REVIEW

Genetic metabolic polymorphisms and the risk of cancer: a review of the literature

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The purpose of this paper is to systematically analyse the design and results of epidemiological studies on the association between various types of cancer (lung, bladder, breast, colon, stomach) and four genetically-based metabolic polymorphisms, involved in the metabolism of several carcinogens (glutathione-S-transferase M1, debrisoquine hydroxylase, N-acetyltransferase, aryl hydrocarbon hydroxylase). These inherited polymorphisms usually cause modifications in the quality or quantity of the relevant enzymes. Such enzymes are involved in the activation/inactivation of known carcinogens and seem to modify the extent to which carcinogens interact with DNA in target tissues. Two enzymes, debrisoquine hydroxylase and aryl hydrocarbon hydroxylase, activate procarcinogens to carcinogens (phase I enzymes). The other two, glutathione-S-transferase M1 and N-acetyltransferase, mainly detoxify carcinogenic substances (phase II enzymes). Because of their role as host factors (modulating the action of carcinogens), it has been hypothesized that subjects presenting a specific phenotype for such polymorphisms could be at a greater risk of developing various types of cancer. A number of epidemiological studies have investigated such associations, often with discordant results. We examine and discuss the design of the studies, and present a meta-analysis of the available data.

Keywords: *cancer, metabolic polymorphisms, cancer susceptibility genes, GSTM1, NAT2, CYP2D6, CYP1A1, AHH.*

Abbreviations: *AHH, Aryl hydrocarbon hydroxylase; GST, Glutathione-S-transferase; NAT, N-acetyltransferase.*

Introduction: description of the polymorphisms

Glutathione-S-transferase (GSTM1)

Cytosolic glutathione-*S*-transferases are a large family of isozymes involved in detoxification of many electrophilic substrates (Mannervik 1985), by their conjugation with reduced glutathione. They can be divided by electrophoresis into four classes; α, θ, μ, π (Board *et al.* 1990). The class μ contains a specific isozyme, designated GSTM1 or GST1, that is polymorphically distributed, being present only in about 40% of Caucasians (Seidegard *et al.* 1984). The μ form has been found in high concentrations in the liver, adrenal gland and leucocytes. The absence of the isozyme is due to an inherited deletion of both paternal and maternal alleles of the GSTμ1 gene (Seidegard *et al.* 1984), transmitted in an autosomic dominant way. GSTM1 has been shown to play a role in the metabolism of organic epoxides and peroxides and in particular to conjugate known carcinogens as epoxides of polycyclic aromatic hydrocarbons (PAHs) (Warholm *et al.* 1981), suggesting that people who lack the gene are at greater risk of developing cancers associated with exposure to PAHs.

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Debrisoquine hydroxylase (CYP2D6)

Debrisoquine hydroxylase belongs to the cytochromes P450 family, enzymes involved in the oxidative metabolism of many lipophilic substances (Nebert and Gonzalez 1987). Usually, these enzymes catalyse hydroxylation of the substrate, enhancing its hydrophilicity and improving its metabolism and excretion. Many carcinogens, including PAHs and some carcinogenic aromatic amines, have been shown to be hydroxylated by cytochrome P450 enzymes; this group of enzymes activates carcinogens from their parent form to a more reactive state with higher carcinogenic activity (Wolf 1986).

It has been observed that a small proportion (10% in Caucasian populations) of subjects treated with debrisoquine, an antihypertensive drug, experienced a stronger and more prolonged hypotensive effect than other subjects. These subjects had a lower capability to hydroxylate the drug to 4-hydroxydebrisoquine, debrisoquine's major metabolite (Mahgoub *et al.* 1977, Idle *et al.* 1978, Ayesh *et al.* 1984). The low activity of debrisoquine hydroxylase in these subjects has been found to be due to an inherited autosomal recessive trait in the P450 gene CYP2D6, located on chromosome 22 (Mahgoub *et al.* 1977). In recent years many other drugs, which are substrates of debrisoquine hydroxylase—such as neuroleptics, tricyclic antidepressants, β-blocking agents and dextrometorphan (Meyer *et al.* 1990)—have been identified. NNK—a tobacco-specific nitrosamine and a metabolite of nicotine—has been proposed as one of the substrates of the enzyme (Crespi *et al.* 1991).

N-acetyltransferase

The *N*-acetyltransferase polymorphism was discovered during the 1950s through the observation of inter-individual variability in the metabolism of isoniazid (Evans 1989). Such variability was found to be due to the presence, in about one-half of Caucasian populations, of a low activity rate of *N*-acetyltransferase in metabolizing isoniazid (Evans and White 1964). Through the administration of this drug and the measure of ratios of acetylated to non-acetylated metabolites, researchers were able to classify subjects as fast and slow acetylators. Family studies have demonstrated that this metabolic polymorphism depends on a mutation in the NAT2

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gene, transmitted in an autosomal dominant way. Slow acetylators are homozygous recessive individuals, while fast acetylators are both wild homozygous or heterozygous (Evans *et al.* 1960). Other drugs (sulphamethazine, dapsone, hydralazine, aminoglutethimide, procainamide) have been shown to be substrates of the polymorphic *N*-acetyltransferase. This enzyme has also been shown to polymorphically acetylate arylamines, including well known bladder carcinogens, to arylamides (Hearse and Weber 1973). *N*-acetyltransferase competes in this reaction with *N*-hydroxylase, which transforms arylamines into active carcinogens. This finding has suggested that *N*-acetylation is a detoxifying step in the metabolism of arylamines and that individuals who are slow acetylators are at higher risk of developing bladder cancer. On the other hand, fast acetylators have been found to be at increased risk of developing colorectal cancer (Ilett *et al.* 1987), apparently because some carcinogenic heterocyclic aromatic amines (HAA) derived from food are *N*-hydroxylated rather than *N*-acetylated as a detoxifying step. HAAs show high enzymatic affinity for hepatic *N*-hydroxylase; *N*-hydroxylated compounds can then be acetylated by the colonic mucosal *N*-acetyltransferase to *N*-acetoxyarylamines, which are able to form adducts to DNA (Kadlubar *et al.* 1992).

Recently, another *N*-acetyltransferase, expressed by the NAT1 gene, has been found to be polymorphic (Vatsis and Weber 1993) and to participate in the *N*-acetylation of some carcinogenic aromatic amines which are also substrates of NAT2 enzyme (Grant 1993).

CYP1A1

The enzyme superfamily, cytochrome P450, plays multiple roles in the maintenance of body homeostasis including metabolism of prostaglandins, steroids, hormones, and aliphatic fatty acids. It also has the unique capability to activate numerous xenobiotics, including known procarcinogenic components of tobacco (Kawajiri *et al.* 1990, Amos *et al.* 1992). The cytochrome P4501A (CYP1A) genes are located on chromosome 15q22-qter (Jaiswal *et al.* 1985). P4501A1 (CYP1A1) gene product aryl hydrocarbon hydroxylase (AHH) catalyses the first step in the metabolism of polycyclic aromatic hydrocarbons (PAH) to carcinogens. Three CYP1A1 polymorphisms have been identified. These include MspI, Exon 7 and AA MspI, which is a race-specific polymorphism only found in African–American and African populations (Crofts *et al.* 1993, Garte *et al.* 1996). The MspI polymorphism is closely linked with the Exon 7 polymorphism in Caucasians and Asians, but not in African–Americans. The frequency of the three polymorphisms shows racial differences (Hayashi *et al.* 1992, Hirvonen *et al.* 1992, Cosma *et al.* 1993). It has been reported that Exon 7 polymorphism is related to an elevated level of CYP1A1 catalytic activity (Crofts *et al.* 1994).

