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Influence of *GSTM1*, *GSTT1*, *GSTP1*, *NAT1*, *NAT2*, *EPHX1*, *MTR* and *MTHFR* polymorphism on chromosomal aberration frequencies in human lymphocytes

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We have studied the influence of genetic polymorphisms in the xenobiotic-metabolizing genes *GSTM1*, *GSTP1*, *GSTT1*, *EPHX1*, *NAT1* and *NAT2* and the folate-metabolizing genes *MTR* and *MTHFR* on the frequencies of cells with chromosomal aberrations (CAs) in peripheral lymphocytes of Norwegian men. Log-linear Poisson regression models were applied on 357 subjects of whom data on all the polymorphisms examined were available. Total CAs and chromosome-type aberrations (CSAs) were significantly increased by higher age alone, whereas chromatid-type aberrations (CTAs) were elevated by the *GSTT1*-null genotype and *MTHFR* codon 222 variant allele and chromatid gaps (CTGs) by *EPHX1* high activity genotype and occupational exposure. Stratification by smoking and age (<40 and ≥40 years) showed that the effect of the *GSTT1* null and *EPHX1* high activity genotypes only concerned (older) smokers, in agreement with the roles of the respective enzymes in detoxification and metabolic activation. The *MTHFR* codon 222 variant allele was associated with high CTGs in smokers, the *MTR* codon 919 variant allele with high CTAs in older smokers and the *NAT2* fast acetylator genotype with high CTGs in older subjects. Among younger nonsmokers, however, carriers of the *MTHFR* codon 222 and *MTR* codon 919 variant alleles showed a decrease in the level of CTGs and total CAs, respectively. In conclusion, polymorphisms of *GSTT1*, *EPHX1*, *MTHFR*, *MTR* and *NAT2* differentially affect the frequency of CTAs, CSAs and CTGs, showing interaction with smoking and age. It appears that CA subtypes rather than total CAs should be considered in this type of studies.

Introduction

Structural chromosomal aberrations (CAs) in peripheral blood lymphocytes have been used as a biomarker for cellular damage caused by exposure to genotoxic carcinogens since the late 1960s. Using the conventional technique based on uniform staining (usually Giemsa) of chromosomes, CAs visible in arrested metaphase-stage lymphocytes are traditionally divided in two main classes: chromosome-type aberrations (CSAs), which are primarily associated with exposure to ionizing radiation (but may also reflect effects of radiomimetic chemicals), and chromatid-type aberrations (CTAs), which are mostly produced by chemical genotoxicants (1,2). This approach is well suited for recording of chromosomal breakage, dicentric chromosomes, ring chromosomes and chromatid exchanges, but inefficient in detecting some chromosome-type rearrangements such as translocations and inversions.

A great number of studies exist on the effect of various occupational exposures on lymphocyte CAs using the conventional technique without the aid of chromosomal banding or *in situ* hybridization. These studies have made it feasible to evaluate the possible association between the level of CAs in lymphocytes and subsequent cancer outcome. A number of cohort studies have indicated that a high level of lymphocyte chromosome damage is associated with an increased risk of cancer (3–6) irrespective of occupational exposure to carcinogens or tobacco smoking (3). Besides undetected exposure, this may suggest a role for individual susceptibility factors.

Several investigations have indicated that some genetic polymorphism in genes coding for xenobiotic-metabolizing enzymes, DNA repair or folate metabolism are associated with increased cancer risk, depending upon carcinogenic exposure, tumor type and ethnicity (7–9). Information is emerging that polymorphism of xenobiotic-metabolizing enzymes could also influence cytogenetic end points (2,10).

In the present paper, we have explored potential links between genetic polymorphisms in several xenobiotic-metabolizing enzyme genes and CAs in a population of Norwegian men previously studied for CAs. The study concerned polymorphic genes coding for glutathione *S*-transferases (GSTs) mu 1 (*GSTM1*), pi 1 (*GSTP1*) and theta 1 (*GSTT1*), *N*-acetyltransferases 1 (*NAT1*) and 2 (*NAT2*) and microsomal epoxide hydrolase (*EPHX1*)—which are among the main metabolic polymorphic enzymes studied so far for associations with cancer or cytogenetic end points. Due to our previous findings suggesting a role for methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase (*MTR*) polymorphisms in determining CA level (11–13), we also included these folate-metabolizing genes in the present study.

Materials and methods

Subjects

The population consisted of 651 healthy Norwegian males of Caucasian descent studied for CAs between 1974 and 1998. The mean age of the subjects at the time of the analysis was 41 years (range 18–71). Nine subjects were <20 and eight >65 years of age. Fifty-one percent of the participants were smokers, and 46% were included because of possible exposure to clastogenic/carcinogenic agents at work, such as stainless steel welding fumes, vinyl chloride, vapors of naphthenic oils of low and high viscosity, electromagnetic fields, acrylamide, mercury vapor, aluminum, lead, styrene and various solvents (for details see, ref. 11). Written informed consent was obtained from all subjects. The Regional Ethics Committee and the Data Inspectorate approved the study.

Cytogenetic analysis

Phytohemagglutinin-stimulated lymphocyte cultures from heparinized whole blood were used according to methods described earlier (14). CAs were scored by the same three microscopists in a double-blind fashion on coded slides from 100 cells per subject in the earliest studies and from 200 cells per subject in more recent studies. Different types of CAs were recorded, using scoring criteria described by Brøgger *et al.* (15) and Savage (16) and harmonized among the scorers. Fixed cell suspensions and slides were stored from all subjects.

DNA isolation

Cells that had been stored in methanol–acetic acid fixative at −20°C for 3–22 years were used for DNA extraction, as described earlier (11). When cell suspensions were not available, DNA was extracted from unstained slides that had been kept at room temperature for 3–26 years, as explained previously

Abbreviations: CA, chromosomal aberration; CSA, chromosome-type aberration; CTA, chromatid-type aberration; CTG, chromatid gap; EPHX1, epoxide hydrolase; FR, frequency ratio; GST, glutathione S-transferases; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; PCR, polymerase chain reaction; SD, standard deviation.

