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Polymorphism of glutathione S-transferase M3: interaction with glutathione S-transferase M1 and lung cancer susceptibility

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GSTM3 is one of five mu-class genes (M1–M5) belonging to a cluster located in chromosome 1. *GSTM3* has been found to be polymorphic in humans with a number of individuals presenting a 3 bp deletion within intron 6 (*GSTM3*B*). In this study we have addressed the possible role of the *GSTM3* polymorphism on lung cancer susceptibility. *GSTM3* was genotyped in a group of lung cancer patients ($n = 176$) and in a control group of healthy smokers ($n = 175$). The frequency distribution of *GSTM3*A*/*GSTM3*A*, *GSTM3*A*/*GSTM3*B* and *GSTM3*B*/*GSTM3*B* showed no significant differences between patients and controls. Allelism at *GSTM3* was also analysed in combination with the *GSTM1* polymorphism. The χ^2 analysis confirmed that *GSTM3*B* allele is in linkage desequilibrium with *GSTM1*A*. The over-representation of *GSTM1* null detected in previous studies, appeared to be restricted to those individuals with both *GSTM1* null and *GSTM3*A*/*GSTM3*A* (48.3 % in patients versus 36.0 % in controls). The application of a second order logistic regression model revealed a significant adjusted odds ratio for the interaction term between *GSTM1* null and *GSTM3*A*/*GSTM3*A* (OR: 2.14 95% CI 1.08–4.25) suggesting that this combined genotype may increase lung cancer risk. The analysis of transcription factor binding sites near the deleted sequence suggests that the heat-shock transcription factor 1 (HSTF1) could be involved in an enhanced expression of *GSTM3*B*, thus providing a possible mechanistic basis for a protective role of this allele.

Keywords: *GSTM3*, *GSTM1*, lung cancer, susceptibility.

Introduction

The study of cancer risk as a consequence of a genetic predisposition and the interaction between genotype and environmental carcinogens is rapidly evolving as a major scientific issue (Gonzalez 1995). Biomarkers of susceptibility and earlier genotoxic events are increasingly used in molecular epidemiology studies to assess cancer risk among exposed or general populations. Within this field, a number of studies are focused on polymorphic genes involved in the modulation of bioactivation/detoxification reactions (Miller *et al.* 1997). Since most of the well known carcinogens require metabolic activation previous to the formation of adducts in DNA hotspots (Denissenko *et al.* 1996) the genes encoding for phase I and II isoenzymes may regulate the amount of reactive intermediates finally reaching and binding to DNA. The glutathione S-transferases (GST; EC 2.5.1.18) constitute a major family of isoenzymes involved in detoxification of reactive

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intermediates (Hayes and Pulford 1995). Substrates of GSTs include tobacco-derived electrophiles, epoxides and products of oxidative stress and most of the GSTs show a broad cellular and tissue distribution. Microsomal and cytosolic forms are known to exist in mammals, with the different cytosolic isoenzymes being currently assigned to four major classes designated α, μ, π, θ (Mannervik *et al.* 1992). Further classes (σ,κ, ζ) have been recently identified (Pemble *et al.* 1996, Blackburn *et al.* 1998) The genes encoding for GSTs are distributed in the human genome: *GSTM* genes encoding for GST μ isoenzymes in 1p13; GSTA (α isoenzymes) in 6p12; GSTP (π) in 11q3; and GSTT (θ) in 22q 11.2

Several of the *GST* genes are polymorphic in humans and are currently being investigated as possible cancer risk modifiers. Two of the *GST* genes (*GSTM1* and *GSTT1*) are frequently deleted in human populations. About 50% of individuals in Caucasian populations show an homozygous *GSTM1* deletion and about 15–20 % an homozygous *GSTT1* deletion. Several groups have studied whether the individuals presenting a homozygous deletion of one or two *GST* genes are at an increased risk of developing lung, bladder, skin or colorectal cancer (London *et al.* 1995, McWilliams *et al.* 1995, d'Errico *et al.* 1996, To-Figueras *et al.* 1997) . With a major world-wide meta-analysis going on, the present status of *GSTM1* and *GSTT1* deletions as cancer risk modifiers is still controversial. Other polymorphisms in the same family (*GSTP1*A/B*) have been studied, also with conflicting results (Ryberg *et al.* 1997, Harris *et al.* 1998).

