Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers

P. Vineis[1,2*], F. Veglia[2], S. Garte[3], C. Malaveille[4], G. Matullo[2], A. Dunning[5], M. Peluso[6], L. Airoldi[7], K. Overvad[8], O. Raaschou-Nielsen[9], F. Clavel-Chapelon[10], J. P. Linseisen[11], R. Kaaks[11], H. Boeing[12], A. Trichopoulou[13], D. Palli[14], P. Crosignani[15], R. Tumino[16], S. Panico[17], H. B. Bueno-De-Mesquita[18], P. H. Peeters[19], E. Lund[20], C. A. Gonzalez[21], C. Martinez[22], M. Dorronsoro[23], A. Barricarte[24], C. Navarro[25], J. R. Quiros[26], G. Berglund[27], B. Jarvholm[28], N. E. Day[29], T. J. Key[30], R. Saracci[31], E. Riboli[32] & H. Autrup[33]

[1]Imperial College London, London, UK, and University of Torino, Italy; [2]Institute for Scientific Interchange Foundation, Turin, Italy; [3]Genetics Research Institute, Milano, Italy; [4]International Agency for Research on Cancer, Lyon, France; [5]Department of Oncology, University of Cambridge, Cambridge, UK; [6]Cancer Risk Factor Branch, Molecular Biology Laboratory, CSPO-Scientific Institute of Tuscany, Florence, Italy; [7]Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy; [8]Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark, and Department of Epidemiology and Social Medicine, Institute of Public Health, University of Aarhus, Aarhus, Denmark; [9]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; [10]Institut National de la Santé et de la Recherche Médicale, ERI 20, EA 4045, and Institut Gustave Roussy, Villejuif, France; [11]Division of Clinical Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg, Germany; [12]German Institute of Human Nutrition, Potsdam-Rehbücke, Germany; [13]Department of Hygiene and Epidemiology, Medical School, University of Athens, Greece; [14]Molecular and Nutritional Epidemiology Unit, CSPO-Scientific Institute of Tuscany Region, Florence, Italy; [15]Environmental Epidemiology Unit, National Cancer Institute, Milan, Italy; [16]Ragusa Cancer Registry, Sicily, Italy; [17]Department of Clinical and Experimental Medicine, University Federico II, Naples, Italy; [18]Centre of Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; [19]Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands; [20]Institute of Community Medicine, University of Tromso, Norway; [21]Department of Epidemiology, Catalan Institute of Oncology, Consejería de Sanidad y Servicios Sociales, Barcelona, Spain; [22]Andalusian School of Public Health, Granada, Spain; [23]Department of Public Health of Guipuzkoa, San Sebastian, Spain; [24]Public Health Institute, Navarra, Spain; [25]Department of Epidemiology, Murcia Health Council, Murcia, Spain; [26]Dirección General de Salud Pública, Consejería de Salud y Servicios Sanitarios Asturias, Oviedo, Spain; [27]Malmö Diet and Cancer Study, Lund University, Malmö, Sweden; [28]Department of Nutritional Research, University of Umeå, Sweden; [29]MRC Dunn Human Nutrition Unit, Cambridge, UK; [30]Cancer Research UK Epidemiology Unit, University of Oxford, UK; [31]IFC-National Research Council, Pisa, Italy; [32]Imperial College London, London, UK; [33]Department of Epidemiology and Social Medicine (KO) and Environmental and Occupational Medicine (HA), Institude of Public Health, University of Aarhus, Aarhus, Denmark

Background: We chose a set of candidate single nucleotide polymorphisms (SNPs) to investigate gene–environment interactions in three types of cancer that have been related to air pollution (lung, bladder and myeloid leukemia).
Patients and methods: The study has been conducted as a nested case–control study within the European Prospective Investigation into Cancer and Nutrition cohort (409 cancer cases and 757 matched controls). We included never and ex-smokers. SNPs were in genes involved in oxidative stress, phase I metabolizing genes, phase II metabolizing genes and methylenetetrahydrofolate reductase (MTHFR).
Results: The most notable findings are: GSTM1 deletion and bladder cancer risk [odds ratio (OR) = 1.60; 95% confidence interval 1.00–2.56]; CYP1A1 and leukemia (2.22, 1.33–3.70; heterozygotes); CYP1B1 and leukemia (0.47, 0.27–0.84; homozygotes); MnSOD and leukemia (1.91, 1.08–3.38; homozygotes) and NQO1 and lung cancer (8.03, 1.73–37.3; homozygotes). Other statistically significant associations were found in subgroups defined by smoking habits (never or ex-smokers), environmental tobacco smoke or gender, with no obvious pattern. When gene variants were organized according to the three main pathways, the emerging picture was of a strong involvement of combined phase I enzymes in leukemia, with an OR of 5 (1.63–15.4) for those having three or more variant alleles. The association was considerably stronger for leukemias arising before the age of 55.
Key words: bladder cancer, leukemia, lung cancer, metabolic genes, nonsmokers

introduction

rationale

Cigarette smoking, a mixture of numerous chemical carcinogens, e.g. polycyclic aromatic hydrocarbons (PAHs),

*Correspondence to: P. Vineis, Department of Epidemiology and Public Health, Norfolk Place, Imperial College London, London W2 1PG, UK.
Tel: +44-20-75943372; Fax: +44-20-75943196; E-mail: p.vineis@mperial.ac.uk

is the leading cause of several types of cancers, including lung, bladder, upper respiratory tract cancers and myeloid leukemias [1]. Exposure of nonsmokers to environmental tobacco smoke (ETS) and air pollution is also associated with an increased risk of lung cancer, and, possibly, of other forms of cancer [2, 3]. Gene–environment interactions are believed to play an important role in the etiology of common human cancers. Constituents of many of the known environmental risk factors, including ETS and ambient air pollution, are metabolized in the organism to products that are either more carcinogenic or are detoxified. Genetic polymorphisms have been identified in metabolic genes, and the biological consequence of such changes is an altered enzyme activity which may influence the ratio between activation and deactivation, and thus the cancer risk. One of the consequences of carcinogen metabolism is the formation of carcinogen DNA adducts. We have previously suggested that people with high levels of adducts at a time when they were healthy had a subsequent higher risk of developing lung cancer [4]. Altered enzymic activity has also been found to influence the effect of chemopreventive agents, e.g. isothiocyanate, and thus cancer risk [5] .

We have hypothesized that the role of genetic susceptibility could be greater at low levels of exposure [6, 7]. We have chosen a set of candidate single nucleotide polymorphism (SNP) to investigate gene–environment interactions in three types of cancer that have been related to air pollution (lung, bladder and myeloid leukemia). We have investigated both the role of single genes and of pathways to which they belong. The study has been conducted as a nested case–control study within the large European Prospective Investigation into Cancer and Nutrition (EPIC) cohort and included only never and long-term ex-smokers.

candidate genes and SNPs

oxidative stress. Oxidative stress *in vivo* is modulated by enzymes such as myeloperoxidase (MPO), catechol-*O*-methyltransferase (COMT), manganese superoxide dismutase (MnSOD) and NAD(P)H:quinone oxidoreductase (NQO1). MPO is a lysosomal enzyme that activates procarcinogens in tobacco smoke, such as benzo[*a*]pyrene [8]. A *G to A* base transition in *MPO* has been identified at the SP1 binding site; the *A* allele is associated with reduced messenger RNA expression and consequently lower activity. COMT catalyzes the methylation of various substances, preventing quinone formation and redox cycling, and thus it protects DNA from oxidative damage [9]. A *G* to *A* transition, which results in amino acid change from valine to methionine at codon 108, leads to lower COMT activity. MnSOD catalyzes the dismutation of superoxide radicals in mitochondria by converting anion superoxide into hydrogen peroxide and oxygen. It plays a key role in protecting cells from oxidative stress, especially in people with a low intake of natural antioxidants [10]. A *C* to *T* substitution was identified in MnSOD, which results in an amino acid change from alanine to valine at 9 position, thus modulating MnSOD transport into mitochondria [11]. NQO1 is an important flavoenzyme in xenobiotic metabolism. It protects cells from oxidative damage [12]. The 690C>T SNPs has been associated with lower enzyme activity as compared with wild type [13, 14], and thus a higher sensitivity against some carcinogens present in ETS or ambient air, e.g. benzene [15].