(5). The success rate depended upon the amount of cells and the age of the slides. Slides prepared in 1982 and later gave good results. Slides from 1974–77 gave variable results both for DNA quality and quantity. In the present study, DNA extracted from old slides was used in the analysis of *GSTM1* genotype for 29 subjects. The quality of DNA from old slides was checked by including a control fragment in the polymerase chain reactions (PCRs). All other genotype analyses were based on DNA extracted from fixed cell suspensions.

Genotype analysis

Polymorphisms of the *GSTM1* (null), *GSTT1* (null) and *GSTP1* (Ile^{105}Val) genes were analyzed simultaneously by multiplex PCR, as described by Nedelcheva Kristensen *et al.* (17) with minor modifications.

Genotype analysis of the *EPHX1* Tyr^{113}His polymorphism (rs1051740) was performed as described by Skjelbred *et al.* (18). The *EPHX1* His^{139}Arg polymorphism (rs2234922) was analyzed by a PCR–restriction fragment length polymorphism method as described by Hassett *et al.* (19). The expected *EPHX1* activity was deduced from the combined codon 113 (alleles *1/*3) and codon 139 (alleles *1/*4) polymorphisms as described earlier (20), with *1/*1+*1/*4 and *1/*1 + *4/*4 defined as high *EPHX1* activity combinations, *1/*1+*1/*1, *1/*3+*1/*4 and *1/*3+*4/*4 as medium activity combinations and *3/*3+*1/*1, *3/*3+*1/*4, *1/*3+*1/*1 and *3/*3+*4/*4 as low activity combinations.

NAT1 genotyping was performed by single-track sequencing as described earlier (21). The *NAT1* alleles analyzed were *3, *4, *10, *11 and *14. All subjects carrying the *10 and *11 alleles were defined as fast acetylators (22). The remaining subjects were classified as slow acetylators. *NAT2* was also analyzed by single-track sequencing. PCR was performed as described by Cascorbi *et al.* (23). Single-track sequencing was then carried out as described for *NAT1* by Soucek *et al.* (21) but with the following sequencing primers forward 5′-CAATCAACTTCTGTACTGGGCTC-3′ and reverse 5′-TTGGGTGATACATACACAAGGG-3′. The *NAT2* alleles analyzed were *4, *5A, *5B, *5C, *6A, *6B, *7B and *12A. The following alleles were also assessed but were not found in this population: 5D, 5E, 6B, 6C, 6E, 7A, 10, 11, 12B, 12C, 13 and 18. All subjects carrying the *4 allele were considered fast acetylators. The remaining subjects were regarded as slow acetylators (23).

The *MTHFR* Ala^{222}Val (rs1801133) genotype analysis was performed as described by Skjelbred *et al.* (11). The *MTR* Asp^{919}Gly (rs1805087) genotype analysis was carried out using the TaqMan allelic discrimination assay on the Sequence Detection System ABI 7000 (Applied Biosystems, Netherlands) with the following primers and probes: forward, 5′-GGATGAATACTTTGAGGAAATCATGGAAGA-3′ and reverse, 5′-TGTTTCTACCACTTACCTTGAGAGACT-3′ and A-allele, 5′-VIC-ATTAGACAGGACCATTATG-MGB-3′ and G-allele, 5′-6-FAM-ACAGGGCCATTATG-MGB-3′ (ordered through assay by design; Applied Biosystems).

For all the genotype analyses, the samples were run in parallel, and controls were included in each run. Repeated genotyping was performed with identical results. We did not succeed in getting genotype results for the whole population for all the genes tested, owing to different sensitivity of the different assays and the quality of the DNA.

Statistical analysis

The primary objective of the statistical analysis was to assess whether subjects with different genotypes differed from each other with respect to the frequency of different types of CAs. The Mann–Whitney test was used for the comparison of the mean frequencies of CAs. The number of subjects in different tests varied as all information was not available for everybody. For the subcohort of 357 subjects for whom all information was available, an adjusted frequency ratio (FR) with 95% confidence interval was calculated to compare the mean CA frequencies for the different genotypes, using a log-linear Poisson regression model, as described earlier (24). The calculations used the quasi-Poisson function rather than Poisson to correct for overdispersion. The homozygous and heterozygous carriers of the variant allele were combined in the statistical analysis, owing to the small numbers of homozygote variants. A model with interaction between genotype and smoking and genotype and age was also tested. Statistical tests for the interaction among genotype, smoking and age were difficult to interpret and added little to results obtained by studying the main effects separately in smokers and nonsmokers and in two age groups ($<$40 and $\geq$40 years). SPSS 12.0 for Windows (SPSS, Chicago, IL) and R 2.2.1 were used for the statistical analyses. The proportion of variance explained by the model was calculated from the difference between null deviance and residual deviance divided by null deviance. The values used were collected from the output of the quasi-Poisson regression model in R.

The observed genotype frequencies were compared with those expected from the Hardy–Weinberg equilibrium using the Chi-square test. All statistical tests were two sided.

Results

Whole cohort

The mean frequencies of the various types of CAs for the different genotypes were studied in the whole cohort of 651 subjects. Allele frequencies were within Hardy–Weinberg equilibrium and in line with expectations for a Caucasian population for all genotypes tested (25,26). The Mann–Whitney test (two tailed) showed a significantly elevated mean frequency in *GSTT1*-null subjects for CTAs [mean 0.96, standard deviation (SD) 0.96 versus mean 0.76, SD 0.90 in GSTT1 positive subjects; $P < 0.05$] and for chromatid gaps (CTGs) in subjects carrying the *EPHX1* codon 139 variant allele (mean 4.53, SD 3.30 versus mean 3.85, SD 2.96 in wild-type homozygotes; $P < 0.05$) or with the *EPHX1* high activity genotype (mean 4.72, SD 3.36 versus mean 3.99, SD 3.37 in carriers of the low activity genotype; $P < 0.05$). As reported earlier (11,12), there was a statistically significant impact of occupational exposure on CSAs (increasing effect) and CTGs (decreasing effect) and of age on the mean frequencies of total CAs and CSAs but no clear effect of smoking. The rest of the parameters tested in the present study showed no significant results (results not shown).

