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SHORT COMMUNICATION

Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers

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Allelism in glutathione S-transferase GSTM1 and GSTT1 has been suggested as a risk factor in various cancers. Accordingly, we describe a group of case-control studies carried out to identify associations between GSTT1 genotypes and susceptibility to lung, oral, gastric and colorectal cancers. The frequencies of the putatively high risk GSTT1 null genotype were not increased in the lung, oral or gastric cancer cases compared with controls but the frequency of this genotype was significantly increased ($P = 0.0011$, odds ratio = 1.88) in the colorectal cancer cases. No significant interactions between the GSTT1 and GSTM1 null genotypes were identified in the cancer groups studied. Indeed, no significant associations between GSTM1 genotypes and susceptibility were identified though further evidence was obtained that the protective effect of GSTM1*A and GSTM1*B is not equal. The data complement studies showing that GSTT1 null is associated with an increased susceptibility to total ulcerative colitis and suggests that this enzyme is important in the detoxification of unidentified xenobiotics in the large intestine.

Introduction

Accumulating evidence indicates susceptibility to cancer is mediated by genetically determined differences in the effectiveness of detoxification of potential carcinogens (1,2). Thus, allelism at the glutathione S-transferase (GST*) GSTM1 locus is associated with an altered risk of certain cancers. Three alleles, GSTM1*0, GSTM1*A and GSTM1*B are identified. GSTM1*0 is deleted and homozygotes (GSTM1 null) express no protein and appear at increased risk, presumably because GSTM1 catalyses the detoxification of genotoxins including hydrocarbon epoxides and products of oxidative stress such as DNA hydroperoxides (1–4). However, while GSTM1 appears a useful candidate, data in several pathologies including lung cancer are conflicting (1,5–10). The reason for these discrepancies is unclear but may reflect the significant but relatively small effect of any genotype on risk. This effect may be masked by interactions with confounding factors such as other polymorphic loci encoding detoxifying enzymes. Thus, Japanese homozygotes for GSTM1*0 and a rare CYP1A1 allele have an enhanced risk of lung cancer (7).

The theta class, GSTT1 locus is relevant in this context as homozygosity for a null allele is common (2,11). GSTT1 utilizes potential carcinogens including constituents of cigarette smoke such as alkyl halides (11,12). Further, like GSTM1, the rat homologue of GSTT1, has activity towards epoxides, suggesting individuals null at both GSTM1 and GSTT1 may be at particularly high risk of cancer. While *in vitro* data suggest GSTT1 is a candidate, there are few sets of data on GSTT1 genotypes in patient groups, though Chenevix-Trench *et al.* (13) found the frequency of the null genotype was increased in patients diagnosed before 70 years of age with colorectal cancer, and we showed GSTT1 null was associated with an increased susceptibility to ulcerative colitis (14). Accordingly, we describe further case control studies to determine if GSTT1 influences susceptibility to lung, oral, gastric and colorectal cancers. Interactions between GSTT1 and GSTM1 were also studied. These tumours were selected because they occur in tissues exposed to environmental carcinogens though the importance of factors such as cigarette smoking differs.

GSTT1 and GSTM1 genotypes were studied in lymphocyte DNA from unrelated cases and controls recruited with ethical approval in the North Staffordshire Hospital between 1990–1994. Only English Caucasians who could give informed consent were studied. The case groups comprised 40 patients with squamous cell cancer of the mouth (28 men, 12 women; mean age 64 years), 108 patients with lung cancer (79 men, 29 women; mean age 68 years), 136 patients with gastric cancer (96 men, 40 women; mean age 68 years) and 252 patients with colorectal cancers (129 men, 123 women; mean age 66 years). All lung cancer patients were cigarette smokers (mean pack years 55, range 5–162). Pack years is the number of years of smoking 20 cigarettes/day. Two control groups were recruited; firstly, 129 patients with obstructive lung disease (87 men, 42 women; mean age 62 years). All were cigarette smokers (mean pack years 46, range 5–150). The second control group comprised 577 patients (277 men, 300 female; mean age 70 years) of whom 283 (49%) had never smoked, 138 (24%) were current smokers and 156 (27%) were ex-smokers. 30% of these controls suffered a variety of non-malignant diseases including varicose veins, hernias, haemorrhoids, mild iron deficiency anaemia, mild hyperlipidaemia and benign ovarian cysts. The remainder suffered tension headaches (~25%), benign skin papillomas (~20%), benign breast lumps

*Abbreviations: GST, glutathione S-transferase; PCR, polymerase chain reaction.