phase I enzymes

CYP1A1 is a phase I, predominantly extrahepatic, microsomal enzyme involved in the bioactivation of carcinogenic PAHs including benzo(*a*)pyrene. Early work [16, 17] suggested that the risk of lung cancer is linked to the inducibility of the CYP1A1 enzyme. However, other studies failed to reproduce the association with cancer risk [17]. The *CYP1A1*2A* allele has a *T* to *C* mutation in the 3′ region, whereas an *A* to *G* transition in exon 7 creates a second allelic variant, *CYP1A1*2B* (also known as exon 7 polymorphism), which leads to an amino acid substitution of Val for Ile in the heme-binding region, and consequently results in an increase in microsomal enzyme activity. The variant *CYP1A1*3* has a polymorphism in intron 7 and is African–American specific, but the biological consequence of this polymorphism has not been established [18]. This SNP has no effect on inducibility of the gene or on the function of the gene product.

CYP1B1 activates tobacco carcinogens such as PAH-dihydrodiols [19]. A *G* to *A* polymorphism resulting in an amino acid change from valine to leucine at codon 432 has been identified [20] with the 432Leu allele having a lower catalytic efficiency than the wild type [21] indicating a reduced risk of cancer.

phase II enzymes

Glutathione-*S*-transferases play important roles in the detoxification of the activated carcinogenic form of PAH and in the detoxification of reactive molecules formed by reactive oxygen species. The common deletion polymorphism in GSTM1 and GSTT1 renders these enzymes inactive. Impaired glutathione conjugation has also been shown to alter the mutation spectrum in P53 in bladder cancer cases [22] Two SNPs in the GSTP1 gene that lead to an amino acid substitution in the enzyme's electrophile-binding site, GSTP1-105 (Ile Val) and GSTP1-114 (Ala Val), are known to change the affinity and activity of GSTP1 for electrophilic substances, thus altering the risk of PAH-induced cancer [23, 24]. The role of genetic polymorphisms in GSTM3 in cancer risk is not entirely clear, but it has been suggested that the expression of GSTM3 is significantly correlated with GSTM1 in the lung [25].

Sulfotransferase 1A1 (SULT 1A1) belongs to a gene superfamily, that is involved in sulfate conjugation of primary environmental toxicants, i.e. involved in the metabolism of aromatic amines and PAH. A genetic polymorphism (*G* to *A*) has been identified in codon 213 resulting in amino acid change Arg to His. The His variant (SULT1A1*2/*2) has lower thermostability and thus decreased enzyme activity [26, 27].

N-acetyltransferases (NAT) play a key role in the detoxification of aromatic amines. Two different classes of NAT have been identified of which the NAT2 is mostly involved in *N*-acetylation. Several genetic polymorphisms have been

identified in NAT2, some of which have been associated with an increased cancer risk [28–30].

MTHFR

Methylenetetrahydrofolate reductase (MTHFR) provides the methyl group required for *de novo* methionine synthesis, and indirectly, for DNA methylation, therefore it controls DNA stability and mutagenesis [31–33]. MTHFR has two common polymorphisms (677C→T and 1298A→C). For both SNPs, the variant allele is associated with reduced enzyme activity *in vitro* which, in the case of 677C→T for example, affects the metabolism of folate, consequently increasing homocysteine levels and the risk of cancer [31].

patients and methods

EPIC cohort

EPIC is a multicenter European study, coordinated by the International Agency for Research on Cancer (IARC) (Lyon) (currently at Imperial College, London), in which >520 000 healthy volunteers have been recruited in 10 European countries (Sweden, Denmark, The Netherlands, UK, France, Germany, Spain, Italy, Greece, Norway) [34]. The cohort includes subjects of both genders, in the age range 35–74 at recruitment. Recruitment took place in 1993–1998. Dietary information on the frequency of consumption of >120 foods and drinks has been obtained by a self-administered questionnaire, validated in a pilot phase. At enrollment, weight, height, waist and hip circumferences have been measured for each participant. Detailed information has been collected on reproductive history, physical activity, smoking and alcohol drinking history, medical history, occupation, education level and other socioeconomic variables. A computerized central database has been developed after checking, coding and quality-control procedures.

Follow-up for cancer incidence and mortality is based on population cancer registries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden, UK) and other methods such as health insurance records, pathology registries and active contact of study subjects or next of kin (France, Germany, Greece). In all centers, cancer diagnosis required confirmation through comprehensive pathology review. A detailed protocol entitled 'Guidelines for Collection of End-point Data in the EPIC study' for the collection and standardization of clinical and pathological data for each cancer site have been prepared by a special EPIC working group.

GenAir is a case–control study nested within the EPIC cohort, aiming at studying the relationship between some types of cancer and air pollution or ETS. Cases are subjects with bladder, lung, oral, pharyngeal or laryngeal cancer or myeloid leukemia, all newly diagnosed after recruitment. Only never smokers or ex-smokers since at least 10 years have been included in GenAir. The cut-off has been set at 10 years to allow a reasonably long time as to decrease the possible confounding effect of smoking in ex-smokers. Matching criteria were gender, age (±5 years), smoking status, country of recruitment and time elapsed between recruitment and diagnosis. Half of the EPIC centers (11/22) (in France, Italy, Denmark, Sweden, The Netherlands and Potsdam, Germany) included questions on ETS in the questionnaire.

GenAir has been approved by the Ethical Committee of the IARC, and by all the local Ethical Committees at the participating centers. We have identified 1662 subjects (568 cases and 1094 controls) from whom all information and DNA were available. Here we consider only lung and bladder cancers and myeloid leukemias (total: 409 cases, including 116 lung cancers, 124 bladder cancers and 167 myeloid leukemias and 757 controls). Numbers of oral and pharyngeal cancers were too limited for a meaningful analysis.

DNA extraction and purification and genotyping

DNA was isolated from blood samples (straws in liquid nitrogen) as previously described [4] and distributed to the laboratories responsible for the genotyping: University of Aarhus for GST, NQO1 and NAT; GRI, Milan, for CYP1A1, CYP1B1 and MTHF; and IARC, Lyon, for MnSOD, COMT, MPO and SULT1A1.

GSTT1 and GSTM1 deletion polymorphisms as well as a SNP in the GSTP1 gene resulting in an amino acid substitution at codon 105 (Ile→Val) were determined by PCR-based assays as previously described [35]. The 3 base deletion in intron 6 of GSTM3 was detected by PCR-restriction fragment length polymorphism (RFLP) as previously described [36]. The NAT2 genotypes were categorized into fast and slow acetylators as described by Okkels et al. [37]. The 638G→A polymorphism of SULTA1 was detected as described by Ozawa et al. [38]. Light Cycler was used at IARC, Lyon, and PCR-RFLP at GRI in Milan and at the Aarhus University.

statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were computed using conditional logistic regression models for matched pairs. Analyses of each polymorphism stratified by gender, passive smoking (ETS) and previous smoking (never or former smokers) were carried out. Information on air pollution was available for a subset of the cases and controls [2]. Interaction under the multiplicative model between gender, smoking (ETS and former versus never smokers) and each genotype was tested by including an interaction term in the logistic regression analysis. Also chi-square tests for heterogeneity were carried out. All analyses were carried out by the SAS package for personal computers (SAS Inc., Cary, NC).

results

The general characteristics of the study population are shown in Table 1. The genotypes were all in Hardy–Weinberg equilibrium.