Subcohort

When only those 357 subjects were included in the analysis of whom information was available for all genotypes studied, a significant increase was seen in the Mann–Whitney test (two tailed) for CTA mean frequency among *EPHX1* codon 139 variant Arg allele carriers (mean 1.00, SD 1.04) in comparison with wild-type His/His homozygotes (mean 0.74, SD 0.88; $P = 0.029$). In addition, an increase was observed in CTGs among subjects with *EPHX1* high activity genotype (mean 4.76, SD 3.23) in comparison with *EPHX1* low activity genotype (mean 3.52, SD 2.79; $P = 0.013$). CSAs were clearly increased by age ($\geq$40 years: mean 1.25, SD 1.35; $<$40 years: mean 0.85, SD 1.29; $P < 0.001$).

When the log-linear Poisson regression model was used, with adjustment for age, to study the effects of the gene polymorphisms, smoking and occupational exposure on various types of CAs among the 357 subjects (Table I), the effects of the *EPHX1* codon 139 variant allele on CTAs and CTGs and the *EPHX1* high activity genotype on CTGs could be confirmed. Furthermore, significantly increased FRs were found for CTAs in carriers of the *MTHFR* codon 222 variant allele (Val allele) and for CTGs in *GSTT1*-null subjects and for occupationally exposed subjects. No significant associations were found for CSAs or total CAs.

Smoking

The influence of the polymorphisms was also studied separately for smokers and nonsmokers in the subcohort, and Table II shows the main results of this approach. In smokers, the Mann–Whitney test indicated a statistically significant increase in CTAs for *GSTT1*-null subjects and in CTAs and CTGs for *EPHX1* high activity genotype (versus *EPHX1* low activity); a borderline effect was seen in total CAs for the *NAT1* fast acetylator genotype ($P = 0.05$). The log-linear Poisson regression analysis on smokers, adjusted for age, could verify the increasing effect of the *EPHX1* high activity genotype on CTAs and CTGs (Table II). An increased FR was also observed among *GSTT1*-null subjects for CTGs.

For nonsmokers, the Mann–Whitney test suggested a significant effect on the mean frequency only for age on CSAs (data not shown). Borderline increases were observed in CSAs ($P = 0.055$) among *EPHX1* low activity subjects (versus high activity) (Table II). The log-linear Poisson regression analysis on nonsmokers, adjusted for age, confirmed the increase in CSAs among *EPHX1* low activity subjects (versus medium activity subjects) and additionally showed an elevated level of FR for total CAs in *MTHFR* codon 222 variant allele carriers and CTGs among exposed subjects (Table II).

When comparing smokers with nonsmokers, an effect of smoking on CTAs and CTGs was only seen in *EPHX1* high activity genotype;

Table I. Effect of exposure, smoking and genetic polymorphisms on the frequency of main categories of CAs according to log-linear Poisson regression models[a] in 357 individuals with all information available

Group	No. of subjects	Reference group[b]		FR (95% confidence interval) for CA categories[c]			
		Name	No. of subjects	Total CAs	CSAs	CTAs	CTGs
Exposed	193	Controls	164	1.02 (0.87–1.20)	1.07 (0.83–1.38)	0.91 (0.73–1.14)	**1.16 (1.00–1.34)**
Current smokers	182	Nonsmokers	175	1.16 (0.99–1.37)	0.95 (0.74–1.23)	1.24 (0.99–1.56)	1.01 (0.87–1.17)
GSTM1 null	181	*GSTM1* positive	176	1.01 (0.86–1.19)	0.84 (0.65–1.09)	1.17 (0.93–1.47)	1.02 (0.88–1.18)
GSTT1 null	67	*GSTT1* positive	290	1.13 (0.91–1.38)	0.96 (0.67–1.34)	1.30 (0.97–1.71)	**1.26 (1.05–1.51)**
GSTP1 Ile/Val + Val/Val	200	*GSTP1* Ile/Ile	157	1.07 (0.91–1.26)	1.22 (0.94–1.58)	1.03 (0.82–1.29)	0.98 (0.85–1.14)
EPHX1 codon 113 Tyr/His + His/His	165	*EPHX1* Tyr/Tyr	192	0.93 (0.79–1.10)	1.08 (0.83–1.39)	0.94 (0.74–1.18)	0.89 (0.76–1.03)
EPHX1 codon 139 His/Arg + Arg/Arg	122	*EPHX1* His/His	235	1.01 (0.86–1.20)	0.85 (0.64–1.11)	**1.29 (1.03–1.63)**	**1.19 (1.02–1.39)**
EPHX1 medium activity	179	*EPHX1* low activity	113	1.04 (0.86–1.25)	0.88 (0.66–1.17)	1.29 (0.98–1.69)	1.07 (0.90–1.27)
EPHX1 high activity	65	*EPHX1* low activity	113	1.09 (0.86–1.37)	0.76 (0.51–1.12)	1.38 (0.99–1.92)	**1.35 (1.10–1.66)**
NAT1 fast (*10,*11)	122	*NAT1* slow	235	1.06 (0.84–1.33)	0.96 (0.65–1.39)	0.97 (0.70–1.34)	1.12 (0.90–1.37)
NAT2 fast (*4)	134	*NAT2* slow	223	1.13 (0.91–1.40)	1.14 (0.81–1.59)	1.16 (0.85–1.56)	1.20 (0.99–1.45)
NAT1/NAT2 fast/fast	58	Other combinations	299	1.05 (0.89–1.24)	1.13 (0.87–1.49)	1.07 (0.84–1.37)	0.93 (0.79–1.08)
MTR Asp/Gly + Gly/Gly	127	*MTR* Asp/Asp	230	1.02 (0.86–1.21)	1.02 (0.78–1.33)	0.99 (0.78–1.26)	0.95 (0.81–1.12)
MTHFR Ala/Val + Val/Val	194	*MTHFR* Ala/Ala	163	1.16 (0.98–1.37)	1.10 (0.85–1.43)	**1.32 (1.05–1.67)**	1.02 (0.88–1.19)