GSTM3 is one of the five mu-class genes (M1–M5) on chromosome 1 (Pearson *et al.* 1993) and has also been found to be polymorphic with a number of individuals presenting a three-base deletion in intron 6 (Inskip *et al.* 1995). Since this mutation may generate a recognition sequence and *GSTM3* has been found to be variably expressed in human lung, the possibility has been raised that this gene may also play a role in lung cancer susceptibility (Anttila *et al.* 1995, Hand *et al.* 1996, Yengi *et al.* 1996, Jahnke *et al.* 1997, Saarikoski *et al.* 1998, Strange *et al.* 1998).

In this study, we genotyped the *GSTM3* polymorphism in two groups of North-West Mediterranean Caucasians: (a) a group of lung cancer patients and (b) a group of healthy volunteers of similar age, gender and smoking history. The results have been studied in relation to other *GSTM* polymorphisms genotyped in the same population.

Material and methods

The study involved 176 patients with a diagnosed bronchogenic carcinoma and 175 healthy volunteers with a known smoking history. Some of the patients ($n = 160$) and some of the healthy volunteers ($n = 120$) had participated in a previous genotyping study of *GSTM1* and *GSTT*. Some newly diagnosed lung cancer patients and some new healthy smokers were added to the present study. The lung cancer group were patients consecutively diagnosed at the 'Hospital Clínic' of Barcelona (Spain). They were 161 men and 15 women with a mean age of 60 years (range 32–87). The criteria for inclusion in the case group were: (a) North-West Mediterranean Caucasians (Catalonia) as judged by their names and places of birth; (b) residence in the area of Barcelona (minimum 10 years); (c) available clinical history including unequivocal histological diagnostic of lung cancer. The distribution of histological cancer types were as follows: 50 squamous-cell carcinoma, 56 small-cell carcinoma, 42 adenocarcinoma and 12 large cell carcinoma. The patients were interviewed for a detailed occupational and smoking history, dietary and drinking habits, and cancer in family members. Pack-years (PY) were calculated as usual from daily cigarette consumption and the number of years of smoking (1 PY = daily consumption of 20 cigarettes for 1 year). Mean PY in the group was 55 (range 0–170). The control group comprised healthy current smokers who fitted criteria (a) and (b) of the case group. They were selected to match as close as possible the gender and age distribution of the case group. The group

finally selected comprised 143 men and 32 women with a mean age of 50 years (range 28–82); mean PY was 45 (range: 7–196). They were interviewed as the case group and those presenting any diagnosed pathology were excluded. In all cases, patients and healthy controls were informed about the objectives of the study and they submitted a written consent for inclusion in the protocol, blood extraction and DNA genotyping. The whole study design was approved by the ethical committees (Hospital Clínic, IDIBAPS, UB).