Table 2 shows ORs and CIs for the genes involved in oxidative stress, Table 3 for phase I genes, Table 4 for phase II genes and MTHFR. In spite of a large number of comparisons, we find few statistically significant associations. The most notable ones are: GSTM1 deletion and bladder cancer risk (OR = 1.60; 95% CI 1.00–2.56); CYP1A1 and leukemia (2.22, 1.33–3.70; heterozygotes); MnSOD and leukemia (1.91, 1.08–3.38; homozygotes); NQO1 and lung cancer (8.03, 1.73–37.3; homozygotes) and a protective effect of CYP1B1 for leukemia (0.47, 0.27–0.84; homozygotes).

The investigated NAT2 SNPs were used to classify individuals into NAT2 fast and NAT2 slow phenotypes. The NAT2 slow phenotype, however, was not associated with any of the cancers we have considered (Table 4).

Subgroup analyses have been carried out. An increased risk for GSTM1 was indicated in ex-smokers for lung cancer (1.66, 95% CI 0.84–3.31; $P = 0.15$) and for bladder cancer (1.47, 95% CI 0.76–2.84; $P = 0.25$). Although not statistically significant, a higher risk of lung cancer was observed for GSTM1 in people exposed to ETS compared with people not exposed (5.55, 95% CI 0.62–49.7) versus 1.27 95% CI 0.33–4.87, P for interaction 0.41). GSTM3 showed a stronger association

Table 1. Cases and controls stratified by country, gender, educational level and smoking status (cases and controls were matched)

	Cases (n = 409) (%)		Controls (n = 757) (%)	
Age (mean ± SD)	60.3 ± 7.7		60.7 ± 7.7	
Country				
France	9	(2.2)	17	(2.3)
Italy	47	(11.5)	96	(12.7)
Spain	34	(8.3)	70	(9.3)
UK	131	(32)	203	(26.8)
The Netherlands	35	(8.6)	69	(9.1)
Greece	21	(5.1)	40	(5.3)
Germany	76	(18.6)	141	(18.6)
Sweden	1	(0.2)	1	(0.1)
Denmark	55	(13.5)	120	(15.9)
Gender				
Male	196	(47.9)	367	(48.5)
Female	213	(52.1)	390	(51.5)
Highest school level				
None	18	(4.4)	45	(5.9)
Primary school completed	138	(33.7)	231	(30.5)
Technical/professional school	104	(25.4)	201	(26.6)
Secondary school	55	(13.5)	108	(14.3)
University degree	67	(16.4)	134	(17.7)
Not specified	22	(5.4)	31	(4.1)
Missing	5	(1.2)	7	(0.9)
Smoking status				
Never	227	(55.5)	424	(56)
Former	182	(44.5)	333	(44)

SD, standard deviation.

with bladder cancer among never smokers (OR = 4.90, 95% CI 1.15–20.9 for homozygotes) than in ex-smokers. An association of COMT with leukemia was limited to ex-smokers, with much higher ORs (4.77, 95% CI 1.28–17.8, for heterozygotes; 3.88, 0.99–15.3 for homozygotes) than in never smokers (0.91 and 0.74, respectively). Also CYP1B1 showed a stronger, protective, association with leukemia in ex-smokers (OR = 0.26, 95% CI 0.08–0.86 for homozygotes). No interaction was identified with indicators of traffic-related air pollution.

None of the tests for heterogeneity (by smoking status, gender or ETS exposure) was statistically significant.

analysis by pathways

In Table 5 we show the effects of the combination of gene variants belonging to different metabolic pathways (oxidative damage scavenging, phase I or phase II). We have postulated a codominant effect, i.e. a dose–response relationship of at risk alleles (as defined a priori). A recessive model was also tested; it gave results that were overall weaker than those shown here. The results show a strong effect of phase I gene variants on the risk of myeloid leukemia, with an OR of 5 (1.63–15.4) for those having three or more variant alleles in the pathway, and a clear dose–response relationship (Figure 1). The association was considerably stronger for leukemias arising before the age of 55 (Table 5).

discussion

Genetic polymorphisms in several genes coding for enzymes involved in biotransformation of environmental toxicants and defense against oxidative stress have been associated with increased risks for various cancers [39, 17]. Most of the previous studies have been limited case–control studies including both smokers and nonsmokers, whereas this study focused on cancers in nonsmokers (defined as never smokers and people who had not smoked for at least 10 years before joining the cohort). The aim of the study was to verify the hypothesis that genetic susceptibility could be more relevant at low exposure levels than at high levels, and to study the contribution of gene variants according to metabolic pathways [6, 7].

oxidative stress

Free radicals, which are produced naturally in the body, can cause oxidative damage of DNA, lipids, proteins and other cell constituents, contributing to the onset of cancer and other chronic diseases [40]. Several enzymes, including MnSOD, NQO1, MPO and COMT, are involved in the scavenging of free radicals and prevention of oxidative damage. We have found an association between genetic polymorphisms in MnSOD and myeloid leukemia, and between NQO1 and lung cancer, indicating that oxidative stress can play a role in both types of cancer. Some evidence for an involvement of oxidative stress is available for hemopoietic malignancies [41]. The evidence is stronger, however, for lung cancer [42].

phase I genes

Studies on the association between lung cancer and *CYP1A1* polymorphisms have been published [43, 44], with overall positive results. The sparse literature on bladder cancer suggests no association with CYP1A1 polymorphisms [45], while the studies on myeloid leukemias are conflicting [46–49]. In one study on lung cancer among nonsmokers [50], an interaction of CYP1A1 polymorphism and ETS has been observed in ETS-exposed people [51]. CYP1B1 Leu432Val was significantly associated with lung cancer susceptibility (OR for at least one valine allele = 2.87, 95% CI 1.63–5.07), while no association was found in another study [52].

The reasons for involvement of the phase I bioactivating enzymes in leukemogenesis are not clear, but it can be hypothesized that leukemias in the nonsmoking population could be due to compounds in the environment that undergo such metabolic pathways, e.g. aromatic compounds as benzene. Exposure to benzene occurs both in ETS and through filling of cars. Our observation indicates that future studies should look in more detail into the gene–environment interactions between the phase I pathway and environmental exposures in leukemia.

Table 2. Genes involved in oxidative stress—odds ratios for matched pairs and 95% CI from logistic regression