[a]The model included the shown variables (*EPHX1* as activity genotype) with adjustment for age. The effects of *EPHX1* codon 113 and 139 polymorphisms are from another model, replacing *EPHX1* activity for *EPHX1* codon 113 and codon 139 genotypes separately did not alter effects seen for the other variables.
[b]FR was set at 1.00 for each reference group.
[c]FRs and 95% confidence intervals indicating a statistically significant effect are shown in bold.

the mean frequencies of CTAs and CTGs increased from *EPHX1* low through medium to high activity genotype in smokers, whereas no such effect was seen in nonsmokers (Table II). Similarly, a smoking effect on CTAs and CTGs was obvious in *GSTT1* null but not in *GSTT1*-positive subjects, and the *GSTT1* genotype effect was only present in smokers (Table II). The effect of smoking on total CAs was primarily seen in *NAT1* fast acetylators (Table II).

Age and smoking

The results for the subcohort were then viewed separately in two age groups (<40 and ≥40 years) for the smokers and nonsmokers (Table III). The number of subjects in each group thus diminished, and no significant effects were demonstrated for the mean of any of the cytogenetic parameters tested with the Mann–Whitney test.

However, the log-linear Poisson regression analysis on the older age group of smokers showed significantly increased FRs for total CAs in *MTR* codon 919 variant allele carriers, for CTAs in subjects with the *GST*-null genotype and *MTR* codon 919 variant allele and for CTGs in the *EPHX1* high activity genotype. These effects were not observed among the nonsmokers in any of the two age groups.

The FRs of the younger age group of nonsmokers were significantly decreased for CAs in carriers of the *MTR* codon 919 variant allele, for CSAs in the *EPHX1* medium activity genotype and for CTGs in *MTHFR* codon 222 variant allele carriers but significantly increased for the older age group.

When looking at the older age group without stratifying for smoking, FR was significantly increased for CTAs in carriers of the *MTHFR* codon 222 variant allele (FR 1.47, 95% confidence interval 1.04–2.08; results not shown). The same was true for CTGs in the *NAT2* fast acetylators (FR 1.42, 95% confidence interval 1.07–1.87; versus slow acetylators). The increasing effect of occupational exposure on CTGs was only present among nonsmokers in the older age group (results not shown).

Discussion

CSAs are formed by different agents, in different phases of interphase and by different cellular mechanisms than CTAs. When only total CA frequency is viewed, differences that are specific to CSAs, CTAs or CTGs may be masked, and possible exposure-specific effects may be lost. In the present study, we examined the effects of polymorphisms in eight metabolic genes on the levels of the various types of CAs independently to see if their influence could specifically concern certain types of CAs. Our findings underline the importance of looking at CTAs, CSAs and CTGs separately and evaluating the modifying effects of smoking habits and age when assessing the influence of genetic polymorphisms on CAs. Some discrepancies in published literature on genotypes and chromosome damage may be due to these factors.

Smoking represents the most common exposure to a genotoxic carcinogen in human populations, and the effect of genetic polymorphisms on smokers and nonsmokers were therefore considered separately. As the possible effects of exposure may not become manifest until later in life, we also examined the influence of age by dividing the study population in two groups according to age. The discussion below is primarily based on the results of the log-linear Poisson regression analysis, as this approach could simultaneously take into account the various variables.

Our results indicated a modifying effect of genetic polymorphisms in *GSTT1*, *EPHX1*, *MTR*, *MTHFR* and *NAT2* on the mean frequencies of the different types of CAs. Among subjects in the subcohort, total CAs and CSAs were significantly increased by higher age alone, in agreement with a number of studies (1), whereas CTAs were affected by *EPHX1* and *MTHFR* genotypes and CTGs by *GSTT1* and *EPHX1* genotypes and occupational exposure. However, further stratification by tobacco smoking and age revealed associations with *NAT2* and *MTR*. The effect of occupational exposure on CTGs was restricted to older nonsmokers. Although the exposures were quite variable and some of the separate studies were positive and some negative, the finding suggests that an exposure effect can more easily be detected when smoking is not interfering with the results, especially in older workers who may have experienced longer and higher exposures than younger subjects.

Significant associations with genotypes and CAs mainly concerned CTAs (CTGs), which may indicate—besides the observed links with smoking—interactions with other ongoing exposures due

Table II. Effect of some genetic polymorphisms and exposure on CAs among 357 subjects stratified by smoking