MATERIALS AND METHODS

A search through Medline and in personal archives identified relevant epidemiological studies on GSTM1, CYP2D6, CYP1A1, and NAT published until June 1995. Only case-control studies have been selected; the relationship between a polymorphism and a specific site of cancer has been included when at least two studies on that topic had been published.

In order to compare the design of the epidemiological studies, information was collected for the following categories: number and type of cases and controls, response rate, phenotyping and genotyping techniques, relevant exposures, covariates examined, therapies. Information on the study design is contained in Tables 1–4.

Statistical analysis

Cases and controls have been compared for the frequency of the relevant phenotype/genotype by means of the χ^2 test.

Meta-analysis has been performed using the Mantel–Haenszel technique for stratified data, testing each group for heterogeneity. All statistical analyses have been conducted using the EPISTAT statistical package.

Results of the meta-analysis are reported in Figures 1–9, showing overall meta-Odds Ratios (OR) and their corresponding 95% confidence limits. Meta-ORs are given separately by ethnic group and occasionally by phenotype/genotype. Confidence limits have been computed according to Cornfield's method (Cornfield 1956).

The Odds Ratios (ORs) shown have been computed on the basis of crude data and of stratified data, when available. The power of the study has been computed as the probability of finding a statistically significant association between the relevant polymorphism and disease, assuming that the true OR is 2 and that the proportion of the relevant phenotype/genotype in the population is the same as found in controls. Estimates of power of the studies have been performed by using the statistical program POWER 3, elaborated by R. Shore, at the Department of Epidemiology of NYU, on the basis of the method published by Fleiss *et al.* (1980), with $\alpha = 0.05$.

Results

Study design

Cases

The use of incident cases is usually preferable, since in prevalent cases the distribution of the polymorphism could be affected by various factors, whose importance increases with time since diagnosis. Cases could change their phenotype with disease progression. Metabolic impairment has been observed in advanced stages of different types of cancer. In addition, treatment with drugs may modify the enzyme activity. Cases who are carriers of a particular polymorphic gene could experience better survival than others.

In the studies we have reviewed, the proportion of investigations based on prevalent cases was 10/21 for GSTM1, 21/25 for NAT, 9/16 for CYP2D6 (debrisoquine hydroxylase) and 9/32 for CYP1A1.

The distribution of allelle frequencies has been shown to vary widely between different ethnic groups; the variable 'ethnic group', in that it is associated with the polymorphism and is a risk factor for the disease, plays a potential confounding role. Such a role should be controlled in data analysis or by restriction techniques.

Controls

The choice of healthy rather than hospital controls is usually preferable, since in hospital controls the frequency of a

specific phenotype could be associated with the occurrence of the disease leading to hospitalization.

Hospital controls were used in 3 out of 16 studies on the CYP1A1 genotype, and in 11 out of 14 studies on the AHH phenotype; several studies on AHH were based on controls affected by lung disease. Hospital controls were used in 6 out of 16 studies on CYP2D6, in 12 out of 25 studies on NAT and in 11 out of 21 studies on GSTM1.

Response rates

A low response rate could imply a selection bias; lower response rates occurred particularly in the choice of outpatients. If phenotyping requires administration of drugs with possible adverse reactions, such as debrisoquine, it is possible that a higher proportion of poor metabolizers, who are more likely to have experienced side effects, will deny their participation in the study, causing an underrepresentation of PM in controls (Shaw *et al.* 1995). Unfortunately, response rates were reported in a limited number of investigations; in the case of different studies on CYP1A1 they were 80.5% in cases and 87.8% in controls (Hamada *et al.* 1995); 95% in cases (Taioli *et al.* 1995a); 40% in cases and 52% in controls (Ambrosone *et al.* 1995); 72.4% in cases and 53% in controls (Sivaraman *et al.* 1994); 90.7% in cases and 80% in controls (Petruzzelli *et al.* 1988); and 97% in cases (Yoshikawa *et al.* 1994). For CYP2D6 the response rates were 26% in one study (Caporaso *et al.* 1990); 80.5% in cases, 59% in outpatient and 98% in inpatient controls in another study (Shaw *et al.* 1995). For GSTM1 they were 59% in cases, 80% in random-digit dialling controls and 67% in other controls (Heckbert *et al.* 1992). No response rates are apparently available for NAT studies.

Phenotyping and genotyping

Since the techniques for genotyping are relatively standardized, we have shown in Tables 1–4 only the names of such techniques. For phenotyping we have collected information on: the name and dosage of the substance administered, the kind of analysis and, when relevant, the metabolic ratio. The latter is the ratio of the parent compound and of a specific metabolite, and represents the criterion to separate phenotypic categories.

Sources of error in metabolic phenotyping can be due to variation in subject compliance and dose of the substance administered, to different ways of sample collection and storage, to exposure to drugs or to other factors able to inhibit or induce enzyme activity (Brosen *et al.* 1987) and to measurement errors.

Genotyping is affected by errors related to DNA techniques such as contamination during the analysis, false priming in PCR assays (Heim and Mayer 1991), and detection of a pseudogene.

The measurement error in the characterization of both phenotypes and genotypes is given by the accuracy of the test performed, i.e. by its sensitivity and specificity, defined respectively by the proportion of susceptibles correctly classified and the proportion of non-susceptibles correctly classified as such. The prevalence of the polymorphism must be considered in evaluating the bias introduced by misclassification in the measure of effect. In fact, when a polymorphism is widespread (over 85%), sensitivity is important, since small decreases cause the misclassification of many subjects, while decreasing specificity produces little bias. Conversely, when the prevalence is very low (under 15%), even small variation in specificity may cause important variation of the Odds Ratio (OR), while variation in sensitivity does not affect much the value of the true OR (Rothman *et al.* 1993).

Different metabolic ratios have been used as thresholds between phenotypic categories (Tables 1–4). For example, for the NAT phenotype six studies used dapsone, with a threshold of 0.3 in five studies and 0.4 in one; 13 studies used sulphamethazine, with a threshold of 0.6 in two studies and 0.3 in the others. The dose administered ranged between 50 and 100 mg for dapsone and between 500 mg and 1 g, or 10 mg kg^{-1} and 40 mg kg^{-1} for sulphamethazine. Two studies used isoniazid.

Exposures

Since polymorphic enzymes are believed to interact with carcinogens, activating or inactivating them, assessment of the relevant exposures is essential. In the absence of exposure no difference in risk should be found between phenotypes/genotypes. Unfortunately we do not know the relevant carcinogenic substrates for all the polymorphisms. Components of tobacco smoke are frequently involved: smoking was the relevant exposure in 12 out of 21 studies on GSTM1, in 11 out of 25 studies on NAT, in 8 out of 16 studies on CYP2D6, in 7 out of 17 studies on CYP1A1 and in 4 out of 14 studies on AHH. Occupation was frequently considered for NAT (9/25).