MnSOD			
Smoking status	Lung (116)[a]	Bladder (124)[a]	Leukemia (167)[a]
All			
Het	1.41 (0.76–2.65)	0.79 (0.46–1.37)	1.11 (0.69–1.8)
Var	1.21 (0.61–2.4)	0.77 (0.4–1.47)	1.91 (1.08–3.38)
Ex-smokers			
Het	1.17 (0.54–2.51)	0.89 (0.42–1.91)	1.4 (0.55–3.6)
Var	0.68 (0.28–1.64)	0.81 (0.35–1.92)	1.85 (0.63–5.46)
Never smokers			
Het	2.27 (0.7–7.38)	0.7 (0.32–1.54)	1.03 (0.58–1.81)
Var	3.11 (0.92–10.5)	0.71 (0.26–1.92)	1.93 (0.99–3.77)
Gender			
Females			
Het	1.15 (0.48–2.78)	1.1 (0.44–2.78)	1.06 (0.59–1.92)
Var	1.87 (0.71–4.98)	0.89 (0.28–2.75)	1.97 (0.94–4.1)
Males			
Het	1.9 (0.76–4.79)	0.66 (0.33–1.31)	1.21 (0.53–2.77)
Var	0.75 (0.27–2.07)	0.71 (0.32–1.57)	1.89 (0.76–4.7)
MPO			
Smoking status	Lung (116)[a]	Bladder (124)[a]	Leukemia (167)[a]
All			
Het	0.97 (0.58–1.61)	0.64 (0.39–1.04)	1.06 (0.7–1.61)
Var	1.08 (0.36–3.23)	0.78 (0.23–2.66)	1.69 (0.63–4.48)
Ex-smokers			
Het	1.03 (0.53–2.02)	0.82 (0.43–1.58)	0.62 (0.27–1.39)
Var	1.08 (0.33–3.57)	1.01 (0.23–4.37)	0.33 (0.03–3.56)
Never smokers			
Het	0.89 (0.4–1.97)	0.45 (0.21–0.98)	1.29 (0.79–2.13)
Var	1.3 (0.07–23.15)	0.49 (0.05–4.95)	2.62 (0.86–7.99)
Gender			
Females			
Het	0.7 (0.36–1.38)	0.43 (0.18–1.04)	0.98 (0.57–1.68)
Var	0.96 (0.2–4.59)	0.48 (0.05–4.9)	0.99 (0.24–4.04)
Males			
Het	1.58 (0.69–3.6)	0.78 (0.43–1.41)	1.21 (0.62–2.37)
Var	1.51 (0.32–7.19)	0.98 (0.23–4.23)	3.12 (0.71–13.7)
COMT			
Smoking status	Lung (116)[a]	Bladder (124)[a]	Leukemia (167)[a]
All			
Het	0.99 (0.57–1.72)	0.53 (0.3–0.94)	1.26 (0.77–2.07)
Var	0.99 (0.49–1.99)	0.73 (0.4–1.34)	1.04 (0.58–1.84)
Ex-smokers			
Het	0.86 (0.39–1.9)	0.43 (0.2–0.94)	4.77 (1.28–17.8)
Var	0.74 (0.29–1.9)	0.48 (0.2–1.13)	3.88 (0.99–15.3)
Never smokers			
Het	1.13 (0.52–2.49)	0.66 (0.27–1.59)	0.91 (0.52–1.61)
Var	1.44 (0.5–4.14)	1.17 (0.48–2.83)	0.74 (0.37–1.45)
Gender			
Females			
Het	1.15 (0.54–2.44)	0.36 (0.13–0.98)	1.02 (0.54–1.9)
Var	1.47 (0.56–3.86)	0.57 (0.21–1.55)	0.76 (0.37–1.58)
Males			
Het	0.81 (0.35–1.86)	0.65 (0.32–1.32)	1.78 (0.77–4.14)
Var	0.63 (0.22–1.79)	0.85 (0.39–1.83)	1.74 (0.67–4.54)

Table 2. (*Continued*)

NQO1			
Smoking status	Lung (108)[a]	Bladder (119)[a]	Leukemia (160)[a]
All			
Het	0.79 (0.45–1.38)	0.61 (0.34–1.09)	1.09 (0.72–1.66)
Var	8.03 (1.73–37.3)	0.65 (0.2–2.11)	0.55 (0.15–2.06)
Ex-smokers			
Het	1.07 (0.48–2.37)	0.55 (0.24–1.25)	0.94 (0.39–2.24)
Var	5.62 (0.6–52.8)	0.6 (0.16–2.27)	–
Never smokers			
Het	0.57 (0.25–1.31)	0.68 (0.3–1.54)	1.14 (0.7–1.86)
Var	10.5 (1.25–87.8)	0.89 (0.08–9.92)	2.14 (0.42–10.8)
Gender			
Females			
Het	0.71 (0.34–1.51)	0.36 (0.12–1.09)	0.94 (0.54–1.63)
Var	6.01 (1.23–29.35)	(–)	0.65 (0.13–3.27)
Males			
Het	0.91 (0.38–2.15)	0.78 (0.39–1.55)	1.36 (0.7–2.65)
Var	–	0.84 (0.25–2.84)	0.44 (0.05–4.06)

[a]Number of cases. When not specified otherwise, the reference category is the wild type.
CI, confidence interval; Het, heterozygous; Var, variant homozygous.

phase II genes

Meta-analyses indicate that the deletion of GSTM1 and GSTT1 is associated with a slightly increased risk of lung cancer [53, 54] and acute leukemia [24]; bladder cancer has been clearly associated with GSTM1 [28], but not GSTT1 deletions [55, 56]. The effect of some of these genotypes appears to be dependent on the exposure levels, but the evidence is controversial [36, 57, 58]. In a previous investigation in never smokers, the GSTT1 and GSTM1 genotype had no overall effect, but in people exposed to high levels of environmental tobacco smoke [50], the GSTM1*2/*2 genotype was associated with an increased risk.

In one study, the association of the expression of GSTM3 with the GSTM1 polymorphism was dependent on smoking habits, being higher in GSTM1*1 subjects who were smokers, compared with GSTM1*2/*2 smokers [59]. This effect was not seen in long-term nonsmokers, as is the case in this study. In our study, a slightly increased risk of bladder cancer was associated with the GSTM3*2/*2 genotype, similar to the observation by Schnakenberg et al. [60].

The NAT2 slow genotype was associated in several studies with an increased risk for bladder cancer, a risk that was especially pronounced in current smokers [61] and former smokers [28], the latter result being similar to the present observation. A higher, but not statistically significant risk for lung cancer was seen in our study for the NAT2 slow genotype in subjects exposed to ETS. In contrast to our results, a recent Taiwanese study showed that the risk of lung cancer in never smokers was increased in the NAT2 fast genotype [62]. Adult acute leukemia has been associated with exposure to tobacco smoke, but no interaction was evident between NAT2 acetylator status and smoking [63]. In the present study we did not detect, in fact, any association between NAT2 and any type of cancer.

Sulfotransferase IA1 plays an important role in the detoxification of hydroxylated metabolites of PAHs and aromatic amines. Both NAT2 and SULT1A1 are involved in the metabolism to genotoxic metabolites of 3-nitrobenzanthrone, one of the carcinogenic compounds found in diesel exhaust [64]. No clear role for the Sulfotransferase IA1 polymorphism was seen in our study. An increased risk of lung cancer was previously linked to the variant allele especially in current smokers [65].

MTHFR

Only few studies have been carried out on the cancer types included in the present analyses, e.g. lung and bladder cancers and the data are conflicting. The 1298CC genotype was associated with a significantly increased risk of lung cancer in women in one study [66]. In another study, three SNPs (Ala360Ala, 222Val, Pro232Pro) were associated with increased risk of lung cancer in both sexes [67], while the polymorphisms 677C→T and 1298A→C SNP did not show any association [68]. No evidence of an effect of 677C→T variants on bladder cancer was found in two studies [55, 69]; however, the same variants appeared protective in a third investigation [56]. A significantly 3.51-fold increased risk of bladder cancer (95% CI 1.59–6.52 was described in subjects with the variant genotype (CT or TT) reporting a low folate intake) [70]. In the case of myeloid leukemias, previous studies are consistent in showing a protective effect of MTHFR variants. In an investigation in Korea, 1298A→C variants significantly decreased the risks of acute lymphoblastic leukemia (ALL) and chronic myeloid leukaemia (CML) compared with 1298AA. ORs and 95% CIs of 1298AC and 1298AC + CC were 0.53 (0.31–0.93) and 0.54 (0.31–0.93) in ALL and 0.34 (0.14–0.80) and 0.40 (0.18–0.89) in CML, respectively, compared with 1298AA [71]. Similar results were reported from a study in Caucasian adults [72].