CA category, group of subjects	Nonsmokers			Smokers		
	No. of subjects	Mean (SD) no. of CAs/100 cells	FR (95% confidence interval)[a]	No. of subjects	Mean (SD) no. of CAs/100 cells	FR (95% confidence interval)[a]
Total CAs						
MTHFR codon 222						
Ala/Ala	83	1.14 (0.93)	1.00	80	1.50 (1.30)	1.00
Ala/Val + Val/Val	92	1.38 (1.05)	**1.26 (1.00–1.59)**	102	1.53 (1.21)	1.08 (0.85–1.37)
NAT1 acetylator genotype						
Slow	118	1.30 (1.03)	1.00	117	1.37 (1.16)	1.00
Fast (*10,*11)	57	1.20 (0.95)	0.78 (0.54–1.11)	65	1.77[b] (1.37)	1.26 (0.91–1.72)
CSAs						
EPHX1 activity genotype						
Low	57	1.32[c] (1.42)	1.00	56	1.04 (1.47)	1.00
Medium	86	0.86 (1.07)	**0.67 (0.46–0.96)**	93	1.13 (1.45)	1.12 (0.71–1.80)
High	32	1.05 (1.44)	0.72 (0.44–1.14)	33	0.77 (1.08)	0.76 (0.38–1.45)
CTAs						
GSTT1						
Positive	141	0.74 (0.87)	1.00	149	0.83 (0.97)	1.00
Null	34	0.75 (0.79)	1.15 (0.73–1.74)	33	**1.29**[d] (1.15)	1.29 (0.85–1.92)
EPHX1 activity genotype						
Low	57	0.69 (0.75)	1.00	56	0.65 (0.83)	1.00
Medium	86	0.80 (0.84)	1.18 (0.80–1.76)	93	0.88 (1.03)	1.39 (0.94–2.10)
High	32	0.69 (1.04)	0.86 (0.50–1.45)	33	**1.44**[e] (1.09)	**1.85 (1.16–2.98)**
CTGs						
Exposure						
Controls	92	3.52 (2.46)	1.00	101	3.94 (2.61)	1.00
Exposed	83	4.11 (2.77)	**1.25 (1.00–1.55)**	81	3.86 (3.12)	1.04 (0.84–1.28)
GSTT1						
Positive	141	3.81 (2.63)	1.00	149	3.62 (2.43)	1.00
Null	34	3.75 (2.62)	1.06 (0.80–1.39)	33	5.20 (4.03)	**1.39 (1.06–1.80)**
EPHX1 activity genotype						
Low	57	3.71 (3.01)	1.00	56	3.33 (2.56)	1.00
Medium	86	3.85 (2.41)	1.05 (0.82–1.33)	93	3.63 (2.45)	1.08 (0.84–1.41)
High	32	3.83 (2.49)	1.06 (0.77–1.44)	33	**5.67**[f] (3.63)	**1.57 (1.16–2.12)**

[a]According to log-linear Poisson regression model including exposure and the genotypes studied, with adjustment for age. FRs and 95% confidence intervals indicating a statistically significant effect are shown in bold. Probabilities shown below according to Mann–Whitney test (two tailed; statistically significant effects are shown in bold).
[b]$P = 0.050$ versus smoking slow acetylators.
[c]$P = 0.055$ versus nonsmokers with high activity genotype.
[d]$P = 0.024$ versus *GSTT1*-positive smokers.
[e]$P < 0.001$ versus smokers with low activity genotype.
[f]$P = 0.001$ versus smokers with low activity genotype.

to environmental and lifestyle factors such as diet (e.g. folate intake). These factors probably varied with time and among the separate studies included, which might have influenced the results. However, except for smoking, such factors could not be controlled for in the present study, which was based on historical samples. Occupational exposure could be considered only in general (yes/no).

GSTT1 and EPHX1

An effect of *GSTT1* and *EPHX1* activity genotypes on CTAs and CTGs was only present in smokers and, conversely, a smoking effect was only seen in *GSTT1*-null subjects and *EPHX1* high activity genotype. These findings agree with the roles of these xenobiotic-metabolizing enzyme polymorphisms in the metabolism of genotoxic components of tobacco smoke.

Our results on the *GSTT1* polymorphism are consistent with the idea that *GSTT1*-null subjects, devoid of GST theta 1 activity, are more sensitive than *GSTT1*-positive subjects to various reactive compounds present in (or derived from) tobacco smoke and detoxified by GST theta 1. We observed a clear effect of smoking on CTAs among *GSTT1*-null subjects only in the older age group (≥40 years). In an earlier pooled study, the effect of *GSTT1* polymorphism was found to be age dependent also on lymphocyte micronucleus frequency, *GSTT1*-null subjects showing decreased values in younger subjects but increased values in older subjects (27). The *GSTT1*-null genotype has previously been linked with increased susceptibility to smoking in several papers (9). Although the present data indicated that *GSTT1*-null smokers have a higher level of chromatid-type damage (CTAs and CTGs), two earlier studies (24,28) found that smokers with the *GSTT1*-null genotype show an increase in CSAs. Furthermore, the *GSTT1*-null genotype was associated with an increased frequency of CSAs in workers exposed to nitrotoluene (28) and of chromosome 21 monosomy and chromosome 8 and 21 trisomy in benzene-exposed workers (29).

Our findings on the elevated induction of CTAs by smoking in subjects with the *EPHX1* high activity genotype agrees with the known role of EPHX1 in the metabolic activation of carcinogenic polyaromatic hydrocarbons, such as benzo[*a*] pyrene, found in tobacco smoke. High EPHX1 activity is expected to enhance the formation of reactive polyaromatic hydrocarbon metabolites and thereby increase the genotoxic effects of smoking. Our results suggest that the effect is primarily due to the codon 139 variant allele which has been associated with an increased enzyme activity, as *EPHX1* codon 113 variant allele, reducing enzyme activity, showed no influence alone.

The *EPHX1* activity genotype showed a differential effect on chromatid- and chromosome-type CAs. Although the *EPHX1* high activity genotype resulted in a clear increase in CTAs and CTGs in

Table III. Effect of some genetic polymorphisms on CAs among 357 subjects stratified by smoking and age