Covariates

We show in Tables 1–4 variables, such as age, gender, ethnicity, tumour grade, place of residence and others, that have been controlled for in data analysis, according to a stratified or multivariate analysis. These variables are expected to play different roles in the study of the association between polymorphism and cancer: some of them are potential confounders, while others are effect modifiers. Age is usually not regarded as a confounder in genotype-based studies, since the genetic make-up does not change during the life of a subject. However, there are several ways in which age can exert an indirect confounding role: (1) several exposures, e.g. occupational exposures, are strongly age-dependent; (2) the population prevalence of some genotypes may differ at different ages if that genotype is associated with survival.

Therapy

We have included in Tables 1–4 information on chemotherapy, radiotherapy, or surgery to which patients were submitted before phenotyping, for the possible interference that therapies could exert on polymorphic enzyme activity.

Statistical power

A statistical power greater than 80% (for an Odds Ratio equal or greater than 2.0 and $\alpha = 0.05$, two-tails), was attained in 12/21 studies on GSTM1, 1/16 studies on CYP2D6 (considering intermediate metabolizers with extensive metabolizers), 4/25 studies on NAT, 4/16 studies on the

Author	Number of cases	Number of controls	Phenotyping/ genotyping	Exposures	Covariates	Therapy	Slow acetylators in controls	Power ($\alpha = 0.05$, OR > 2)
Bulovskaya *et al.* 1978 (USSR)	41 prevalent **breast cancer** (Caucasian)	38 healthy controls matched for age (Caucasian)	sulphamethazine: < 51 kg–500 mg, 51–83 kg–750 mg, > 83 kg–1000 mg; blood and urine collected after 6 h; analysis performed by spectrophotometry				63%	24.8%
Lower *et al.* 1979 (Sweden, Denmark)	186 prevalent **bladder cancer** histologically confirmed (Caucasian)	192 hospital and healthy controls (Caucasian)	sulphamethazine 10 mg kg^{-1} orally, blood and urine collected after 4.5 h; analysis performed by spectrophotometry	**smoking** for cases (light smokers < 1 pack/day; heavy smokers > pack/day)	**residence** (rural, urban)		51%	89%,
Cartwright *et al.* 1982 (UK)	111 prevalent **bladder cancer** histologically confirmed (Caucasian)	95 hospital, 112 healthy controls (Caucasian)	dapsone 50 mg orally, blood collected after 2–6 h; metabolic ratio monoacetyl dapsone/ dapsone = 0.3; analysis performed by HP thin-layer chromatography	**smoking** for cases (> 5 years, 5 years before diagnosis); **occupation** for cases (engineers, clinical, dye workers)	**gender, age, tumour grade**	no radio- or chemotherapy before phenotyping, nor therapy with: sulphonamides, isoniazid, procainamide, hydralazine	57%	75.4%
Miller and Cosgriff 1983 (USA)	26 prevalent **transitional cell bladder cancer** histologically confirmed (Caucasian)	26 healthy controls (Caucasian)	sulphamethazine: < 51 kg–500 mg, 51–83 kg–750 mg, > 83 kg–1000 mg; blood and urine collected after 6 h; analysis performed by spectrophotometry	**smoking** for cases (smokers = > 1 pack/day for > 5 years, 15 years before diagnosis); **occupation** for cases (list of jobs involving exposure to bladder carcinogens)		no chemotherapy before phenotyping	69%	10.8%
Mommsen and Maggold 1985 (Denmark)	228 incid. **bladder cancer** histologically confirmed from a rural area (Caucasian)	100 cancer-free urology patients matched for age from a rural area (Caucasian)	sulphamethazine 1 g orally, blood and urine collected after 4.5 h; analysis performed by spectrophotometry		**residence** (rural)	no chemotherapy before phenotype, nor therapy with: sulphonamides, isoniazid, hydralazine	54%	76.5%
Ladero *et al.* 1985 (Spain)	130 prevalent **transitional cell bladder cancer** histologically confirmed (Caucasian)	157 healthy controls (Caucasian)	sulphamethazine 10 mg kg^{-1} orally; blood and urine collected after 6 h; analysis performed by spectrophotometry	**smoking** and **occupation** for cases (list of jobs involving exposure to bladder carcinogens)	**age, tumour grade**		57.4%	74.8%
Hanssen *et al.* 1985 (Germany)	105 prevalent **bladder cancer** histologically confirmed (Caucasian)	42 healthy controls (Caucasian)	sulphamethazine, MR = 0.3	**smoking** for cases, **occupation** for cases (exposure to bladder carcinogens)	**drug abuse** (analgesics, artificial sweeteners, **tumour grading, tumour staging** (superficial, infiltrating)	no chemotherapy before phenotyping	42.9%	39.8%
Burgess and Trafford 1985 (UK)	53 prevalent **lung cancer** histologically confirmed	31 healthy controls (Caucasian?)	sulphamethazine 40 mg kg^{-1} orally; blood collected after 6 h		**cancer histology**		58%	23.2%

Karakaya *et al.* 1986 (Turkey)	23 prevalent **bladder cancer** histologically confirmed (Caucasian)	109 healthy volunteers (Caucasian)	sulphamethazine 40 mg kg^{-1} orally; blood collected after 6 h; analysis performed by spectrophotometry			no chemotheraphy before phenotyping	61.5%	17.1%
Lang *et al.* 1986 (USA)	43 prevalent **colorectal cancer**, male, 45–75 years old (mixed)	41 hospital controls, male, 45–75 years old (mixed)	sulphamethazine 10 mg kg^{-1} orally, blood and urine collected after 4.5 h; analysis performed by HPLC				68.3% (mixed)	25.6%
Kaisary *et al.* 1987 (USA, UK)	98 prevalent **transitional cell bladder cancer** histologically confirmed (Caucasian)	110 urology patients (Caucasian)	dapsone 100 mg orally, blood collected after 8 h; MR = 0.4; analysis performed by HPLC	**smoking, alcohol, occupation** (no exposure to known bladder carcinogens)	**gender, age, tumour grade**	no drugs known to influence the dapsone metabolism before phenotyping	49%	63.4%
Ladero *et al.* 1987 (Spain)	81 prevalent **breast cancer** histologically confirmed (Caucasian)	75 healthy or suffering from diseases not related to the acetylator phenotype (Caucasian)	sulphamethazine 10 mg kg^{-1} orally, blood and urine collected after 6 h; analysis performed by spectrophotometry		**menopausal status** (pre- or post-)	no hormonal, radio- or chemotherapy before phenotyping nor drugs known to influence the sulphamethazine metabolism	60% (Caucasian)	50.8%
Ilett *et al.* 1987 (Australia)	49 prevalent **colorectal cancer** histologically confirmed (Caucasian)	41 old hospital controls or volunteers matched for age, gender, smoking; 45 young healthy volunteers (Caucasian)	sulphamethazine 15 mg kg^{-1} orally, blood collected after 5, 6, 7, 8, 9 h; urine collected after 5–6 and 7–8 h; MR = 0.6; analysis performed by HPLC	**occupation** (no exposure to known carcinogens)		no chemotherapy before phenotyping	66.3% (Caucasian)	40.7%
Philip *et al.* 1987 (UK)	181 incid. **breast cancer** and 136 incid. **benign breast disease** histologically confirmed (mixed)	337 healthy controls (Caucasian)	dapsone 50 mg orally, blood collected after 2–6 h; MR = 0.3; analysis performed by HPLC		**age, tumour grade, sex hormones receptor status**		55.2% (mixed)	95.4% for breast cancer, 90.8% for benign disease
Philip *et al.* 1988 (UK)	126 prevalent **lung cancer** histologically confirmed (Caucasian)	82 hospital controls, 191 hospital controls > 65 years old (Caucasian)	dapsone 100 mg orally, blood collected after 1.5–4 h; MR = 0.3; analysis performed by HPLC	**smoking** for cases and for 82 controls	**cancer histology**	no therapy before phenotyping	50.5%	85.8%
Horai *et al.* 1989 (Japan)	51 prevalent **transitional cell bladder cancer** histologically confirmed (Asians)	203 healthy controls (Asians)	dapsone 100 mg orally, blood collected after 2–6 h; metabolic ratio monoacetyl dapsore/ dapsone = 0.3; analysis performed by HPLC	**smoking** for cases (none, < 20, > 20 cigarettes/day), **occupation** for cases (no exposure to β-naphthylamine, benzidine, 4-aminobiphenyl)	**gender, age, tumour grade**	no drugs known to influence the dapsone metabolism before phenotyping	6.4%	22.4%
Webster *et al.* 1989 (UK)	100 prevalent **breast cancer** histologically confirmed (Caucasian)	32 breast lumps and 68 healthy controls (Caucasian)	isoniazid 200 mg orally, blood collected after 3 h; analysis performed by HPLC				59% (Caucasian)	62.9%

