 0.01$). The scatter diagram of the population frequencies and the least-squares regression line are shown in Figure 2. The estimated regression equation is *GSTT1* "null" homozygote frequency (%) = –1.751 + 0.388 *GSTM1* "null" homozygote frequency (%).

Single-Linkage Cluster Analysis. Mainly, four clusters were obtained on the basis of *GSTM1* and *GSTT1* data. Austro-Asiatic-speaking tribals (Lodha and Santal) sampled from the same geographical location are grouped together. Jamatia (Mongoloid tribal) and Chamar (low-caste Hindu) were sampled from distant geographical locations, but they are clustered together. Brahmin–West Bengal do not group with Brahmin–Uttar Pradesh, who are of the same social rank but were sampled from different geographical habitats, but rather are grouped with other castes from the same locations and with Rajput and Muslim from different geographical locations.

Discussion

Considerable variations were observed in the frequencies of the homozygous "null" genotypes at the *GSTM1* and *GSTT1* loci among 10 ethnic populations of various linguistic, social, and geographical backgrounds of India (Table

1). This may be in part due to their differing evolutionary histories and in part to differential selection arising from differing exposures to toxic substances, such as diet and tobacco and alcohol consumption. Our finding that the Mongoloid tribal Jamatias have high frequencies of "null" homozygotes is consistent with an earlier report on other Mongoloid populations, such as Japanese, Chinese, and Korean (Nelson et al. 1995; Lee et al. 1995). This feature of Mongoloid populations is surely a reflection of their common ancestral backgrounds. Similarly, the near-equal frequencies of these mutations among Austro-Asiatic-speaking tribals (Lodha and Santal) are also probably a reflection of their common ancestry. On the other hand, the various populations of Uttar Pradesh with differing social ranks have quite dissimilar frequencies, but those of West Bengal have similar frequencies (with the exception of the Bagdi population for the *GSTT1* "null" frequency).

The reason for high frequencies of homozygous "null" mutations at these two loci in the Chamar population is not clear to us at present. Unfortunately, data on the prevalence of various diseases, particularly those that are related to exposure to toxic substances, in these populations are unavailable. Therefore, it is difficult to ascertain either the causes of ethnic variation in the frequencies of these "null" mutations, or the implications of this variation on epidemiological profiles of diseases. Cluster compositions based on the allele-frequency data at the two GST loci among the populations have been compared with those obtained from same individuals (Majumder et al. 1999), based on the frequencies of eight "neutral" insertion/deletion (Indel) polymorphic markers. Four clusters obtained on the basis of data on GST loci were: (Lodha, Santal), (Brahmin–West Bengal, Rajput, Mahishya, Muslim, Bagdi), (Brahmin–Uttar Pradesh), and (Chamar, Jamatia). The clusters that were obtained on the basis of Indel markers were (Majumder et al. 1999): (Brahmin–West Bengal, Mahishya, Bagdi), (Brahmin–Uttar Pradesh, Muslim), (Rajput), (Santal), (Chamar), and (Lodha). Jamatia was not included in the Indel polymorphism study. The above results show that the observed patterns of variation in frequencies of homozygous "null" genotypes at the *GSTM1* and *GSTT1* loci among the study populations are explained only partially by the population structure. This implies that in addition to ancestral histories of the study populations, the "null" genotype frequencies may have been modulated by natural selection.

The significant positive correlation, observed between population frequencies of homozygous *GSTM1* and *GSTT1* "null" genotypes, is both interesting and intriguing. Both are detoxification enzymes and are located on different chromosomes: *GSTM1* on chromosome 1 (Pearson et al. 1993) and *GSTT1* on chromosome 22 (Tan et al. 1995). Assuming redundancy in their functions, one would have expected an inverse relationship in the frequencies of "null" mutations at these two loci. But our finding of a positive correlation raises an interesting possibility that the two enzymes are working in tandem, instead of working in a complementary way. It will be worthwhile to look for the endogenous and/or exogenous factors that could produce positive correlation between these loci.

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