Analysis of polymorphisms

DNA samples were extracted from fresh peripheral leukocytes using phenol–chloroform–isoamyl-alcohol. The polymorphic site in *GSTM3* locus was performed by restriction fragment length polymorphism (RFLP) of polymerase chain reaction (PCR) amplified fragments. Hot start PCR reactions were carried out in a 30 ml volume containing about 100 ng genomic DNA template, 200 mM each dNTP, 30 pmol of each primer, 50 mM KCl, 10 mM Tris–HCl pH 8.3 at 1.5 mM $MgCl_2$, and 0.6 units AmpliTaq Gold™ polymerase. *GSTM3* locus amplification was achieved using oligonucleotides, as described by Inskip *et al.* (1995) : 5´-CCT CAG TAC TTG GAA GAG CT-3´, and 5´-CAC ATG AAA GCC TTC AGG TT-3´ . After 12 min at 93 ºC (hot start and AmpliTaq Gold™ polymerase activation), PCR reactions were processed through 39 temperature cycles of 50 s at 94 ºC (denaturing step), 40 s at 59 ºC (annealing step), and 50 s at 72 ºC (extension step). The last elongation step was extended to 5 min. All reactions were performed in a PTC-200 MJResearch Thermocycler. Negative (without DNA) and positive control samples were included in each amplification series. The PCR product was purified and concentrated by Ultrafree®-MC Centrifugal (Millipore™) filter units. A 10 ml aliquot of the PCR purified product was digested with 3 units *MnlI* (5´...CCTC(N)7↓...3´ ; 3´...GGAG(N)6↑...5´) at 37 ºC overnight (about 18 h).The detection of the different alleles was carried out routinely by horizontal submarine ethidium bromide 3% NuSieve 3:1 FMC™ agarose gel electrophoresis, along with a 100-bp ladder. Control sample genotypes were kindly provided by Drs J Alldersea and RC Strange (North Staffordshire Hospital, UK). *GSTM3*A*/*GSTM3*A* homozygotes presented the expected 11, 51, 86 and 125 bp fragments. The *GSTM3*A*/ *GSTM3*B* pattern demonstrated the additional 134 bp fragment, and the *GSTM3*B*/*GSTM3*B* homozygotes gave the expected 11, 125, and 134 bp fragments. Additional *GSTM1* genotyping was carried out as previously described (To-Figueras *et al.*1997).

Intron sequence analysis

The intron sequence for both alleles, *GSTM3*A* and *GSTM3*B*, have been screened for putative targets recognized by transcription factors. The software TESS, created by CBIL (Computational Biology and Informatics Laboratory) of the University of Pennsylvania has been used (Schug and Overton 1987). This public and free application searches a given nucleic acid sequence for potential transcription factors binding sites from the TRANSFAC database (http://www.cbil.upenn.edu).

Statistical analysis

A χ^2 test was used to compare the frequency distribution of *GSTM3* and *GSTM1* alleles between patients and controls. χ^2 tests were also used in order to examine the independence of the genotype distributions of both GST loci. Because some genotype frequencies were small, the exact *P* values for the Pearson's *R* statistic were also calculated. The influence of *GSTM1* and *GSTM3* genotypes on cancer susceptibility was determined as adjusted odds ratios by logistic regression analysis. Gender, age and smoking status (quantified as pack-years) were included as variables in the regression model. Age and pack-years were used as numerical variables. The possible interaction between *GSTM1* and *GSTM3* was studied carrying out different logistic regression models: (a) introducing *GSTM1* or *GSTM3* alone, as variables; (b) introducing both genes simultaneously; (c) including an interaction between them (second order regression model). The odds ratio for the interaction variable can be interpreted as the risk associated wiith the simultaneous presence of risk genotypes in both loci. The calculations were made using the Statgraphics Plus software.

Results

The frequencies of the different *GSTM3* genotypes among patients and healthy controls are shown in table 1 and fitted the Hardy–Weinberg equilibrium ($\chi^2 = 0.04$; $P = 0.98$). The comparison showed no significant statistical differences between patients and controls ($\chi^2 = 1.76$; $P = 0.41$). Frequencies (calculated) of *GSTM1* alleles among the controls were as follows: *GSTM1**0: 0.708;

Table 1. Frequency distribution of GSTM3 genotypes and alleles in healthy controls and lung cancer patients.

	*GSTM3*AA*	*GSTM3*AB*	*GSTM3*BB*	*GSTM3*A*	*GSTM3*B*
Healthy controls	114 (65.1%)	55 (31.4%)	6 (3.4%)	283 (80.9%)	67 (19.1%)
Cancer patients	124 (70.5%)	49 (27.8%)	3 (1.7%)	297 (84.4%)	55 (15.6%)

Table 2. Frequency distribution of *GSTM1* and *GSTM3* genotypes in healthy controls and lung cancer patients. Expected values calculated assuming no linkage between them are shown in parentheses.