Table I. GSTT1 and GSTM1 genotype frequencies in controls and patients with lung, oral and gastric cancers

	Lung controls	Lung cases	General controls	Oral cases	Gastric cases
GSTT1 pos	111 (86.0%)	91 (84.3%)	415 (81.5%)	30 (88.2%)	93 (81.6%)
GSTT1 null	18 (14.0%)	17 (15.7%)	94 (18.5%)	4 (11.8%)	21 (18.4%)
	129	108	509	34	114
GSTM1 A	40 (31.3%)	32 (30.2%)	162 (28.1%)	8 (20.0%)	34 (25.0%)
GSTM1 B	12 (9.4%)	20 (18.9%)	76 (13.2%)	7 (17.5%)	25 (18.4%)
GSTM1 A/B	6 (4.7%)	4 (3.8%)	23 (4.0%)	3 (7.5%)	5 (3.7%)
GSTM1 null	70 (54.7%)	50 (47.2%)	316 (54.8%)	22 (55.0%)	72 (52.9%)
	128	106	577	40	136

Table II. (a) GSTT1 and GSTM1 genotype frequencies related to site of colorectal cancer

	Colorectal	Right	Left	Rectum
GSTT1 pos	148 (70.1%)	53 (74.6%)	36 (65.5%)	52 (70.3%)
GSTT1 null	63 (29.9%)	18 (25.4%)	19 (34.5%)	22 (29.7%)
	211	71	55	74
GSTM1 A	62 (24.6%)	21 (25.6%)	16 (25.0%)	20 (21.3%)
GSTM1 B	42 (16.7%)	15 (18.3%)	10 (15.6%)	14 (14.9%)
GSTM1 A/B	13 (5.2%)	6 (7.3%)	2 (3.1%)	5 (5.3%)
GSTM1 null	135 (53.6%)	40 (48.8%)	36 (56.3%)	55 (58.5%)
	252	82	64	94

Table II. (b) Interactions between GSTT1 and GSTM1 null genotypes in patients with colorectal cancer

	M1/T1 null	M1 null	T1 null	Neither
Controls	42 (9.4%)	207 (46.2%)	37 (8.3%)	162 (36.1%)
Colorectal cases[a]	26 (11.9%)	89 (40.8%)	38 (17.4%)[b]	65 (29.8%)

[a]Frequency distribution in colorectal cases and controls; $\chi^2_3 = 14.58$, Yates corrected $P = 0.0022$.
[b]Frequency of GSTT1 null only in colorectal cases and controls; $\chi^2_1 = 11.44$, odds ratio = 2.35, 95% confidence interval 1.40, 3.92, $P = 0.0072$.

(~5%) and cerebrovascular accidents (~20%). Patients with inflammatory pathologies such as ulcerative colitis, diabetes or asthma were excluded. The GSTM1 null, A, B and A/B genotypes were identified using an amplification refractory mutation system-based polymerase chain reaction method (PCR*) with primer sets to intron 6/exon 7 and exon 4/exon 5. The assay identifies GSTM1*0 homozygotes, GSTM1*A/GSTM1*B heterozygotes and subjects with the GSTM1 A and GSTM1 B phenotypes. It does not distinguish GSTM1*0/GSTM1*A and GSTM1*A/GSTM1*A or the equivalent GSTM1 B phenotypes (15). GSTT1 null and expressing subjects were identified using the primer set and reaction conditions described (11,15). χ^2 tests were used to examine for homogeneity between cases and controls. As some genotype frequencies were small, the StatXact-Turbo statistical package was used to obtain exact *P*-values. Corrected values were used. The influence on susceptibility of the combined GSTT1 null/GSTM1 null genotypes was studied by comparing frequency distributions over mutually exclusive categories (15). This allows identification of factors (alone and in combination) that contribute most to observed differences between cases and controls.