Table 1. Study design: *N*-acetyltransferase.

Author	Number of cases	Number of controls	Phenotyping/ genotyping	Exposures	Covariates	Therapy	Slow acetylators in controls	Power ($\alpha = 0.05$, OR > 2)
Ilet *et al.* 1990 (Australia)	45 incid. **breast cancer** histologically confirmed (Caucasian)	28 healthy and 20 hospital controls (Caucasian)	sulphamethazine 500 mg orally, blood collected after 6 h; MR = 0.6; analysis performed by HPLC	**smoking**	**age** (5 years grouping), **tumour grade, sex hormones receptor status**		64.6% (Caucasian)	29.6%
Hanke and Krajewska 1990 (Poland)	67 prevalent **bladder cancer** (Caucasian)	22 healthy controls, 90 workers exposed to benzidine (Caucasian)	isoniazid	**occupation** for cases (exposed or not to aromatic amines)			45.4% healthy controls, 67% workers exposed to benzidine	21.2% vs healthy controls, 12.4% vs workers exposed to benzidine
Wohlleb *et al.* 1990 (USA)	same as Lang *et al.* 1986	same as Lang *et al.* 1986	same as Lang *et al.* 1986	**occupation, food, home heating**	**age** (< 64, > 64 years), **marital status** (single, married), **race** (white, non-white), **exercise** (< 1, > 1 time/week)		68.3% (mixed)	25.6%
Ladero *et al.* 1991 (Spain)	61 prevalent **colon cancer** and 48 prevalent **rectal cancer** histologically confirmed (Caucasian)	96 healthy controls (Caucasian)	sulphamethazine 10 mg kg^{-1} orally, blood and urine collected after 6 h; analysis performed by spectrophotometry		**age, gender, surgery treatment** (yes or no), **tumour site** (colon, rectum)	no drugs known to influence the sulphamethazine metabolism before phenotyping; surgery treatment for 74 cases	58.3%	63.9%
Hayes *et al.* 1993 China	38 prevalent **bladder cancer** histologically confirmed, exposed to benzidine (Asians)	43 controls with negative cytology exposed to benzidine (Asians)	dapsone 100 mg orally, blood collected after 3 h; MR = 0.3; analysis performed by HPLC; PCR	**smoking** (none, < 20, > 20 cigarettes/day); **occupation** (benzidine exposure estimated; low, medium, high)	**gender, age** (50–59, 60–69, >69 years), **residence, weight**		23.2%	21.5%
Shibuta *et al.* 1994 (Japan)	234 prevalent **colorectal cancer** histologically confirmed (Asians)	329 healthy volunteers (Asians)	PCR		**age** (< 60, > 60 years) **tumour site** (colon, rectum), **histological type** (well, moderately, poorly differentiated)		9.6%	44%
Oda *et al.* 1994 (Japan)	36 prevalent **colorectal cancer** histologically confirmed (Asians)	36 autopsied controls matched for age (Asians)	RFLP				8.3%	4%
Risch *et al.* 1995 (UK)	19 prevalent **bladder cancer** (Caucasian)	59 urological controls matched for age (Caucasian)	RFLP	**smoking** for 186 cases and 43 controls, **occupation** for all cases and 43 controls	**gender**		44.1%	58.2%
Agundez *et al.* 1995 (Spain)	160 incid. **breast cancer** histologically confirmed (Caucasian)	132 healthy controls (Caucasian)	PCR		**cancer histology, menopausal status** (pre- or post-)		50.8% (Caucasian)	79.3%

Table 1. (*cont.*)

Author	Number cases	Number of controls	Phenotyping/ genotyping	Exposures	Covariates	Therapy	Slow acetylators in controls	Powerof (α = 0.05, OR > 2)
Seidegard *et al.* 1966 (USA, Sweden)	66 incid. **lung cancer** histologically confirmed; > 45 years; smokers > 20 cigarettes/day (Caucasian?)	7 healthy controls randomly from a cohort study, matched for age and smoking; > 45 years; smokers > 20 cigarettes/day (Caucasian?)	Trans-stilbene oxide conjugation in leucocytes	**smoking** (heavy, light smokers)	**age** (> 65, < 65 years) **gender, cancer histology**		41.6%[a]	90.6%[a]
Seidegard *et al.* 1990 (USA)	125 incid. **lung cancer** histologically confirmed; smokers > 20 pack-years (Caucasian?)	114 healthy controls from a cohort study, matched for age and smoking; smokers > 20 pack-years (Caucasian?)	Trans-stilbene oxide conjugation in leucocytes	**smoking**	**age** (< 65, > 65 years), **gender, cancer histology**		41.6%[a]	90.6[a]
Zhong *et al.* 1991 (U.K.)	228 prevalent **lung cancer** histologically confirmed, smokers (Caucasian)	225 controls chosen randomly from two hospitals and a group of volunteers (Caucasian)	RIA in leucocytes RFLP		**cancer histology**		41.8%	94.7%
Strange *et al.* 1991 (UK)	26 prevalent operated **colon adnocarcinoma** and 19 **stomach adeno-carcinoma** histologically confirmed (Caucasian)	49 controls deceased for cardiovascular diseases (Caucasian)	Gel electrophoresis in liver tissue		**tumour stage**		40.8%	21.3% (colon), 16.7% (stomach)
Heckbert *et al.* 1992 (USA)	66 incid. **lung cancer** (Caucasian)	120 healthy controls selected by random-digit dialing and randomly from Social Security list (Caucasian)	Trans-stilbene oxide conjugation in leucocytes carcinognens	**smoking** (<20, > 20 pack-years), **occupation** (exposure to lung carcinogens), **alcohol** (never, ex-, current drinkers)	**age, gender**	no change after chemotherapy, radiation therapy, surgery	58%	48.8%
Hayashi *et al.* 1992 (Japan)	212 prevalent **lung cancer** histologically confirmed (Asians)	358 healthy controls, chosen randomly from a cohort study (Asians)	PCR		**cancer histology, CYP1A1 polymorphism**		46.6%	97.3%
Harada *et al.* 1992 (Japan)	19 prevalent **stomach cancer**, 32 prevalent **hepatocellular carcinoma**, 65 prevalent, **breast cancer** (Asians)	84 healthy blood donors (Asians)	PCR				47.6%	27.8% (stomach), 47.1% (breast)
Brockmoller *et al.* 1993 (Germany)	117 incid. **lung cancer** histologically confirmed < 85 years (Caucasian)	155 hospital controls, mainly pulmonary; 200 hospital controls from an intensive care unit (Caucasian)	Trans-stilbene oxide conjugation in leucocytes ELISA; PCR	**smoking** for cases and 155 controls (non-smokers, 1–20, > 20 pack-years)	**age** (<70, > 70 years), **gender, cancer histology**	no surgical chemotherapeutic or radiation treatment	52.9%	86.4%