	GSTM1 null	*GSTM1 A*	*GSTM1 B*	*GSTM1 A,B*
Healthy controls				
*GSTM3*AA*	63 (56.7)	24 (34.5)	26 (18.9)	1 (3.9)
*GSTM3*AB*	21 (27.3)	26 (16.7)	3 (9.1)	5 (1.9)
*GSTM3*BB*	3 (3.0)	3 (1.8)	0 (1.0)	0 (0.2)
Lung cancer patients				
*GSTM3*AA*	85 (72.6)	21 (36.6)	17 (13.4)	1 (1.4)
*GSTM3*AB*	18 (28.7)	28 (14.5)	2 (5.3)	1 (0.6)
*GSTM3*BB*	0 (1.8)	3 (0.9)	0 (0.3)	0 (0.0)

*GSTM1*A*: 0.186; *GSTM1*B*: 0.106. The genotype frequencies were in Hardy–Weinberg equilibrium ($\chi^2 = 0.13$; $P = 0.99$). The comparison showed no significant statistical differences between patients and controls ($\chi^2 = 5.44$; $P = 0.14$).

The *GSTM3* genotype frequencies were analysed in combination with *GSTM1* genotypes (table 2). χ^2 analysis revealed a strong association between *GSTM1* and *GSTM3* both in the patients ($\chi^2 = 36.06$; 6 d.f.; $P<0.0001$) and in the controls ($\chi^2 = 26.7$; 6 d.f. ; $P = 0.0003$). Since the *p*-value is less than 0.01, the hypothesis that rows and columns are independent must be rejected at the 1% significance level. Therefore, the observed value of *GSTM1* on a particular case is related to its value for *GSTM3*. The comparison with the expected values shows that among patients and controls, there is an over-representation of individuals with both a *GSTM1*A* and a *GSTM3*B* allele (patients plus controls: observed 66; expected 36.4). Therefore, *GSTM1*A* is associated with an increased frequency of *GSTM3*B* suggesting a linkage desequilibrium between both GSTM polymorphic genes. This should be confirmed by an haplotype analysis carried out in a family study.

Comparison between patients and controls in table 1 did not show statistical significant differences ($\chi^2 = 11.52$; $P = 0.24$). However, *GSTM1 null* tends to be over-represented among the patients. This tendency, detected in previous studies (To-Figueras *et al*. 1997), now appears to be restricted to those individuals with both *GSTM1 null* and *GSTM3*A/GSTM3*A* (48.3 % in patients versus 36.0 % in controls). Therefore, no increased frequency of GTSM1 *null* + *GSTM3*A/GSTM3*B* or *GSTM1 null+GSTM3*B/GSTM3*B* was found among the patients compared with the controls. Another observed tendency is found for individuals with a *GSTM1*B* allele that tends to be less frequent among the patients (19.9% in controls versus 11.9 % in patients).

The risk of lung cancer associated with several genotype combinations of *GSTM1* and/or *GSTM3* was studied by logistic regression analysis. The most relevant results are shown in table 3 as adjusted odds ratios. The *GSTM1* variable was codified in two alternative ways, considering as a risk factor either (a) *GSTM1*

Table 3. Odds ratios for different genotypes or genotype combinations. ORs were adjusted for sex, age and smoking habit (pack-years).

Risk factor	Adjusted OR	95% CI
1 *GSTM1 null*	1.31	0.82–2.10
2 *Absence of GSTM1*B*	1.64	0.86–3.13
3 *Absence of GSTM3*B*	1.24	0.75–2.0
4 *Absence of GSTM1*B*[a]	1.73	1.04–2.87
5 *Absence of GSTM3*B*[b]	1.32	0.79–2.19
6 *GSTM1null × absence of GSTM3*B*[c]	2.14	1.08–4.25

[a] Estimated including the *absence of GSTM3*B* as a variable in the regression model.
[b] Estimated including the *absence of GSTM1*B* as a variable in the regression model.
[c] Interaction term (second order regression) estimated including both individual factors in the model.