Table 3. Phase I genes—odds ratios for matched pairs and 95% CI from logistic regression

CYP1A1			
Smoking status	Lung (110)[a]	Bladder (119)[a]	Leukemia (166)[a]
All			
Het	1.03 (0.53–2)	1.23 (0.67–2.26)	2.22 (1.33–3.7)
Var	3.02 (0.5–18.2)	–	–
Ex-smokers			
Het	0.67 (0.28–1.64)	2.31 (1.02–5.2)	1.14 (0.42–3.11)
Var	2 (0.13–31.97)	–	–
Never smokers			
Het	1.94 (0.69–5.45)	0.49 (0.17–1.4)	2.78 (1.52–5.06)
Var	5.2 (0.45–60.5)	–	–
Gender			
Females			
Het	1.22 (0.48–3.11)	0.55 (0.2–1.5)	1.64 (0.86–3.1)
Var	2.11 (0.29–15.21)	–	–
Males			
Het	0.86 (0.34–2.19)	2.22 (0.98–5.03)	3.75 (1.54–9.11)
Var	–	–	–
CYP1B1			
Smoking status	Lung (111)[a]	Bladder (119)[a]	Leukemia (163)[a]
All			
Het	1.03 (0.5–2.12)	0.94 (0.5–1.77)	0.59 (0.35–1)
Var	1.18 (0.55–2.56)	0.75 (0.38–1.5)	0.47 (0.27–0.84)
Ex-smokers			
Het	1.29 (0.51–3.26)	1.18 (0.51–2.74)	0.3 (0.1–0.92)
Var	1.23 (0.45–3.37)	0.73 (0.29–1.86)	0.26 (0.08–0.86)
Never smokers			
Het	0.72 (0.22–2.31)	0.69 (0.26–1.8)	0.73 (0.4–1.36)
Var	1.09 (0.33–3.65)	0.78 (0.27–2.2)	0.57 (0.29–1.12)
Gender			
Females			
Het	0.65 (0.22–1.92)	1.07 (0.33–3.44)	0.87 (0.45–1.68)
Var	0.62 (0.20–1.91)	0.62 (0.18–2.13)	0.57 (0.28–1.16)
Males			
Het	1.57 (0.57–4.33)	0.89 (0.42–1.9)	0.28 (0.11–0.72)
Var	2.18 (0.71–6.69)	0.84 (0.36–1.95)	0.31 (0.11–0.88)

[a]Number of cases. When not specified otherwise, the reference category is the wild type.
CI, confidence interval; Het, heterozygous; Var, variant homozygous.

strengths and limitations

Our study has several strengths. Since the a priori hypothesis was that genetic susceptibility from low-penetrant gene variants could be larger among people exposed to low levels of environmental exposure, we were able to identify >400 cases of cancer (suspected of an association with ETS or traffic-related air pollution) among nonsmokers. The design is particularly strong because the case–control study nested in the cohort guarantees that the estimates of association are not biased due to case or control selection, a very common phenomenon in usual (hospital- or population-based) case–control studies. Weaknesses include the relatively small number of cases for each cancer type, and the even smaller numbers with information on ETS and air pollution. This limited the possibility of investigating interactions. According to our power calculations, we had 80% power to detect a statistically significant OR of 1.5 for the main effect of common gene variants, but much lower for the study of interactions (although this is one of the largest studies in nonsmokers).

conclusions

Among many comparisons we have made, the only firm associations that we found with single genes, in accordance with previous investigations, were between GSTM1 and bladder cancer and between CYP1B1 and leukemias, whereas the association between leukemia and CYP1A1 has been conflicting in the literature. Another observation that has been repeatedly suggested in the literature is the association between CYP1A1 and lung cancer; we found an OR of 5.2 in never smokers (not statistically significant). New associations that we have detected include MnSOD and leukemias and NQO1 and lung cancer, both indicating a role of oxidative stress in such cancer types.

Table 4. Phase II genes and MTHFR—odds ratios for matched pairs and 95% CI from logistic regression

GSTM1 deletion			
Smoking status	Lung (114)[a]	Bladder (123)[a]	Leukemia (168)[a]
All	1.14 (0.69–1.87)	1.60 (1.00–2.56)	1.25 (0.82–1.89)
Ex-smokers	1.66 (0.84–3.31)	1.47 (0.76–2.84)	1.26 (0.59–2.70)
Never smokers	0.72 (0.34–1.50)	1.75 (0.89–3.43)	1.24 (0.75–2.04)
Gender			
Female	0.85 (0.44–1.62)	1.43 (0.69–2.96)	0.96 (0.57–1.64)
Male	1.73 (0.78–3.84)	1.73 (0.93–3.23)	1.86 (0.94–3.68)
GSTM3 (GSTM3*1/*1 = reference category)			
Smoking status	Lung (109)[a]	Bladder (121)[a]	Leukemia (163)[a]
All			
1/2	0.63 (0.36–1.09)	0.90 (0.53–1.54)	0.84 (0.52–1.36)
1/2	0.42 (0.09–1.99)	1.45 (0.49–4.28)	1.29 (0.36–4.59)
Ex-smokers			
1/2	0.68 (0.33–1.42)	0.61 (0.30–1.27)	1.07 (0.49–2.36)
2/2	0.45 (0.05–4.04)	–	–
Never smokers			
1/2	0.55 (0.23–1.32)	1.89 (0.78–4.60)	0.74 (0.41–1.36)
2/2	0.38 (0.04–3.53)	4.90 (1.15–20.9)	1.50 (0.40–5.63)
Gender			
Female			
1/2	0.63 (0.30–1.28)	1.45 (0.59–3.56)	0.72 (0.38–1.36)
2/2	0.58 (0.12–2.92)	3.12 (0.55–17.81)	2.74 (0.46–16.55)
Male			
1/2	0.60 (0.25–1.47)	0.68 (0.35–1.33)	1.08 (0.52–2.24)
2/2	–	0.76 (0.17–3.45)	0.50 (0.06–4.51)
GSTT1 deletion			
Smoking status	Lung (114)[a]	Bladder (123)[a]	Leukemia (169)[a]
All	0.82 (0.46–1.46)	1.17 (0.68–2.02)	0.84 (0.53–1.33)
Ex-smokers	0.86 (0.40–1.84)	0.95 (0.42–2.13)	0.77 (0.34–1.73)
Never smokers	0.77 (0.32–1.87)	1.40 (0.67–2.94)	0.88 (0.51–1.52)
Gender			
Female	0.94 (0.47–1.90)	1.21 (0.47–3.12)	1.14 (0.64–2.03)
Male	0.63 (0.23–1.71)	1.15 (0.59–2.24)	0.52 (0.24–1.11)
GSTP1 (codon 105) (reference GSTP1*1/*1 = 1)			
Smoking status	Lung (114)[a]	Bladder (123)[a]	Leukemia (166)[a]
All			
*1/*2	1.37 (0.82–2.31)	0.80 (0.49–1.30)	0.83 (0.54–1.25)
*2/2	1.84 (0.86–3.94)	0.76 (0.35–1.68)	1.04 (0.58–1.87)
Ex-smokers			
*1/*2	0.94 (0.33–1.42)	0.70 (0.35–1.38)	1.50 (0.67–3.33)
*2/2	2.31 (0.82–6.53)	0.75 (0.28–2.01)	1.66 (0.51–5.48)
Never smokers			
*1/*2	2.07 (0.97–4.42)	0.92 (0.45–1.85)	0.65 (0.40–1.08)
*2/2	1.31 (0.40–4.32)	0.78 (0.21–2.92)	0.88 (0.45–1.74)
Gender			
Female			
*2/2	1.19 (0.61–2.32)	0.76 (0.34–1.69)	0.85 (0.50–1.46)
*2/2	1.60 (0.57–4.55)	1.00 (0.28–3.63)	0.66 (0.30–1.44)
Male			
*2/2	1.69 (0.75–3.84)	0.81 (0.44–1.50)	0.77 (0.39–1.50)
*2/2	2.16 (0.70–6.64)	0.66 (0.25–1.79)	2.12 (0.81–5.53)

Table 4. (*Continued*)