Table 2. Study design: GSTM1.

[a] Results of Seidegard *et al.* 1986 and 1990 are combined.

Author	Number of cases	Number of controls	Phenotyping/ genotyping	Exposures	Covariates	Therapy	Slow acetylators in controls	Power ($\alpha = 0.05$, OR > 2)
Nakachi *et al.* 1993 (Japan)	85 incid. **squamous cell carcinoma of the lung** histologically confirmed (Asians)	170 healthy controls randomly from a cohort study, > 40 years, matched for gender and age (Asians)	PCR	**smoking** (< 32.1×10^4, > 32.1×10^4 cumulative cigarette dose)	**CYP1A1 polymorphism**		49.4%	67.4%
Nazar-Stewart *et al.* 1993 (USA)	35 incid. deceased or operat. **lung cancer** histologically confirmed; > 30 years; > 100 cigarettes in lifetime (mixed: 2 non-white cases)	43 controls deceased or operated for causes unrelated to smoking; 30–80 years; > 100 cigarettes in lifetime (mixed: 3 non-white controls)	TSO conjugation in leucocytes; immunological assay with GST-μ antibodies; RFLP	**smoking** (< 54, > 54 pack-years)	**age** (< 64, > 64 years), **gender, race, cancer histology**		48.3% (mixed)	23.8%
Hirvonen *et al.* 1993c (Finland)	138 incid. **lung cancer** histologically confirmed (Caucasian)	142 healthy controls, 36 hospital controls having a benign lung tumour (Caucasian)	PCR	**smoking** (non-smokers, < 40, > 40 pack-years) for cases and 36 controls	**cancer histology**		43.7%	83.2% vs all controls. 78.8% vs healthy controls, 38.3% vs hospital controls
Daly *et al.* 1993 (UK)	53 prevalent **bladder transitional cell carcinoma** (Caucasian?)	52 urology clinic patients with negative cystoscopy; 5 healthy volunteers (Caucasian?)	PCR				57.4%	41.8%
Bell *et al.* 1993 (USA)	229 incid.+prevalent **bladder transitional cell carcinoma** histologically confirmed (Caucasian, African–Americans)	211 urology clinic patients matched on race, gender, age; 466 healthy controls (Caucasian, African–Americans)	PCR	**smoking** (non-smokers, 1–50, > 50 pack-years) for cases and clinic controls	**race, tumour grade**		49% (Caucasian), 35% (African–American)	93.7%, 92.1% (Caucasian), 6.7% African–Americans
Zhong *et al.* 1993 (UK)	97 prevalent **bladder cancer**, 197 prevalent **breast cancer**, 196 prevalent **colon cancer** (Caucasian)	225 randomly from two hospitals and a group of volunteers (Caucasian)	PCR		**tumour site** (proximal, distal) for colon cancer		41.8%	77.6% (bladder), 93.1% breast 93% (colon)
Lafuente *et al.* 1993 (Spain)	75 incid. **bladder transitional cell carcinoma**, histologically confirmed; smokers (Caucasian)	127 healthy controls, smokers, matched for age and smoking history (Caucasian)	ELISA in leucocytes	**smoking**	age (< 70, > 70 years), **tumour grade and penetration**	no chemo-therapeutic or radiation treatment	44.9%	57.8%

Kihara *et al.* 1994 (Japan)	178 incid. **lung cancer** histologically confirmed, < 69 years, smokers (Asians)	201 healthy controls, < 69 years, smokers, from a general health check-up (Asians)	PCR	**smoking** (< 800, 800–1200, > 1200 cigarettes × day × years), **occupation** (no exposure to lung carcinogens)	**gender, cancer histology**		45.3%	89.9%
Alexandrie *et al.* 1994 (Sweden)	296 incid. **lung cancer** histologically confirmed (Caucasian)	329 healthy controls from laboratory personnel, chimney sweeps and welders < 65 years; 79 COPD patients (Caucasian)	Trans-stilbene oxide conjugation in leucocytes; PCR		**age** (< 65, > 65 years), **gender, cancer histology**		52.2% all, 52.9% healthy, 49.4% hospital (Caucasian)	99.1% vs all controls, 98.4% vs. healthy controls, 73.4% vs hospital controls
Brockmoller *et al.* 1994 (Germany)	296 prevalent **bladder cancer** histologically confirmed (Caucasian)	400 hospital controls (Caucasian)	Trans-stilbene oxide conjugation and ELISA in serum; PCR	**smoking** (non smokers, 1–20, 20–50, > 50 pack-years), **occupation** (exposure to bladder carcinogens) for cases and 296 controls	**age** (< 55, 55–65, 65–75, > 75 years), **gender, tumour grade and stage, cancer histology**		50.7%	99.2%
Lin *et al.* 1994 (USA)	114 incid. **bladder cancer** histologically confirmed (mixed)	1104 healthy controls (mixed)	PCR		**race**		49% (Caucasian), 45% (African–Americans), 48% (Asians)	92.2%, 80.7% Caucasian, 11.7% African–Americans
Nakajima *et al.* 1995 (Finland)	27 prevalent **lung cancer** histologically confirmed (Caucasian)	11 non-lung cancer controls (Caucasian)	immunoblot analysis, enzyme assay	**smoking**		no chemotherapy or radiotherapy before phenotyping	64%	8.1%
Katoh *et al.* 1995 (Japan)	83 prevalent **transitional cell carcinoma** histologically confirmed (65 of the bladder, 12 renal pelvis, 6 ureter) (Asians)	101 healthy controls from a community (Asians)	PCR				42.6%	58.3%

Table 2. (*cont.*)