null or (b) the absence of the *GSTM1*B* allele. The *GSTM3* variable was codified assuming the *GSTM3*A/GSTM3*A* genotype (in other words, the absence of the *GSTM3*B* allele) as the risk factor. In a first approach, the effect of each marker alone was estimated. An increased risk (but not reaching significance) for *GSTM1 null* alone was observed (table 3; row 1) confirming previous observations (To-Figueras *et al.* 1997). The *absence of GSTM1*B* and the *absence of GSTM3*B* both showed, when analysed separately, a slight tendency to increase lung cancer risk (table 3, rows 2 and 3).

To take into account possible relationships between *GSTM1* and *GSTM3* loci, it was necessary to include both simultaneously in the regression model. The previous prospective analysis of results suggested two kinds of relationship: a genetic linkage between *GSTM1*A* and *GSTM3*B*, and a synergy effect of *GSTM1 null* and *absence of GSTM3*B* allele on lung cancer risk. The genetic linkage between a risk factor (as *GSTM1*A* seems to be) and a protector factor (as *GSTM3*B*) would produce an underestimation of individual odds ratios when calculated separately. In fact, the odds ratios for *absence of GSTM1*B* and *absence of GSTM3*B* increase and the confidence limits improve, when both were considered simultaneously, the first turning out to be statistically significant (table 3, rows 4 and 5). The synergetic interaction between two risk factors would produce a moderate decrease in their respective odds ratios when introduced simultaneously in the regression but a drastic decrease when including an interaction variable (second order regression model); a part of the risk would then be transferred to the interaction term. This kind of effect was observed when estimating the risk for *GSTM1 null* and *absence of GSTM3*B*: a significant odds ratio (OR = 2.14) was found for the interaction term (table 3, row 6), whereas the individual odds ratios fall down to less than 0.8 (not shown). The odds ratio of the interaction term can be interpreted as the risk associated to the simultaneous presence of the *GSTM null* genotype and *absence of the GSTM3*B* allele. Differently, no significant interaction was found between *absence of GSTM1*B* and *absence of GSTM3*B* (results not shown).

Discussion

Our results show that among North-Western Mediterranean Caucasians there appears to exist a strong linkage desequilibrium between *GSTM1*A* and *GSTM3*B*; similar to that reported previously (Inskip *et al.*1995) among English Caucasians. Since *GSTM1* and *GSTM3* belong to a cluster located in chromosome

1, the most probable explanation for this desequilibrium linkage is the low probability of a recombination event separating the alleles.

Previous results in part of the same population had found a borderline increased frequency of *GSTM1 null* among some histological subgroups of lung cancer patients (To-Figueras *et al.* 1997). After genotyping new cases and controls for *GSTM1* and all the cases and controls for *GSTM3*, it appears that the increased frequency of *GSTM1 null* is clearly restricted to those patients with both *GSTM1 null* and *GSTM3*A/A*. A significant interaction between both genes was observed with this combined genotype notably increasing the risk previously calculated for *GSTM1 null*. Therefore, *GSTM3* may play a critical role in carcinogen metabolism and lung cancer susceptibility with the allele *GSTM3*B* being protective. This is in accordance with Anttila *et al.* (1995) who found a major expression of *GSTM3* in human lung tissue and with Yengi *et al.* (1996) who found a low frecuency of *GSTM3*B* among patients with multiple cutaneous basal cell carcinoma.

The biological significance of the 3 bp deletion in the *GSTM3*B* allele is still unclear. This deletion is located within an intron sequence and introns, specially those close to the 5′ end of the gene, may contain transcription factor binding sites, acting as repressors or enhancers. Other authors (Inskip *et al.* 1995) suggested that the YY1 factor could bind to this intron sequence and affect the expression of GSTM3. The putative binding site of YY1 lies at the very beginning of this intron, at nucleotides 2–12, quite apart from the 3 bp-deletion (nucleotides 22–24 of *GSTM3*A* intron sequence). Then, it is difficult to explain how the binding of this factor could affect differently the expression of both alleles, as suggested.