Frequencies of GSTT1 genotypes in the lung cancer cases were not different to those in lung or general controls (Table I). All the lung cases and controls were smokers with similar mean pack years in these groups (54.6 ± 27.7 SD and 46.0 ± 26.0 SD, respectively). Mean pack years were also similar in the lung cancer cases with the GSTT1 null and those with expressing genotypes at this locus (57.13 ± 25.7 SD and 54.21 ± 28.1 SD respectively) indicating GSTT1 genotype does not influence smoking behaviour. The frequency of GSTT1 null in 52 patients with squamous cell cancer of the lung (17.3%) was also similar to that in the lung controls. The numbers of patients with other types of lung cancer (19 patients undifferentiated; 27 patients large cell; 10 patients adenocarcinoma) were too small to analyse. Frequency distributions of GSTM1 genotypes in lung cases and controls were not significantly different, though the frequency of GSTM1 B was lower in lung controls than cases; $\chi^2_1 = 4.426$; exact $P = 0.0378$. Interactive effects between GSTT1 null and GSTM1 null were studied by comparing multinomial frequency distributions over mutually exclusive categories. Frequency distributions in the general or lung controls were not significantly different (data not shown).

Table I shows GSTT1 genotype frequencies in patients with squamous cell cancer of the mouth. The frequencies of both GSTT1 and GSTM1 genotypes in these cases and the general controls were not different. No significant interactions between GSTT1 null and GSTM1 null were identified (data not shown). Frequencies of GSTT1 and GSTM1 genotypes in gastric cancer cases and controls were also not significantly different (Table I). GSTT1 and GSTM1 genotype frequencies were similar in 42 moderately differentiated and 63 poorly differentiated tumours (data not shown). The number of patients with well differentiated gastric tumours ($n = 6$) was too small to allow analysis. No significant interactions between GSTT1 null and GSTM1 null were identified.

Table II shows the frequencies of GSTT1 and GSTM1

genotypes in colorectal cancer patients. The frequency of GSTT1 null was significantly increased in these cases compared with the general controls shown in Table I ($\chi^2_1 = 10.69$; $P = 0.0011$; odds ratio = 1.88, 95% confidence interval 1.28, 2.77). The frequency distribution of GSTM1 genotypes in the controls was not different to that in the colorectal cancer group. Within this case group, distributions of GSTT1 and GSTM1 genotypes in males and females and 87 patients <65 years and 147 patients >65 years were not significantly different (data not shown). Table II also shows GSTT1 and GSTM1 genotypes in patients with tumours in the right and left colon, and rectum. Inspection of the data indicates no obvious trend in GSTT1 null frequencies ($\chi^2_2 = 1.262$; $P = 0.5319$). GSTT1 and GSTM1 genotype frequencies were not significantly different in patients grouped on the basis of the Duke stage (A 27 patients, B 117 patients, C 62 patients or D 27 patients) or tumour differentiation (well differentiated 31 patients, moderately differentiated 167 patients, poorly differentiated 34 patients) (data not shown). Though there were significant differences in the frequency distributions of the combination GSTT1 null/GSTM1 null (Table II) in colorectal cases and controls, these resulted from the increased frequency of GSTT1 null rather than interactions between this genotype and GSTM1 null. Logistic regression was used to select the best set of predictors (GSTM1 null, GSTT1 null, smoking) for susceptibility to colon cancer. A step up routine identified GSTT1 null as the only significant predictor.