CA category, polymorphism, age group, genotype	Nonsmokers			Smokers		
	No. of subjects	Mean (SD) no. of CAs/100 cells	FR (95% confidence interval)[a]	No. of subjects	Mean (SD) no. of CAs/100 cells	FR (95% confidence interval)[a]
Total CAs						
MTR codon 919						
<40 years						
Asp/Asp	60	1.34 (0.98)	1.00	59	1.43 (1.12)	1.00
Asp/Gly + Gly/Gly	31	0.84 (0.78)	**0.66 (0.45–0.94)**	34	1.35 (1.42)	0.86 (0.59–1.25)
≥40 years						
Asp/Asp	51	1.40 (1.01)	1.00	60	1.34 (1.13)	1.00
Asp/Gly + Gly/Gly	33	1.32 (1.14)	0.90 (0.63–1.28)	29	2.26 (1.31)	**2.01 (1.43–2.83)**
CSAs						
EPHX1 activity						
<40 years						
Low	24	1.17 (1.47)	1.00	35	1.00 (1.49)	1.00
Medium	51	0.53 (0.66)	**0.53 (0.31–0.92)**	47	0.87 (1.53)	0.94 (0.48–1.88)
High	16	1.16 (1.41)	0.86 (0.45–1.62)	11	0.55 (0.93)	0.68 (0.15–2.29)
≥40 years						
Low	33	1.44 (1.38)	1.00	21	1.10 (1.46)	1.00
Medium	35	1.34 (1.34)	0.91 (0.56–1.48)	46	1.39 (1.33)	1.51 (0.79–3.02)
High	16	0.94 (1.50)	0.55 (0.26–1.09)	22	0.88 (1.08)	1.01 (0.45–2.26)
CTAs						
GSTT1						
<40 years						
Positive	78	0.79 (0.93)	1.00	78	0.84 (0.88)	1.00
Null	13	1.00 (1.00)	1.52 (0.78–2.79)	15	1.07 (1.12)	0.94 (0.49–1.70)
≥40 years						
Positive	63	0.68 (0.79)	1.00	71	0.82 (1.06)	1.00
Null	21	0.60 (0.60)	0.92 (0.49–1.65)	18	1.47 (1.18)	**2.32 (1.26–4.20)**
MTR codon 919						
<40 years						
Asp/Asp	60	0.89 (0.96)	1.00	59	0.91 (0.87)	1.00
Asp/Gly + Gly/Gly	31	0.68 (0.88)	0.72 (0.39–1.26)	34	0.82 (1.00)	0.81 (0.51–1.27)
≥40 years						
Asp/Asp	51	0.72 (0.78)	1.00	60	0.79 (0.99)	1.00
Asp/Gly + Gly/Gly	33	0.58 (0.70)	0.73 (0.43–1.19)	29	1.28 (1.29)	**2.47 (1.47–4.21)**
CTGs						
EPHX1 activity						
<40 years						
Low	24	3.38 (2.35)	1.00	35	3.67 (2.68)	1.00
Medium	51	3.72 (2.37)	1.09 (0.79–1.54)	47	3.62 (2.68)	0.97 (0.69–1.37)
High	16	4.53 (2.77)	1.39 (0.92–2.10)	11	5.82 (3.45)	1.39 (0.85–2.22)
≥40 years						
Low	33	3.95 (3.43)	1.00	21	2.76 (2.27)	1.00
Medium	35	4.03 (2.50)	1.18 (0.84–1.66)	46	3.63 (2.21)	1.33 (0.87–2.09)
High	16	3.23 (2.03)	0.74 (0.45–1.17)	22	5.60 (3.79)	**1.99 (1.26–3.18)**
MTHFR codon 222						
<40 years						
Ala/Ala	46	4.59 (2.61)	1.00	38	3.97 (2.77)	1.00
Ala/Val + Val/Val	45	2.93 (1.94)	**0.62 (0.46–0.83)**	55	3.85 (2.91)	1.01 (0.73–1.41)
≥40 years						
Ala/Ala	37	3.36 (2.53)	1.00	42	3.64 (2.50)	1.00
Ala/Val + Val/Val	47	4.19 (3.00)	**1.36 (1.00–1.87)**	47	4.15 (3.15)	1.28 (0.94–1.74)

[a]According to log-linear Poisson regression model including exposure and the genotypes studied. FRs and 95% confidence intervals indicating a statistically significant effect are shown in bold.

smokers, the *EPHX1* low activity genotype was associated with a slight increase in CSAs in nonsmokers. When the subjects were stratified for age in addition to smoking, the frequency of CSAs increased from high through medium to low *EPHX1* activity genotype among smokers < 40 years old and nonsmokers ≥ 40 years of age; these differences in CSA levels may be related to other exposures than smoking.

In general, our results agree with a large American study, which indicated that cumulative cigarette smoking alters the effect of the *EPHX1* slow phenotype from a lung cancer risk factor in nonsmokers to a protective factor in heavy smokers (30). Similar results were observed in *EPHX1* codon 139 Arg allele carriers with DNA adducts as the effect parameter (31).

Vodicka *et al.* (32) have earlier reported a significantly higher total CA frequency in tire plant workers with the *EPHX1* low activity genotype, whereas in the present study, this was true for CSAs in nonsmokers. Cajas-Salazar *et al.* (33) showed that carriers of the *EPHX1* codon 139 variant allele among smoking lung cancer patients had a higher chromosome breakage frequency. This is in agreement with the present findings for CTAs and CTGs in smokers. As EPHX1 functions both as a detoxifying and an activating enzyme, the genotoxic response of different *EPHX1* genotypes is expected to vary depending on exposure to compounds that are activated (polyaromatic hydrocarbon like) or detoxified (e.g. aliphatic epoxides) by EPHX1. Complex mixtures often contain both types of compounds.

MTR and MTHFR

The variant alleles of *MTHFR* and *MTR* have been associated with a higher (*MTHFR* Val allele) and a tentatively lower (*MTR* Asp allele) enzyme activity. Genotypes with the *MTHFR* codon 222 Val allele and the *MTR* codon 919 Asp/Asp genotype are expected to result in higher homocysteine levels and lower methionine production than, respectively, the *MTHFR* Ala/Ala genotype and *MTR* genotypes with the Gly variant allele. Low methionine may lead to hypomethylation of DNA and histones (34), and hypomethylation of fragile chromosomal sites may give rise to CAs (35). *MTHFR* codon 222 Val/Val homozygotes have been described to show hypomethylation of genomic DNA (36,37) and promoter DNA (38).