Instead, an exhaustive search in the TRANSFAC database with the complete intron sequence of 88 bp, shows putative binding sites for other transcription factors. According to the matrices for the consensus binding sequence, two target sites for transcription factors stand out as having the maximum likelihood to be recognized: one by HSF1 (heat-shock transcription factor 1) and the other by NF-GMa (nuclear factor for granulocyte/macrophage colony-stimulating factor gene promoter a). Interestingly, both sites are very close to the deletion sequence.

The putative binding site for HSF1, an activator transcription factor, lies in a region surrounding the deletion (nucleotides 6–21). This factor is constitutively expressed in an inactive form and is activated by phosphorylation in response to many different stress stimuli, mainly heat, oxidizing agents and hypoxia (Mivechi *et al.* 1994; and for review, see Sorger (1991)). Recently, it has been reported that tobacco smoke induces activation of HSF (Vayssier *et al.* 1998). Remarkably, several genes activated by HSFs contain essential target sites for this factor within introns (Shen *et al.* 1997). Furthermore, this factor binds cooperatively as trimeric or pentameric sets to clustered arrays of the 3bp target site, AAG or CTT (Kroeger *et al.* 1993, Kroeger and Morimoto 1994, Wang and Morgan 1994). This binding is effectively affected by the flanking sequences. According to our data, although the four AAG target sites for HSF are maintained in both alleles, the deletion brings closer a putative fifth binding site (CTT), which could affect the affinity of the binding, therefore increasing the expression of the *GSTM3*B* allele. However, this hypothesis should be proved and whether these sites are really bound by HSF in human lung remains unknown.

The other transcription factor target site, putatively recognized by NF-GMa is generated in the *GSTM3*B* allelic variant and could be functional only in this

allele. This transcription factor acts as a transcriptional enhancer after stimulation with TNF-a (tumour necrosis factor a) (Shannon *et al*. 1988, Kuczek *et al*. 1991) and recent reports show that tobacco smoke inhibits TNF-a release (Hales *et al*. 1997, Vayssier *et al*. 1998). Therefore, a coordinate contribution of these two factors in the *GSTM3*B* protective phenotype seems highly unlikely.

In conclusion, our findings suggest that the *GSTM3*B* allele could have a protective role and that the combined genotype *GSTM3*A/GSTM3*A + GSTM1 null* may increase lung cancer risk. These preliminary results need to be confirmed with a larger number of individuals and the role of possible transcription factors within the GSTM3 alleles remain an area of further research.

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References

ANTTILA, S., LUOSTARINEN, L., HIRVONEN, A., ELOVAARA, E., KARJALAINEN, A., NURMINEN, T., HAYES, J. D., VAINIO, H. and KETTERER, B. 1995, Pulmonary expression of glutathione S-transferase M3 in lung cancer patients: association with *GSTM1* polymorphism, smoking, and asbestos exposure. *Cancer Research*, **55**, 3305–3309.

BLACKBURN, A. C., WOOLLATT, E., SUTHERLAND, G. R. and BOARD, P. G. 1998, Characterization and chromosome location of the gene GSTZ1 encoding the human Zeta class glutathione transferase and maleylacetoacetate isomerase. *Cytogenetics and Cell Genetics*, **83**, 109–114.

DENISSENKO, M. F., PAO, A., TANG, M. S. and PFEIFER, G. P. 1996, Preferential formation of benzo(*a*)pyrene adducts at lung cancer mutational hotspots in p53. *Science*, **274**, 430–432.

D'ERRICO, A., TAIOLI, E., CHEN, X. and VINEIS, P. 1996, Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers*, **1**, 149–173.

GONZALEZ, F. 1995, Genetic polymorphism and cancer susceptibility: Fourteenth Sapporo Cancer Seminar. *Cancer Research*, **55**, 710–715.

HALES, C. A., ELSASSER, T. H., OCAMPO, P. and EFIMOVA, O. 1997, TNF-alpha in smoke inhalation lung injury. *Journal of Applied Physiology*, **82**, 1433–1437.