GSTP1-114			
Smoking status	Lung (115)[a]	Bladder (124)[a]	Leukemia (167)[a]
All			
Het	0.97 (0.49–1.95)	0.83 (0.42–1.67)	1.40 (0.8–2.44)
Var	0.55 (0.06–5.38)	–	2.74 (0.45–16.7)
Ex-smokers			
Het	0.88 (0.33–2.38)	0.53 (0.17–1.66)	1.33 (0.47–3.79)
Var	–	–	–
Never smokers			
Het	1.19 (0.44–3.2)	1.16 (0.47–2.87)	1.42 (0.74–2.72)
Var	–	–	1.77 (0.24–12.9)
Gender			
Females			
Het	0.79 (0.32–1.94)	1 (0.35–2.88)	1.47 (0.66–3.24)
Var	0.96 (0.09–10.7)	–	0.83 (0.07–9.61)
Males			
Het	1.4 (0.46–4.3)	0.73 (0.29–1.84)	1.3 (0.6–2.83)
Var	–	–	–
SULT1A1 (SULT1A1*1/*1 = reference category)			
Smoking status	Lung (116)[a]	Bladder (124)[a]	Leukemia (167)[a]
All			
*1/*2	0.93 (0.55–1.57)	0.88 (0.55–1.43)	0.84 (0.56–1.28)
*2/*2	0.97 (0.45–2.07)	0.73 (0.35–1.54)	0.84 (0.43–1.63)
Ex-smokers			
*1/*2	0.74 (0.35–1.55)	0.85 (0.44–1.66)	1.09 (0.49–2.47)
*2/*2	0.70 (0.24–2.07)	0.32 (0.08–1.30)	0.52 (0.16–1.68)
Never smokers			
*1/*2	1.20 (0.57–2.54)	0.87 (0.43–1.75)	0.78 (0.48–1.27)
*2/*2	1.38 (0.46–4.08)	1.09 (0.45–2.65)	1.13 (0.49–2.58)
Gender			
Female			
*1/*2	0.71 (0.35–1.42)	0.88 (0.38–2.04)	0.81 (0.46–1.41)
*2/*2	0.83 (0.32–2.17)	2.21 (0.71–6.88)	0.86 (0.38–1.94)
Male			
*1/*2	1.32 (0.61–2.88)	0.85 (0.47–1.52)	0.89 (0.48–1.65)
*2/*2	1.21 (0.35–4.24)	0.30 (0.10–0.95)	0.79 (0.25–2.47)
NAT2 genotype (NAT2 rapid = reference category)			
Smoking status	Lung (114)[a]	Bladder (118)[a]	Leukemia (165)[a]
All	0.85 (0.53–1.36)	0.97 (0.62–1.53)	0.73 (0.48–1.11)
Ex-smoker	1.00 (0.54–1.85)	0.79 (0.42–1.47)	1.01 (0.48–2.12)
Never smokers	0.67 (0.31–1.44)	1.23 (0.63–2.43)	0.63 (0.38–1.05)
Gender			
Female	0.80 (0.42–1.53)	1.72 (0.8–3.70)	0.84 (0.50–1.40)
Male	0.91 (0.45–1.85)	0.70 (0.39–1.25)	0.57 (0.28–1.17)
MTHFR			
Smoking status	Lung (113)[a]	Bladder (116)[a]	Leukemia (165)[a]
All			
Het	0.8 (0.46–1.38)	1.03 (0.6–1.76)	0.84 (0.55–1.3)
Var	1.51 (0.75–3.02)	1.55 (0.75–3.18)	1.31 (0.7–2.46)
Ex-smokers			
Het	0.8 (0.38–1.7)	0.86 (0.41–1.78)	0.81 (0.36–1.8)
Var	2.3 (0.83–6.4)	1.42 (0.54–3.75)	1.26 (0.42–3.75)
Never smokers			
Het	0.79 (0.35–1.79)	1.28 (0.56–2.92)	0.86 (0.51–1.44)
Var	1.04 (0.39–2.77)	1.75 (0.59–5.18)	1.34 (0.62–2.88)

Table 4. (*Continued*)

Gender			
Females			
Het	0.93 (0.44–1.95)	1.34 (0.53–3.44)	0.91 (0.53–1.56)
Var	0.97 (0.36–2.6)	1.09 (0.33–3.62)	1.15 (0.51–2.61)
Males			
Het	0.69 (0.3–1.6)	0.87 (0.45–1.7)	0.74 (0.36–1.52)
Var	2.34 (0.83–6.65)	2.08 (0.82–5.29)	1.58 (0.6–4.19)

[a]Number of cases. When not specified otherwise, the reference category is the wild type.
CI, confidence interval; Het, heterozygous; Var, variant homozygous.

Table 5. Cumulative effect of gene variants according to metabolic pathway (a codominant model is assumed; odds ratios and 95% CI from conditional logistic regression)

	Lung	Bladder	Myeloid leukemia
Phase II enzymes			
Trend[a]	0.96 (0.76–1.21)	1.06 (0.86–1.31)	1.00 (0.82–1.21)
No. of variant alleles			
0–1 (ref)	1	1	1
2	0.81 (0.38–1.75)	0.92 (0.45–1.89)	1.07 (0.59–1.92)
3	1.01 (0.49–2.08)	0.88 (0.42–1.83)	1.12 (0.62–2.01)
4+	0.70 (0.26–1.87)	1.25 (0.57–2.76)	0.77 (0.35–1.67)
Phase I enzymes			
Trend	1.02 (0.73–1.43)	1.22 (0.90–1.65)	1.54 (1.19–2.0)
No. of variant alleles			
0 (ref)	1	1	1
1	0.81 (0.42–1.56)	0.89 (0.49–1.61)	0.97 (0.56–1.66)
2	1.06 (0.49–2.33)	1.27 (0.64–2.52)	1.88 (1.03–3.42)
3+	0.97 (0.23–4.19)	3.09 (0.80–12.0)	5.00 (1.63–15.4)
Phase I enzymes, age <55			
Trend	1.28 (0.50–3.21)	1.03 (0.41–2.42)	3.40 (1.39–8.20)
No. of variant alleles			
0 (ref)	1	1	1
1	1.79 (0.32–10.40)	0.74 (0.20–3.43)	0.39 (0.11–2.23)
2	2.66 (0.30–22.43)	0.91 (0.09–7.56)	21.00 (0.93–496)
3+	(–)	1.26 (0.11–34.12)	1052 (1.90–inf)
Oxidative damage scavengers			
Trend	1.21 (0.97–1.51)	0.89 (0.74–1.07)	1.06 (0.88–1.26)
No. of variant alleles			
0–3 (ref)	1	1	1
4	1.08 (0.57–2.07)	0.58 (0.31–1.06)	0.85 (0.49–1.49)
5	1.57 (0.69–3.55)	0.73 (0.38–1.39)	2.00 (1.12–3.54)
6+	2.31 (0.95–5.60)	0.46 (0.19–1.10)	0.74 (0.31–1.76)

[a]Odds ratio for a unit increment of variant alleles.
CI, confidence interval.

A common problem in the literature about low-penetrant genes is the small size that does not allow a full investigation of gene–environment interactions. Although we collected data on ETS and air pollution, the study of the interactions between gene variants and such exposures was limited by the small numbers of cases. This can also explain the extreme variability of estimates in subgroups in the literature. We believe that subgroup analysis is not particularly informative in this like in most of the previous studies.