We have described further studies into the association of GST allelism with susceptibility to epithelial cancers. While the influence of GSTM1 on susceptibility to lung and gastrointestinal cancers has been evaluated in a number of published studies, some data are conflicting and the significance of the polymorphism remains unclear. The role of GSTT1 and the possibility that it interacts with GSTM1 to mediate susceptibility is also unclear. We did not identify associations between the null genotypes at these loci and risk of lung, oral or gastric cancers. Thus, while we report a significant association between GSTT1 null and susceptibility to colorectal cancer, there appeared to be no significant interaction with GSTM1 null.

The influence of GSTM1 null on risk of lung cancer remains unclear. Initial studies (5) reported increased frequency (63.4%) of GSTM1 null in smokers with lung cancer (particularly adenocarcinoma) compared with controls (41.7%). These data were not supported by studies showing similar frequencies of GSTM1 null in controls and cases; a negative correlation with adenocarcinoma; and a positive association between GSTM1 null and squamous cell cancer (6). Other studies have not found an association with GSTM1 null though recent meta analysis indicates that the genotype confers a small increased risk (16). Our data show no influence of either GST on risk of this cancer. Various factors including exposure and diet will influence the importance of allelism. Indeed, recent studies suggest the effect of GSTM1 null may be more evident in patients who have smoked less than 35 pack years (17) though we found no such relationship. Dietary factors are also likely to be critical because of their effect on DNA damage, mutation and repair. Thus, the influence of GSTM1 null on risk of squamous cell cancer of lung appears enhanced by low intake of vitamin C and other nutrients (18). These data will be important when comparing results from different centres as vitamin intake varies within- and between-countries, with North Staffordshire, UK, recording low intake of vitamin C and carotene (19). The relative levels of protection conferred by GSTM1*A and GSTM1*B is also uncertain; frequencies of GSTM1 B were different in lung cases and controls. The mechanism for this effect is unclear but may be related to recent studies showing firstly, GSTM1*A is in linkage with GSTM3*B, a polymorphic variant at the mu class GSTM3 locus that contains a recognition motif for the YY1 transcription factor (20) and secondly, that expression of GSTM3 in lung is dependent on GSTM1 phenotype (21).

Allelism at GSTM1 and GSTT1 might also be expected to mediate risk of gastrointestinal cancers as substrates including products of inflammation and constituents of cooked meat are implicated as causative factors. Indeed, several studies indicate GSTM1 null confers increased risk. Thus, in a pilot study we found increased frequency of GSTM1 null (67.0%) in 45 colorectal and gastric cancer patients (22). A significantly increased frequency of GSTM1 null (73.7%) was also found in 19 Japanese suffering gastric cancer (23) and 196 British patients with colon cancer (24). However, while significantly increased above the frequency in their controls (41.8%), the genotype frequency in cases (56.1%) found by Zhong *et al.* (24) was similar to that in this study. Further, unlike the present study and other recent reports (13,25), Zhong *et al.* (24) found higher frequencies of GSTM1 null in the proximal compared with distal colon. Allelism at other loci may influence the effect of GSTM1; thus, we found an increased frequency of N-acetyltransferase NAT1*10 alleles in stomach and colorectal cancer patients (26,27).

While the role of GSTM1 in mediating cancer risk has been actively studied, there is less information on GSTT1. Our data show GSTT1 null influences risk of colorectal cancer but not Dukes grade and prognosis. The mechanism is unclear, though our studies showing that the genotype is associated with increased risk of ulcerative colitis, suggest the importance of the gene in the large bowel in the detoxification of unidentified xenobiotics (14). Interestingly, in view of the ability of GSTT1 to metabolise halogenated organic compounds, epidemiological studies suggest an association between colorectal cancer and consumption of chlorination by-products (28). Thus, chloroform and other halogenated compounds are potential contaminants of water. However, because some halogenated hydrocarbons may be detoxified via GSTT1 mediated conjugation while others are activated (12), detailed chemical-specific exposure data are needed to show such a gene-environment interaction.

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