HAND, P. A., INSKIP, A., GILFORD, J., ALLDERSEA, J., ELEXPURU-CAMIRUAGA, J., HAYES, J. D., JONES, P. W., STRANGE, R. C. and FRYER, A. A. 1996, Allelism at the glutathione S-transferase *GSTM3* locus: interactions with *GSTM1* and GSTT1 as risk factors for astrocytoma. *Carcinogenesis*, **17**, 1919–1922.

HARRIS, M. J., COGGAN, M., LANGTON, L., WILSON, S. R. and BOARD, P. G. 1998, Polymorphism of the Pi class glutahione S-transferase in normal populations and cancer patients. *Pharmacogenetics*, **8**, 27–31.

HAYES, J. D. and PULFORD, D. J. 1995, The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviews in Biochemical and. Molecular Biology*, **30**, 445–600.

INSKIP, A., ELEXPERU-CAMIRUAGA, J., BUXTON, N., DIAS, P. S., MACINTOSH, J., CAMPBELL D. JONES, P. W., YENGI, L., TALBOT, J. A., STRANGE, R. C. and FRYER, A. A. 1995, Identification of polymorphism at the glutathione S-transferase, *GSTM3* locus: evidence for linkage with *GSTM1*A*. *Biochemical Journal*, **312**, 713–716.

JAHNKE, V., STRANGE, R., MATHIAS, C. and FRYER, A. 1997, Glutathione S-transferase and cytochrome P450 genotypes as risk factors for laryngeal carcinoma. *European Archives of Otorhinolaryngology Supplements*, **1**, 147–149.

KROEGER, P. E. and MORIMOTO, I. R. 1994, Selection of new HSF1 and HSF2 DNA-binding sites reveals difference in trimer cooperativity. *Molecular Cell Biol*ogy, **14**, 7592–7603.

KROEGER, P. E., SARGE, K. D. and MORIMOTO, R. I. 1993, Mouse heat shock transcription factors 1 and 2 prefer a trimeric binding site but interact differently with the HSP70 heat shock element. *Molecular Cell Biol*ogy, **13**, 3370–3383.

KUCZEK, E. S., SHANNON, M. F., PELL, L. M. and VADAS, M. A. 1991, A granulocyte-colony-stimulating factor gene promoter element responsive to inflammatory mediators is functionally

distinct from an identical sequence in the granulocyte-macrophage colony-stimulating factor gene. *Journal of Immunology*, **146**, 2426–2433.

LONDON, S. J., DALY, A. K., COOPER, J., NAVIDI, W. C., CARPENTER, C. L. and IDLE, J. R. 1995, Polymorphism of glutathione S-transferase M1 and lung cancer risk among African–Americans and Caucasians in Los Angeles County, California. *Journal of the National Cancer Institute*, **85**, 1246-1252.

MANNERVIK, B., AWASTHI, Y. C., BOARD, P. G., HAYES, J. D., DI ILIO, C., KETTERER, B., LISTOWSKY, I., MORGENSTERN, R., MURAMATSU, M., PEARSON, W. R., PICKETT, C. B., SATO, K., WIDERSTEN, M. and WOLF, C. R. 1992, Nomenclature for human glutathione transferases. *Biochemical Journal*, **282**, 305-306.

MCWILLIAMS, J. E., SANDERSON, B. J. S., HARRIS, E. L., RICHERT-BOE, K. E. and HENNER 1995, Glutathione S-tranferase M1 deficiency and lung cancer risk. *Cancer Epidemiology Biomarkers and Prevention*, **4**, 589–594.

MIVECHI, N. F., KOONG, A. C., GIACCIA, A. J. and HANN, G. M. 1994, Analysis of HSF-1 phosphorylation in A459 cells treated with a variety of stresses. *International Journal of Hyperthermia*, **10**, 371–379.