Author	Number of cases	Number of controls	Phenotyping/ genotyping	Exposures	Covariates	Therapy	PM in controls	Power ($\alpha = 0.05$, OR > 2)
Ayesh *et al.* 1984 (UK)	245 prevalent **lung cancer** histologically confirmed, smokers (Caucasian)	234 COPD smokers matched for gender, age, smoking history (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by electron capture GC; MR = 1.0, 12.6	**smoking** (> 20 pack-years)		no surgery, radio- or chemotherapy before phenotyping	9%	39.0%
Cartwright *et al.* 1984 (UK)	122 prevalent **bladder cancer** (Caucasian)	94 hospital controls (Caucasian?)	DBQ 10 mg administration and 8 h urine collection; analysis performed by alkali-flame ionization gas–liquid chromatography; MR = 12.6	**occupation** (exposure to benzidine)			2%	8.8%
Roots *et al.* 1988 (Germany)	270 prevalent **lung cancer** histologically confirmed < 80 years (Caucasian)	270 hospital controls < 80 years (Caucasian)	DBQ 5, 7.5 or 10 mg administration in relation to age and body weight; 5 h urine collection; analysis performed by GC; MR = 12		**age** (< 50, > 50 years) for cases, **cancer histology, NAT2 polymorphism**	no therapy before phenotyping	11.1%	52.5%
Caporaso *et al.* 1989 (UK, USA)	cases from Ayesh *et al.* 1984	controls from Ayesh *et al.* 1984	same as Ayesh *et al.* 1984	**smoking** (> 20 pack-years), **occupation** (exposure to asbestos and PAH)	**gender, age, cancer histology**	no surgery, radio- or chemotherapy before phenotyping	9%	93.8%
Law *et al.* 1989 (UK)	104 incid. **lung cancer** histologically confirmed, smokers > 10 pack-years (Caucasian)	82 healthy and 22 hospital controls, smokers > 10 pack-years, matched by gender, age, smoking history (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by electron catpure GC; MR = 12.6				8.7%	14.9%
Caporaso *et al.* (1990 (USA)	96 incid. **lung cancer** histologically confirmed (mixed)	55 COPD and 37 controls with cancers other than lung and bladder (mixed)	DBQ 10 mg administration and 8 h urine collection; analysis performed by electron capture GC; MR = 4.8, 11.7 (Caucasian) 4.2, 26.4 African–Americans)	**smoking** (< or > median), **alcohol, occupation** (exposure to lung carcinogens)	**age** (< 64, > 64 years), **gender, race, cancer histology, educational level, hospital**	no radio- or chemotherapy before phenotyping	20% (Caucasian) 5 % (African–Americans)	24%
Benitez *et al.* 1990 (Spain)	125 prevalent **transitional cell bladder cancer** histologically confirmed (Caucasian)	556 healthy controls having no use of drugs (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by flame ionization GC; MR = 12.6	**smoking** (< 10, 10–19, 20–24, > 24 cigarettes/day), **alcohol** (< 40, > 40 g/day) **occupation** (exposure to bladder carcinogens) for cases	**gender**		6.1%	14.4%
Duche *et al.* 1991 (France)	153 prevalent **lung cancer** histologically confirmed, smokers > 20 pack-years (Caucasian)	135 COPD, smokers ≥ 20 pack-years, similar to cases for age and healthy controls (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by flame ionization GC; MR = 13.18			no changes in phenotype in 14 cancer patients	6.7%	19.5%

Benitez *et al.* 1991 (Spain)	84 prevalent **lung cancer** histologically confirmed, all ex- or current smokers, except two (Caucasian)	143 healthy male smokers, similar to cases for gender and smoking history (Caucasian)	DBQ 10 mg administration and 8 h urine collectuon; analysis performed by flame ionization GC; MR = 12.6	**smoking**	**cancer histology**		7%	9.6%
Horsmans *et al.* 1991 (Belgium)	91 prevalent **lung cancer** histologically confirmed; < 80 years (Caucasian)	167 healthy controls (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by flame onization GC; MR = 12.6		**cancer histology**	14 cases had radiotherapy and 6 had chemotherapy more than 4 weeks before	7.2%	11.1%
Ladero, *et al.* 1991b (Spain)	9 prevalent **breast cancer** histologically confirmed, mastectomized (Caucasian)	446 healthy controls (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by flame ionization GC; MR = 12.6			no radio- or chemotherapy before phenotyping	5.%	35.9%
Wolf *et al.* 1992 (UK)	361 prevalent **lung cancer**, 184 prevalent **bladder cancer**, 313 prevalent **breast cancer** (Caucasian)	720 healthy and 151 COPD controls (Caucasian)	PCR		**cancer histology** for lung cancer cases	no radio-chemotherapy or surgery before genotyping	4.3%	33.8% (lung), 14.6% (bladder), 68.6% (breast)
Hiryonen *et al.* 1993 (Finland)	106 incid. **lung cancer** (Caucasian)	122 healthy controls (Caucasian)	DBQ 10 mg administration and 6 h urine collection; analysis performed by GC; MR = 12.6; PCR, RFLP	**smoking** (non-smokers, ex-smokers, < 25, 26–50, ≥ 50 pack-years) for cases	**cancer histology**	no radio-, chemotherapy or surgery before genotyping	5.7%	9.7%
Buchert *et al.* 1993 (USA)	167 incid. **breast cancer** (Caucasian)	114 healthy controls (Caucasian)	PCR, RFLP		**age** (< 40, 40–49, 50–59, > 60 years), **menopausal status** (pre-, post-)		6.1%	24.9%
Agundez *et al.* 1994 (Spain)	89 incid. **lung cancer** histologically confirmed (Caucasian)	98 healthy volunteers (Caucasian)	PCR		**cancer histology**		7%	9.9%
Tefre *et al.* 1994 (Norway, UK)	204 incid. non-operable **lung cancer** histologically confirmed (Caucasian)	117 healthy controls (Caucasian)	PCR	**smoking** (non-smokers, < 20, 20–40, > 40 pack-years) for 165 cases	**age, gender, cancer histology**		5.1%	15.2%
Shaw *et al.* 1995 (USA)	335 incid. **lung cancer** histologically confirmed (Caucasian)	135 outpatients and 23 inpatients, matched for 5-years age group and gender (Caucasian)	DBQ 10 mg administration and 8 h urine collection; analysis performed by HPLC; MR = 7.39	**smoking** (non-smokers, 1–30, 31–49, > 50 pack-years), **occupation** (exposure to asbestos)	**age, gender, education, cancer histology, tumour stage, hospital, medication use**	no therapy before phenotyping	7%	43.7%