Our findings among young nonsmokers appeared to conform to this theory as *MTR* codon 919 wild-type homozygotes had higher frequencies of total CAs and CSAs than variant allele (Gly allele) carriers. In earlier studies, *MTR* wild-type homozygotes showed an elevated frequency of CTGs among nonsmoking railroad workers exposed to complex chemical mixtures (13). However, we observed an effect of smoking on total CAs, CSAs and CTAs primarily in *MTR* codon 919 variant allele carriers, suggesting that the variant allele is associated with an increased sensitivity to the clastogenicity of tobacco smoking. CTAs were increased by smoking in *MTR* variant allele carriers only if they belonged to the older age group ($\geq$40 years). This finding is similar as recorded for the *GSTT1*-null genotype and is suggestive of an age-dependent sensitivity of older subjects carrying the *MTR* variant allele. The effect of age on CSAs was particularly clear in carriers of the *MTR* codon 919 variant allele. The highest CSA level was seen in older *MTR* variant allele carriers who were smokers.

In the present study, carriers of the *MTHFR* codon 222 variant allele (Val allele) showed an increase in CTAs only in the older age group. In the younger age group, a slight increase was found for the *MTHFR* wild-type genotype in CTGs among nonsmokers. The *MTHFR* Val allele was previously associated with increased CTAs and decreased CSAs in control subjects but increased CSAs in railroad workers exposed to complex chemical mixtures (13).

Thus, results available on the effects of the *MTR* and *MTHFR* polymorphisms of the two folate-metabolizing enzymes are variable and seem to be modulated by smoking, other exposures and age. A further complication is the possible interference of folate level (34). Folate levels were not available for the present historical cohort.

GSTM1 and GSTP1

Our study showed no significant effects for the *GSTM1* or *GSTP1* polymorphism on CAs. This agrees with a number of earlier studies (10).

NAT1 and NAT2

The *NAT2* fast acetylator genotype was associated with an increase in CTGs in the older age group. In earlier studies, *NAT2* fast genotypes appeared to be associated with elevated frequencies of micronuclei (39) and stable chromosomal exchanges in smokers and micronuclei in patients treated with radioiodine therapy (40) but decreased frequencies of baseline CAs in some (9,24,41) but not all studies (9,28,42). Variation in age, smoking habits and genotoxic exposure could perhaps explain the variable findings for *NAT2* genotypes and CA frequencies.

NAT1 polymorphisms had no significant effect on CAs in the log-linear Poisson regression analysis. A smoking effect on total CAs seemed to be present only in *NAT1* fast acetylators, suggesting that they may have increased sensitivity to smoking. Very little is presently known about the possible effect on *NAT1* polymorphism on CA levels. *NAT1* fast acetylators were previously observed to show an increased level of CTAs and total CAs in workers manufacturing nitrotoluenes (28,42).

General considerations

Although our findings are based on a rather large group of subjects (357 with all parameters available), the numbers of individuals in some subgroups are still relative small. With many parameters tested with the log-linear Poisson regression model, some statistically significant associations may have occurred by chance. Therefore, caution is required in the interpretation of our results.

The present findings and various earlier studies (9,11–13,24, 26,28,32,33,41,42) suggest that genetic polymorphisms of xenobiotic metabolism, folate metabolism and DNA repair affect CA levels and may, therefore, contribute to the association between chromosome damage and cancer. However, the influence of each individual polymorphism is at best rather modest, and a recent study indicated no modifying role for *GSTM1* and *GSTT1* genotypes in the association between CA level and cancer risk (5). In the present study, genetic polymorphisms in the eight genes investigated explained only 4–13% of the variation observed in different CA categories.

Although polymorphisms in DNA repair genes thus far evaluated (*hOGG1*, *XRCC1*, *XRCC3* and *ERCC*2) (11,12) may explain another 4–10%, much of the individual variation in CA frequency is still due to unknown factors.

Conclusions

Our results, based on a large number of subjects, demonstrate that genetic polymorphisms of *GSTT1*, *EPHX1*, *MTHFR*, *MTR* and *NAT2* affect the level of CAs, the associations being modified by smoking and age. The genotype effects are different for CTAs, CSAs or CTGs, suggesting that CA subtypes rather than total CAs should be considered in this type of studies.

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References

1. Albertini,R.J. *et al*. (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat. Res.*, **463**, 111–172.
2. Norppa,H. (2004) Cytogenetic biomarkers. *IARC Sci. Publ.*, **157**, 179–205.
3. Norppa,H. *et al*. (2006) Chromosomal aberrations and SCEs as biomarkers of cancer risk. *Mutat. Res.*, **600**, 37–45.
4. Bonassi,S. *et al*. (2008) Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22,358 subjects in 11 countries. *Carcinogenesis*, **29**, 1178–1183.
5. Rossi,A.M. *et al*. (2009) Association between frequency of chromosomal aberrations and cancer risk is not influenced by polymorphisms in *GSTM1* and GSTT1. *Environ. Health Perspect.*, **117**, 203–208.
6. Musak,L. *et al*. (2008) Chromosomal aberrations in tire plant workers and interaction with polymorphisms of biotransformation and DNA repair genes. *Mutat. Res.*, **641**, 36–42.
7. Agúndez,J.A. (2008) Polymorphisms of human N-acetyltransferases and cancer risk. *Curr. Drug Metab.*, **9**, 520–531.
8. Dong,L.M. *et al*. (2008) Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. *JAMA*, **299**, 2423–2436.
9. McIlwain,C.C. *et al*. (2006) Glutathione S-transferase polymorphisms: cancer incidence and therapy. *Oncogene*, **25**, 1639–1648.
10. Norppa,H. (2004) Cytogenetic biomarkers and genetic polymorphisms. *Toxicol. Lett.*, **149**, 309–334.
11. Skjelbred,C.F. *et al*. (2006) Influence of DNA repair gene polymorphisms of hOGG1, XRCC1, XRCC3, ERCC2 and the folate metabolism gene MTHFR on chromosomal aberration frequencies. *Mutat. Res.*, **602**, 151–162 Erratum Mutat. Res. doi: 10.1016/j.mrfmmm.2007.04.001.