MILLER, M. S., MCCARVER, D. G., BELL, D. A., EATON, D. L. and GOLDSTEIN, J. A. 1997, Genetic polymorphisms in human drug metabolic enzymes. *Fundamental and Applied Toxicology*, **40**, 1–14.

PEARSON, W. R., VORACHEK, W. R., XU, S. J., BERGER, R., HART, I., VANNAIS, D., PATTERSON, D. and AM, J. 1993, Identification of class-mu glutathione transferase genes *GSTM*–-GSTM5 on human chromosome 1p13. *Human Genetics*, **53**, 220–233.

PEMBLE, S. E., WARDLE, A. F. and TAYLOR, J. B. 1996, Glutathione S-transferase class Kappa: characterization by cloning of rat mitochondrial GST and identification of a human homologue. *Biochemical Journal,* **319**, 749–754.

RYBERG, D., SKAUG, V., HEWER, A., PHILLIPS, D. H., HARRIES, L. W., WOLF, C. R., OGREID, D., ULVIK, A., VU, P. and HAUGEN, A. 1997, Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis*, **18**, 1285–1289.

SAARIKOSKI, S. T., VOHO, A., REINIKAINEN, M., ANTTILA, S., KARJALAINEN, A., MALAVEILLE, C., VAINIO, H., HUSGAFVEL-PURSIAINEN, K. and HIRVONEN, A. 1998, Combined effect of polymorphic GST genes on individual susceptibility to lung cancer. *International Journal of Cancer*, **77**, 516–521.

SCHUG, J. and OVERTON, G. C. 1987, TESS: Transcription Element Search Software on the WWW. Technical Report CBIL-TR-1997-1001-v0.0 of the Computational Biology and Informatics Laboratory, School of Medicine, University of Pennsylvania.

SHANNON, M. F., GAMBLE, J. R. and VADAS, M. A. 1988, Nuclear proteins interacting with the promoter region of the human granulocyte/macrophage colony-stimulating factor gene. *Proceedings of the National Academy of Sciences. USA* , **85**, 674–678.

SHEN, Y., LIU, J., WANG, X., CHENG, X., WANG, Y. and WU, N. 1997, Essential role of the first intron in the transcription of hsp90beta gene. *FEBS Letters*, **413**, 92–98.

SORGER, P. K. 1991, Heat shock factor and the heat shock response. *Cell*, **65**, 363–366.

STRANGE, R. C., LEAR, J. T. and FRYER, A. A. 1998, Polymorphism in the glutathione S-transferase loci as a risk factor for common cancers. *Archives of Toxicology Supplement*, **20**, 419–428.

TO-FIGUERAS, J., GENÉ, M., GÓMEZ-CATALÁN, J., GALAN, M. C., FUENTES, M., RAMÓN J. M., RODAMILANS, M., HUGUET, J. and CORBELLA, J. 1997, Glutathione S-transferase M1 (*GSTM1*) and T1 (GSTT1) polymorphisms and lung cancer risk among Northwestern Mediterraneans. *Carcinogenesis*, **8**, 1529–1533.

VAYSSIER, M., FAVATIER, F., PINOTM, F., BACHELETM, M. and POLLAM, B. S. 1998, Tobacco smoke induces coordinate activation of HSF and inhibition of NFkappaB in human monocytes: effects on TNFalpha release. *Biochemical and Biophysical Research Communications*, **252**, 249–256.

WANG, Y. and MORGAN, W. D. 1994, Cooperative interaction of human HSF1 heat shock transcription factor with promoter DNA. *Nucleic Acids Research*, **22**, 3113–3118.

YENGI, L., INSKIP, A., GILFORD, J., ALLDERSEA, J., BAILEY, L., SMITH, A., LEAR, J. T., HEAGERTY, A. H., BOWERS, B., HAND, P., HAYES, J. D., JONES, P. W. and STRANGE, R. C. 1996, Polymorphism at the glutathione S-transferase locus *GSTM3*: interactions with cytrochrome P450 and glutathione S-transferase genotypes as risk factors for multiple cutaneous basal cell carcinoma. *Cancer Research*, **56**,1974–1977.