Table 3. Study design: CYP2D6

Author	Number of cases	Number of controls	Genotype/phenotype	Exposures	Covariates	Therapy	% Variant in controls[a]	Power ($\alpha = 0.05$, OR = 2)
Kawajiri *et al.* 1990 (Japan)	6 (lung cancer) histologically confirmed (Asian)	104 (healthy from a cohort)	MspI		histological type		51% (18%)	51.2% (23.8%)
Tefre *et al.* 1991 (Norway)	221 (lung cancer) incid., histologically confirmed (Caucasian)	212 (healthy, laboratory staff, prefabricated house factory)	MspI				21% (1%)	87.0% (4.8%)
Nakachi *et al.* 1991 (Japan)	151 (lung cancer) incid., histologically confirmed (Asian)	375 (healthy from a cohort)	MspI	Stratified by smoking < 41 pack-years, 41–55 pack-years, > 55 pack-years,[b] in squamous cell carcinoma	histological type		56% (19)	91.4% (63.5%)
Hirvonen *et al.* 1992 (Finland)	87 (lung cancer) incid., histologically confirmed surgical patients (Caucasian)	121 (healthy blood donors, volunteers)	MspI		histological type		21%	53.3%
Hirvonen *et al.* 1993b (Finland)	74 (lung cancer) incid., histologically confirmed; surgical patients (Caucasian)	118 (healthy, blood donors, workers in institute)	MspI		histological type		21%	51.0%
Shields *et al.* 1993 (USA)	56 (lung cancer) incid., histologically confirmed (28 African, 29 Caucasian)	48(hospital) COPD, asthma, cancer other than lung, bladder; comparable in age (23 African, 25 Caucasian)	MspI	stratified by smoking < 40 pack-years, 40–59 pack-years, > 60 pack-years	histological type		39% African American (9%) 24% Caucasian (4%)	14.9% African American (8.8%) 12.9% Caucasian (–)
Sugimura *et al.* 1994 (Brazil)	110 (lung cancer) incid., histologically confirmed (88 White Brazilian, 22 Black Brazilian)	112 (hospital) cancer-free patients matched by sex, age (90 White Brazilian, 22 Black Brazilian)	MspI	stratified by smoking < 40 pack-years, 40–60 pack-years, > 60 pack-years			38% White Brazilian (11%) 36% Black Brazilian (13%)	57.0% White Brazilian (20%) Black Brazilian (4.4%)

Hayashi *et al.* 1992 (Japan)	212 (lung cancer) prevalent histologically confirmed 95 (stomach) 85 (colon) 98 (breast) (Asian)	358 (healthy from a cohort)	Exon7		Stratified by GSTM1 histological type	35% (7%)	97.2% lung (45.4%) 81.8% stomach (27.7%) 78.1% colon (29.4%) 82.7% breast (30.7%)
Nakachi *et al.* 1993 (Japan)	85 (lung cancer) only squamous cell carcinoma incid., histologically confirmed (Asian)	170 (healthy from a cohort)	MspI Exon 7	Stratified by smoking: ≤ 44 - pack-years, > 44 pack-years[b]	Stratified by GSTM1	51% MspI (15 %) 38% Exon7 (5%)	66.9% MspI (30.7%) 33.3% Exon7 (–)
Alexandrie *et al.* 1994 (Sweden)	296 (lung cancer) incid., histologically confirmed (Caucasian)	329 (healthy, laboratory staff, welders and chimney sweeps)	MspI and Exon7		Stratified by GSTM1 histological type	16% MspI (0.4%) 7% Exon7	92.8% MspI 51.0% Exon7%
Hamada *et al.* 1995 (Brazil)	99 (lung cancer) incid., histologically confirmed (79 White Brazilian, 20 Black Brazilian)	108 (hospital) cancer-free patients matched by sex, age, race (87 White Brazilian, 21 Black Brazilian)	Exon7	Stratified by smoking: non-smokers, < 40 pack-years, 40–59 pack-years, > 59 pack-years		17% White Brazilian 10% Black Brazilian	38.4% White Brazilian 4.9% Black Brazilian
Kelsey *et al.* 1994 (USA)	72 (lung cancer) incid., histologically confirmed (African American)	97 (healthy) matched by sex, age	AA	Stratified by smoking: < 25 pack-years, 25–49 pack-years, > 49 pack-years		24%	47.2%
Taioli *et al.* 1995 (USA)	76 (lung cancer) incid., histologically confirmed (African American)	123 (healthy)	AA	Stratified by smoking non-smokers, never smokers in adenocarcinoma	histological type	16%	43.4%
London *et al.* 1995 (USA)	145 (lung cancer) incid. histologically confirmed (African American)	234 (healthy) comparable in age, ethnicity and sex	AA	Stratified by smoking non-smokers, 1–35 pack-years, > 35 pack-years Occupational exposure to motor vehicle exhaust, asbestos	histological type intake of β-carotene, vitamin E and vitamin C	15%	70.6%

Table 4. Studies design: CYP1A1 and AHH inducibility

[a] The numbers are percentage of combined genotypes (heterozygous + homozgyous) in controls and the numbers in brackets are percentage of homozygous genotype in controls.

[b] Numbers of pack-years were calculated from cigarette dose as following: pack-years = cigarette dose / (20 × 365.25).

Author	Number of cases	Number of controls	Genotype/phenotype	Exposures	Covariates	Therapy	% Variant in controls[a]	Power ($\alpha = 0.05$, OR = 2)
Rebbeck *et al.* 1994 (USA)	96 (breast cancer) incid. (Caucasian)	216 (healthy) comparable in socioeconomic and geographical factors	Exon7					
Ambrosone *et al.* 1995 (USA)	176 (post-menopausal breast cancer) incid. (Caucasian)	228 (healthy)	Exon7		OR adjusted for age, education, age at menarche, age at first pregnancy, age at menopause, body mass index and family history of breast cancer. Stratified by GSTM and age		15% (1%)	75.0% (5.6%)
Taioli *et al.* 1995 (USA)	51 (breast cancer) (21 African American, 30 Caucasian)	269 (healthy) matched by race	MspI, Exon7 and AA				MspI: 40% African American (4%) 20% Caucasian Exon7: 6% African American 17% Caucasian AA: 16% African American	MspI: 21.5% African–American (–) 30.8% Caucasian Exon7: 27.7% Caucasian AA: 17.6% African–American
Sivaraman *et al.* 1994 (USA)	43 (colon *in situ*) incid. 23 Japanese 7 Caucasian 5 Hawaiian 7 Filipino 1 Chinese	114 (healthy) including: 59 Japanese 34 Caucasian 21 Hawaiian matched by sex, age, race; comparable in education, weight, height, smoking, alcohol and family history of colon cancer	MspI Exon7				51% MspI (4%) 30% MspI	27.7% 27.3%
Katoh *et al.* 1995 (Japan)	83 (urothelial cancer) prevalent histologically confirmed (Asian)	101 (healthy)	Exon7				40%	58.4%