More rewarding than an analysis by single genes (that is affected by instability of the estimates) was an analysis by pathways, that strongly indicates an involvement of phase I gene variants in myeloid leukemia, with on OR of 5 (1.63–15.4) for those having three or more variant alleles in the pathway. The association was considerably stronger for leukemias arising before the age of 55, a finding which is consistent with a role of genetic susceptibility in carcinogenesis. As suggested before, the analysis of the combined effect of gene

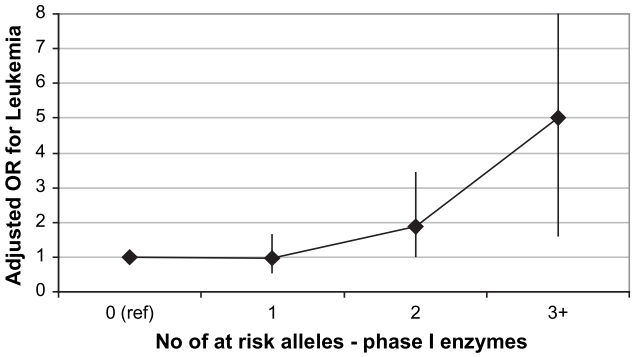

Figure 1. Odds ratios for each unit increment in variant alleles for phase I metabolic genes and risk of leukemia. Vertical bars are confidence intervals.

variants is likely to contribute to the understanding of genetic susceptibility to cancer more than the analysis of single genes [73].

Our analysis by pathway indicates that the distribution of susceptibility to carcinogens in the population should be considered by combining multiple genes and/or multiple SNPs. Under particular circumstances, people with rare combinations of common gene variants have a high risk of cancer and could be assimilated to subjects with highly penetrant mutations.

acknowledgements

Mortality data for The Netherlands were obtained from 'Statistics Netherlands'. This paper was made possible by a grant of the European Community (5th Framework Programme) to PV (grant QLK4CT199900927) and a grant of the Compagnia di San Paolo to the ISI Foundation, and a grant of the EC to the ECNIS Network of Excellence (grant FOOD-CT-2005-513943)(WP4-8). All authors are independent from funders. Also, the work described in the paper was carried out with the financial support of: Europe Against Cancer Program of the European Commission (SANCO); ISCIII, Red de Centros RCESP, C03/09; Deutsche Krebshilfe; Deutsches Krebsforschungszentrum; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra; Cancer Research, UK; Medical Research Council, UK; Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; Wellcome Trust, UK; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skåne, Sweden; Norwegian Cancer Society.

references

1. IARC. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum 2004; 83: 1–1438.
2. Vineis P, Hoek G, Krzyzanowski M et al. Air pollution and risk of lung cancer in a prospective study in Europe. Int J Cancer 2006; 119: 169–174.
3. Vineis P, Airoldi L, Veglia P et al. Environmental tobacco smoke and risk of respiratory cancer and chronic obstructive pulmonary disease in former smokers and never smokers in the EPIC prospective study. BMJ 2005; 330: 277.
4. Peluso M, Munnia A, Hoek G et al. DNA adducts and lung cancer risk: a prospective study. Cancer Res 2005; 65: 8042–8048.
5. Brennan P, Hsu CC, Moullan N et al. Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a mendelian randomisation approach. Lancet 2005; 366: 1558–1560.
6. Vineis P, Bartsch H, Caporaso N et al. Genetically based N-acetyltransferase metabolic polymorphism and low-level environmental exposure to carcinogens. Nature 1994; 369: 154–156.
7. Vineis P, Alavanja M, Garte S. Dose-response relationship in tobacco-related cancers of bladder and lung: a biochemical interpretation. Int J Cancer 2004; 108: 2–7.
8. Stubbins MJ, Wolf CR. Chapter 21. Additional polymorphisms and cancer. IARC Sci Publ 1999; 148: 271–302.
9. Zhu BT. Catechol-O-Methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. Curr Drug Metab 2002; 3: 321–349.
10. McCord JM. Superoxide dismutase in aging and disease: an overview. Methods Enzymol 2002; 349: 331–341.
11. Rosenblum JS, Gilula NB, Lerner RA. On signal sequence polymorphisms and diseases of distribution. Proc Natl Acad Sci USA 1996; 93: 4471–4473.
12. Nebert DW, Roe AL, Vandale SE et al. NAD(P)H:quinone oxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: a HuGE review. Genet Med 2002; 4: 62–70.
13. Siegel D, McGuinness SM, Winski SL, Ross D. Genotype-phenotype relationships in studies of a polymorphism in NAD(P)H:quinone oxidoreductase 1. Pharmacogenetics 1999; 9: 113–121.
14. Ross D, Traver RD, Siegel D et al. A polymorphism in NAD(P)H:quinone oxidoreductase (NQO1): relationship of a homozygous mutation at position 609 of the NQO1 cDNA to NQO1 activity. Br J Cancer 1996; 74: 995–996.
15. Rothman N, Smith MT, Hayes RB et al. Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609C–>T mutation and rapid fractional excretion of chlorzoxazone. Cancer Res 1997; 57: 2839–2842.
16. Kellermann G, Shaw CR, Luyten-Kellerman M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. N Engl J Med 1973; 289: 934–937.
17. Vineis P, Malats N, Cuzick J et al. Metabolic Polymorphisms and Susceptibility to Cancer. Lyon, France: IARC Sci Publ 1999.
18. Crofts F, Cosma GN, Currie D et al. A novel CYP1A1 gene polymorphism in African-Americans. Carcinogenesis 1993: 1729–1731.
19. Shimada T, Watanabe J, Kawajiri K et al. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 1999; 20: 1607–1613.
20. Bailey LR, Roodi N, Dupont WD, Parl FF. Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. Cancer Res 1998; 58: 5038–5041.
21. Hanna IH, Dawling S, Roodi N et al. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res 2000; 60: 3440–3444.
22. Ryk C, Berggren P, Kumar R et al. Influence of GSTM1, GSTT1, GSTP1 and NAT2 genotypes on the p53 mutational spectrum in bladder tumours. Int J Cancer 2005; 113: 761–768.
23. Strange RC, Fryer AA. The glutathione S-transferases: influence of polymorphism on cancer susceptibility. IARC Sci Publ 1999; 148: 231–249.
24. Ye Z, Song H. Glutathione s-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukaemia: a systematic review and meta-analysis. Eur J Cancer 2005; 41: 980–989.
25. Nakajima T, Elovaara E, Anttila S et al. Expression and polymorphism of glutathione S-transferase in human lungs: risk factors in smoking-related lung cancer. Carcinogenesis 1995; 16: 707–711.