12. Skjelbred,C.F. *et al*. (2007) Erratum to ''Influence of DNA repair gene polymorphisms of hOGG1, XRCC1, XRCC3, ERCC2 and the folate metabolism gene MTHFR on chromosomal aberration frequencies'' Mutat. Res., 602 (2006), 151–162. *Mutat. Res.*, **624**, 132–133.
13. Catalán,J. *et al*. (2009) Chromosomal aberrations in railroad transit workers: effect of genetic polymorphisms. *Environ. Mol. Mutagen.*, **50**, 304–316.
14. Skyberg,K. *et al*. (1989) A cytogenetic and haematological investigation of oil exposed workers in a Norwegian cable manufacturing company. *Br. J. Ind. Med.*, **46**, 791–798.
15. Brogger,A. *et al*. (1990) An inter-Nordic prospective study on cytogenetic endpoints and cancer risk. Nordic Study Group on the health risk of chromosome damage. *Cancer Genet. Cytogenet.*, **45**, 85–92.
16. Savage,J.R. (1976) Classification and relationships of induced chromosomal structual changes. *J. Med. Genet.*, **13**, 103–122.
17. Nedelcheva Kristensen,V. *et al*. (1998) Single tube multiplex polymerase chain reaction genotype analysis of GSTM1, GSTT1 and GSTP1: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. *Pharmacogenetics*, **8**, 441–447.
18. Skjelbred,C.F. *et al*. (2007) Meat, vegetables and genetic polymorphisms and the risk of colorectal carcinomas and adenomas. *BMC Cancer*, **7**, 228–239.
19. Hassett,C. *et al*. (1994) Human microsomal epoxide hydrolase: genetic polymorphism and functional expression *in vitro* of amino acid variants. *Hum. Mol. Genet.*, **3**, 421–428.
20. Benhamou,S. *et al*. (1998) Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Res.*, **58**, 5291–5293.
21. Soucek,P. *et al*. (2004) Single-track sequencing for genotyping of multiple SNPs in the N-acetyltransferase 1 (NAT1) gene. *BMC Biotechnol.*, **4**, 28.
22. Fronhoffs,S. *et al*. (2001) Real-time PCR analysis of the N-acetyltransferase NAT1 allele *3, *4, *10, *11, *14 and *17 polymorphism in squamous cell cancer of head and neck. *Carcinogenesis*, **22**, 1405–1412.
23. Cascorbi,I. *et al*. (1995) Arylamine N-acetyltransferase (NAT2) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. *Am. J. Hum. Genet.*, **57**, 581–592.
24. Tuimala,J. *et al*. (2004) Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: effects on levels of sister chromatid exchanges and chromosomal aberrations. *Mutat. Res.*, **554**, 319–333.
25. Garte,S. *et al*. (2001) Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 1239–1248.
26. Pluth,J.M. *et al*. (2000) The relationship between genotype and chromosome aberration frequencies in a normal adult population. *Pharmacogenetics*, **10**, 311–319.
27. Kirsch-Volders,M. *et al*. (2006) The effects of GSTM1 and GSTT1 polymorphisms on micronucleus frequencies in human lymphocytes *in vivo*. *Cancer Epidemiol. Biomarkers Prev.*, **15**, 1038–1042.
28. Sabbioni,G. *et al*. (2006) Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. *Cancer Epidemiol. Biomarkers Prev.*, **15**, 559–566.
29. Kim,S.Y. *et al*. (2004) Chromosomal aberrations in workers exposed to low levels of benzene: association with genetic polymorphisms. *Pharmacogenetics*, **14**, 453–463.
30. Zhou,W. *et al*. (2002) Genetic polymorphisms in N-acetyltransferase-2 and microsomal epoxide hydrolase, cumulative cigarette smoking, and lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 15–21.
31. Georgiadis,P. *et al*. (2004) Impact of phase I or phase II enzyme polymorphisms on lymphocyte DNA adducts in subjects exposed to urban air pollution and environmental tobacco smoke. *Toxicol. Lett.*, **149**, 269–280.
32. Vodicka,P. *et al*. (2004) Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant. *Environ. Mol. Mutagen.*, **44**, 283–292.
33. Cajas-Salazar,N. *et al*. (2003) Effect of epoxide hydrolase polymorphisms on chromosome aberrations and risk for lung cancer. *Cancer Genet. Cytogenet.*, **145**, 97–102.
34. Thomas,P. *et al*. (2008) Methylenetetrahydrofolate reductase, common polymorphisms, and relation to disease. *Vitam. Horm.*, **79**, 375–392.
35. Tuck-Muller,C.M. *et al*. (2000) DNA hypomethylation and unusual chromosome instability in cell lines from ICF syndrome patients. *Cytogenet. Cell Genet.*, **89**, 121–128.
36. Stern,L.L. *et al*. (2000) Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 849–853.
37. Friso,S. *et al*. (2002) A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc. Natl Acad. Sci. USA*, **99**, 5606–5611.
38. van den Donk,M. *et al*. (2007) Dietary folate intake in combination with MTHFR C677T genotype and promoter methylation of tumor suppressor and DNA repair genes in sporadic colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 327–333.
39. Falck,G.C. *et al*. (1999) Micronuclei in blood lymphocytes and genetic polymorphism for GSTM1, GSTT1 and NAT2 in pesticide-exposed greenhouse workers. *Mutat. Res.*, **441**, 225–237.
40. Hernandez,A. *et al*. (2006) Basal and induced micronucleus frequencies in human lymphocytes with different GST and NAT2 genetic backgrounds. *Mutat. Res.*, **606**, 12–20.
41. Knudsen,L.E. *et al*. (1999) Chromosomal aberrations in humans induced by urban air pollution: influence of DNA repair and polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 303–310.
42. Sabbioni,G. *et al*. (2007) Comparison of biomarkers in workers exposed to 2,4,6-trinitrotoluene. *Biomarkers*, **12**, 21–37.

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