26. Engelke CE, Meinl W, Boeing H, Glatt H. Association between functional genetic polymorphisms of human sulfotransferases 1A1 and 1A2. Pharmacogenetics 2000; 10: 163–169.
27. Raftogianis RB, Wood TC, Otterness DM et al. Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. Biochem Biophys Res Commun 1997; 239: 298–304.
28. Garcia-Closas M, Malats N, Silverman D et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 2005; 366: 649–659.
29. Terry PD, Goodman M. Is the association between cigarette smoking and breast cancer modified by genotype? A review of epidemiologic studies and meta-analysis. Cancer Epidemiol Biomarkers Prev 2006; 15: 602–611.
30. Borlak J, Reamon-Buettner SM. N-acetyltransferase 2 (NAT2) gene polymorphisms in Parkinson's disease. BMC Med Genet 2006; 7: 30.
31. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004; 159: 423–443.
32. Eichholzer M, Luthy J, Moser U, Fowler B. Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence. Swiss Med Wkly 2001; 131: 539–549.
33. Kim DH, Ahn YO, Lee BH et al. Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea. Cancer Lett 2004; 216: 199–205.
34. Bingham S, Riboli E. Diet and cancer—the European Prospective Investigation into Cancer and Nutrition. Nat Rev Cancer 2004; 4: 206–215.
35. Autrup JL, Thomassen LH, Olsen JH et al. Glutathione S-transferases as risk factors in prostate cancer. Eur J Cancer Prev 1999; 8: 525–532.
36. Sorensen M, Autrup H, Tjonneland A et al. Glutathione S-transferase T1 null-genotype is associated with an increased risk of lung cancer. Int J Cancer 2004; 110: 219–224.
37. Okkels H, Sigsgaard T, Wolf H, Autrup H. Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. Cancer Epidemiol Biomarkers Prev 1997; 6: 225–231.
38. Ozawa S, Tang YM, Yamazoe Y et al. Genetic polymorphisms in human liver phenol sulfotransferases involved in the bioactivation of N-hydroxy derivatives of carcinogenic arylamines and heterocyclic amines. Chem Biol Interact 1998; 109: 237–248.
39. Autrup H. Gene-Environment interaction in environmental carcinogenesis. In: Cancer as an Environmental disease, Eds. P. Nicolopoulou-Stamati, L. Hens and C.V. Howard. Kluwer Academic Publishers: Dordrecht 2004; 71–101.
40. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutat Res 2004; 567: 1–61.
41. Morgan GJ, Smith MT. Metabolic enzyme polymorphisms and susceptibility to acute leukemia in adults. Am J Pharmacogenomics 2002; 2: 79–92.
42. Alberg AJ, Brock MV, Samet JM. Epidemiology of lung cancer: looking to the future. J Clin Oncol 2005; 23: 3175–3185.
43. Vineis P, Veglia F, Benhamou S et al. CYP1A1 T3801 C polymorphism and lung cancer: a pooled analysis of 2451 cases and 3358 controls. Int J Cancer 2003; 104: 650–657.
44. Larsen JE, Colosimo ML, Yang IA et al. CYP1A1 Ile462Val and MPO G-463A interact to increase risk of adenocarcinoma but not squamous cell carcinoma of the lung. Carcinogenesis 2006; 27: 525–532.
45. Agundez JA. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab 2004; 5: 211–224.
46. Aydin-Sayitoglu M, Hatirnaz O, Erensoy N, Ozbek U. Role of CYP2D6, CYP1A1, CYP2E1, GSTT1, and GSTM1 genes in the susceptibility to acute leukemias. Am J Hematol 2006; 81: 162–170.
47. Joseph T, Kusumakumary P, Chacko P et al. Genetic polymorphism of CYP1A1, CYP2D6, GSTM1 and GSTT1 and susceptibility to acute lymphoblastic leukaemia in Indian children. Pediatr Blood Cancer 2004; 43: 560–567.
48. D'Alo F, Voso MT, Guidi F et al. Polymorphisms of CYP1A1 and glutathione S-transferase and susceptibility to adult acute myeloid leukemia. Haematologica 2004; 89: 664–670.
49. Gallegos-Arreola MP, Batista-Gonzalez CM, Delgado-Lamas JL et al. Cytochrome P4501A1 polymorphism is associated with susceptibility to acute lymphoblastic leukemia in adult Mexican patients. Blood Cells Mol Dis 2004; 33: 326–329.
50. Wenzlaff AS, Cote ML, Bock CH et al. GSTM1, GSTT1 and GSTP1 polymorphisms, environmental tobacco smoke exposure and risk of lung cancer among never smokers: a population-based study. Carcinogenesis 2005; 26: 395–401.
51. Georgiadis P, Topinka J, Vlachodimitropoulos D et al. Interactions between CYP1A1 polymorphisms and exposure to environmental tobacco smoke in the modulation of lymphocyte bulky DNA adducts and chromosomal aberrations. Carcinogenesis 2005; 26: 93–101.
52. Sorensen M, Autrup H, Tjonneland A et al. Genetic polymorphisms in CYP1B1, GSTA1, NQO1 and NAT2 and the risk of lung cancer. Cancer Lett 2005; 221: 185–190.
53. Houlston RS. Glutathione S-transferase M1 status and lung cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 1999; 8: 675–682.
54. Benhamou S, Lee WJ, Alexandrie AK et al. Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. Carcinogenesis 2002; 23: 1343–1350.
55. Karagas MR, Park S, Warren A et al. Gender, smoking, glutathione-S-transferase variants and bladder cancer incidence: a population-based study. Cancer Lett 2005; 219: 63–69.
56. Moore LE, Wiencke JK, Bates MN et al. Investigation of genetic polymorphisms and smoking in a bladder cancer case-control study in Argentina. Cancer Lett 2004; 211: 199–207.
57. Cote ML, Kardia SL, Wenzlaff AS et al. Combinations of glutathione S-transferase genotypes and risk of early-onset lung cancer in Caucasians and African Americans: a population-based study. Carcinogenesis 2005; 26: 811–819.
58. Alexandrie AK, Nyberg F, Warholm M, Rannug A. Influence of CYP1A1, GSTM1, GSTT1, and NQO1 genotypes and cumulative smoking dose on lung cancer risk in a Swedish population. Cancer Epidemiol Biomarkers Prev 2004; 13: 908–914.
59. Anttila S, Luostarinen L, Hirvonen A et al. Pulmonary expression of glutathione S-transferase M3 in lung cancer patients: association with GSTM1 polymorphism, smoking, and asbestos exposure. Cancer Res 1995; 55: 3305–3309.
60. Schnakenberg E, Breuer R, Werdin R et al. Susceptibility genes: GSTM1 and GSTM3 as genetic risk factors in bladder cancer. Cytogenet Cell Genet 2000; 91: 234–238.
61. Vineis P, Marinelli D, Autrup H et al. Current smoking, occupation, N-acetyltransferase-2 and bladder cancer: a pooled analysis of genotype-based studies. Cancer Epidemiol Biomarkers Prev 2001; 10: 1249–1252.
62. Chiou HL, Wu MF, Chien WP et al. NAT2 fast acetylator genotype is associated with an increased risk of lung cancer among never-smoking women in Taiwan. Cancer Lett 2005; 223: 93–101.
63. Rollinson S, Roddam P, Willett E et al. NAT2 acetylator genotypes confer no effect on the risk of developing adult acute leukemia: a case-control study. Cancer Epidemiol Biomarkers Prev 2001; 10: 567–568.
64. Arlt VM, Glatt H, Muckel E et al. Activation of 3-nitrobenzanthrone and its metabolites by human acetyltransferases, sulfotransferases and cytochrome P450 expressed in Chinese hamster V79 cells. Int J Cancer 2003; 105: 583–592.
65. Wang Y, Spitz MR, Tsou AM et al. Sulfotransferase (SULT) 1A1 polymorphism as a predisposition factor for lung cancer: a case-control analysis. Lung Cancer 2002; 35: 137–142.
66. Shi Q, Zhang Z, Li G et al. Sex differences in risk of lung cancer associated with methylene-tetrahydrofolate reductase polymorphisms. Cancer Epidemiol Biomarkers Prev 2005; 14: 1477–1484.
67. Shen M, Rothman N, Berndt SI et al. Polymorphisms in folate metabolic genes and lung cancer risk in Xuan Wei, China. Lung Cancer 2005; 49: 299–309.
68. Shen H, Spitz MR, Wang LE et al. Polymorphisms of methylene-tetrahydrofolate reductase and risk of lung cancer: a case-control study. Cancer Epidemiol Biomarkers Prev 2001; 10: 397–401.
69. Sanyal S, Festa F, Sakano S et al. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 2004; 25: 729–734.

70. Lin J, Spitz MR, Wang Y et al. Polymorphisms of folate metabolic genes and susceptibility to bladder cancer: a case-control study. Carcinogenesis 2004; 25: 1639–1647.
71. Hur M, Park JY, Cho HC et al. Methylenetetrahydrofolate reductase A1298C genotypes are associated with the risks of acute lymphoblastic leukaemia and chronic myelogenous leukaemia in the Korean population. Clin Lab Haematol 2006; 28: 154–159.
72. Skibola CF, Smith MT, Kane E et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA 1999; 96: 12810–12815.
73. Matullo G, Peluso M, Polidoro S et al. Combination of DNA repair gene single nucleotide polymorphisms and increased levels of DNA adducts in a population-based study. Cancer Epidemiol Biomarkers Prev 2003; 12: 674–677.