Kellermann *et al.* 1973 (USA)	50 (lung cancer) incid., histologically confirmed), smokers	85 (healthy)	AHH ratio (induced/basal) (lymphocyte) low < 2.5 intermediate 2.5–3.6 high > 3.6				51% (intermediate) 17% (high)	29.1% 7.5%
Guirigis *et al.* 1976 (USA)	11 (lung cancer) incid., histologically confirmed	11 (healthy) matched by age, sex	induced AHH (lymphocyte)			no treatment prior phenotyping		46.9%[c]
McLemore *et al.* 1977 (USA)	47 (lung cancer) incid., histologically confirmed	56 (hospital) patients with lung diseases	basal AHH (PAM and lymphocyte) AHH ratio (induced/basal) (lymphocyte)	Stratified by smoking non-smokers, smokers		no treatment prior. phenotyping		
Emery *et al.* 1987 (England)	62 (squamous of lung cancer) prevalent histologically confirmed smokers	62 (hospital) (patients with cardiological disease, smokers) matched by age, sex, social class and smoking	AHH Ratio (induced/basal) (lymphocyte) high AHH: ratio > 4.0				34%	39.8%
McLemore *et al.* 1987 (USA)	14 (lung cancer) incid., histologically confirmed smokers	15 (hospital) patients with other lung disease smokers, comparable in age, smoking and diet	basal and induced AHH ratio (PAM and lymphocyte)			no treatment prior phenotyping		
McLemore *et al.* 1979 (USA)	52 (lung cancer) incid., histologically confirmed smokers	52 (hospital) patients undergoing bronchoscopy smokers comparable in age and diet	basal AHH (PAM) high AHH > 100 mU AHH ratio (induced basal) (lymphocyte) high ratio > 2 fold			no treatment prior phenotyping	28% (basal) 30% (ratio)	35.6% 37.3%
Gahmberg *et al.* 1979 (Finland)	90 (lung cancer) incid.,	404 (Healthy)	induced AHH (lymphoblasts) high AHH: relative value > 1.5			no treatment prior phenotyping	15%	64.3%
Kouri, *et al.* 1982 (USA)	21 (lung cancer) incid., histologically confirmed (White and Black)	30 (hospital) patients with lung diseases comparable in age, race and smoking history	induced AHH (lymphocyte) AHH activity corrected by cytochrome c	smoking	no association with age, family history, history type or location in cases	no treatment prior phenotyping		54.0%[c]
Karki *et al.* 1987 (Finland)	30 (lung cancer) prevalent	80 (hospital) 43 non-smokers 37 smokers	basal, induced and AHH ratio (induced/basal) (lymphocyte) AHH activity corrected by ^{3}H-TdR incorporation					97.9% basal[c] 88.5 % induced 99.8% ratio

Table 4. (*cont.*)

Author	Number of cases	Number of controls	Genotype/phenotype	Exposures	Covariates	Therapy	% Variant in controls[a]	Power ($\alpha = 0.05$, OR = 2)
Liu and Wang 1988 (China)	53 (lung cancer) histology confirmed prevalent	61 (hospital) patients with other lung disease comparable in age, sex and occupational exposure to PAH	basal AHH (lung tissue) AHH/GST (lung tissue)	smoking, occupational exposure to PAH adjusted by stepwise regression model	age, sex, living condition and medicines taken adjusted by stepwise regression model			93.0%[c]
Petruzelli *et al.* 1988 (Italy)	49 (lung cancer) incid., histologically confirmed, surgical patient	16 (hospital) patients with thoracic disease	basal AHH (lung tissue, non-tumourous parenchyma)			no treatment prior phenotyping		46.6%[c]
Abe *et al.* 1990 (Japan)	31 (lung cancer) prevalent, histologically confirmed	25, 5 with mediastinal benign tumour and 20 healthy	basal AHH (lymphocyte & PAM)	stratified by smoking non-smokers, current smokers, ex-smokers				99.9%[c] lymphocyte 53.7% PAM
Yoshikawa *et al.* 1994 (Japan)	56 (lung cancer) prevalent histologically confirmed	36 (hospital) tissue samples from autopsy	basal AHH (lung tissue, normal)	stratified by smoking non-smokers, smokers				98.7%[c]
Nakajima *et al.* 1995 (Finland)	27 (lung cancer) incid., histologically confirmed	11 (hospital)	basal AHH (lung tissue, normal)			no treatment prior phenotyping		27.1%[c]

Table 4. (*cont.*)

CYP1A1 polymorphism (genotype), and 4/14 studies on AHH polymorphism (phenotype).

Meta-analyses

The results of meta-analyses are shown in Figures 1–9. The meta-Odds Ratio (Mantel–Haenszel estimate) for *N*-acetyltransferase and bladder cancer is 1.47 in Caucasians (95% c.i. 1.21–1.78) and 0.49 in Asians (based on two studies; 95% c.i. 0.16–1.42) (Figure 1). Three out of 10 studies in Caucasians had a 95% lower confidence limit greater than one, and for five the limit was around 0.9.

In the case of breast cancer (not shown) the meta-OR was 1.1 (0.9–1.45), based on six studies, all in Caucasians.

For colorectal cancer the meta-OR was 1.67 (1.1–2.52) in Caucasians and 0.84 (0.48–1.46) in Asians, for rapid vs slow acetylators (i.e. the slow phenotype was associated with a protective effect) (Figure 2). The studies based on the genotype coincide with studies conducted in Asians.

GSTM1 showed an overall meta-OR for lung cancer of 1.32 (1.12–1.57) in Caucasians and 1.6 (1.26–2.04, based on three studies) in Asians (Figure 3). Five out of 11 studies showed a 95% lower confidence limit greater than one. Genotype-based investigations gave ORs of 1.15 (Alexandrie *et al.* 1994), 3.32 (Nazar-Stewart *et al.* 1993), 1.05 (Zhong *et al.* 1991), 1.44 (Hirvonen *et al.* 1993c), 1.44 (Hayashi *et al.* 1992), 1.61 (Nakachi *et al.* 1993) and 1.86 (Kihara *et al.* 1994). When only studies based on incident cases and healthy controls were considered, the meta-OR was 1.76 (1.4–2.2) (Figure 4), with a range between 1.3 and 2.4. The meta-OR restricted to smokers was 1.77 (1.4–2.2) (range 1.0–3.3). Disaggregation by histotype gave the following ORs: squamous-cell carcinoma 1.5 (1.2–1.8); small-cell carcinoma 1.9 (1.3–2.9); adenocarcinoma 1.2 (1.0–1.5).

For bladder cancer, the meta-OR concerning GSTM1 was 1.54 (1.3–1.8) for Caucasians (range 0.9–4.3) (Figure 5). Five of seven studies had a 95% lower confidence limit greater than 1.0. In Caucasians the meta-OR was 1.54 (1.3–1.8), in Asians 2.4 (1.3–4.45) and in African–Americans 1.41 (0.5–4.1). Among smokers the meta-OR was 1.59 (1.2–2.1) and in non-smokers was 1.61 (0.9–2.8). Both colon and stomach cancer showed strong associations with the GSTM1 null genotype, with meta-ORs of 1.84 (1.27–2.7) and 3.52 (1.5–8.6) respectively (Figure 6). The association with breast cancer was weaker (OR = 1.25; 0.9–1.8).

Figure 7 shows the data for CYP2D6 (debrisoquine polymorphism) and lung cancer, the only site which has been extensively studied. The overall meta-OR is 1.52 (1.18–1.96), with a range between 0.5 and 13.7. Studies restricted to the phenotype gave a meta-OR of 1.73 (1.3–2.3), while genotype-based studies reported ORs of 0.5, 13.7, and 1.15, with a meta-OR of 6.4 (1.0–14.3), which, however, is not justifiable because of the evident heterogeneity of the single estimates. When studies based on healthy controls only are considered, the meta-OR is 1.26 (0.8–2.0) (range 0.5–13.7). In smokers the

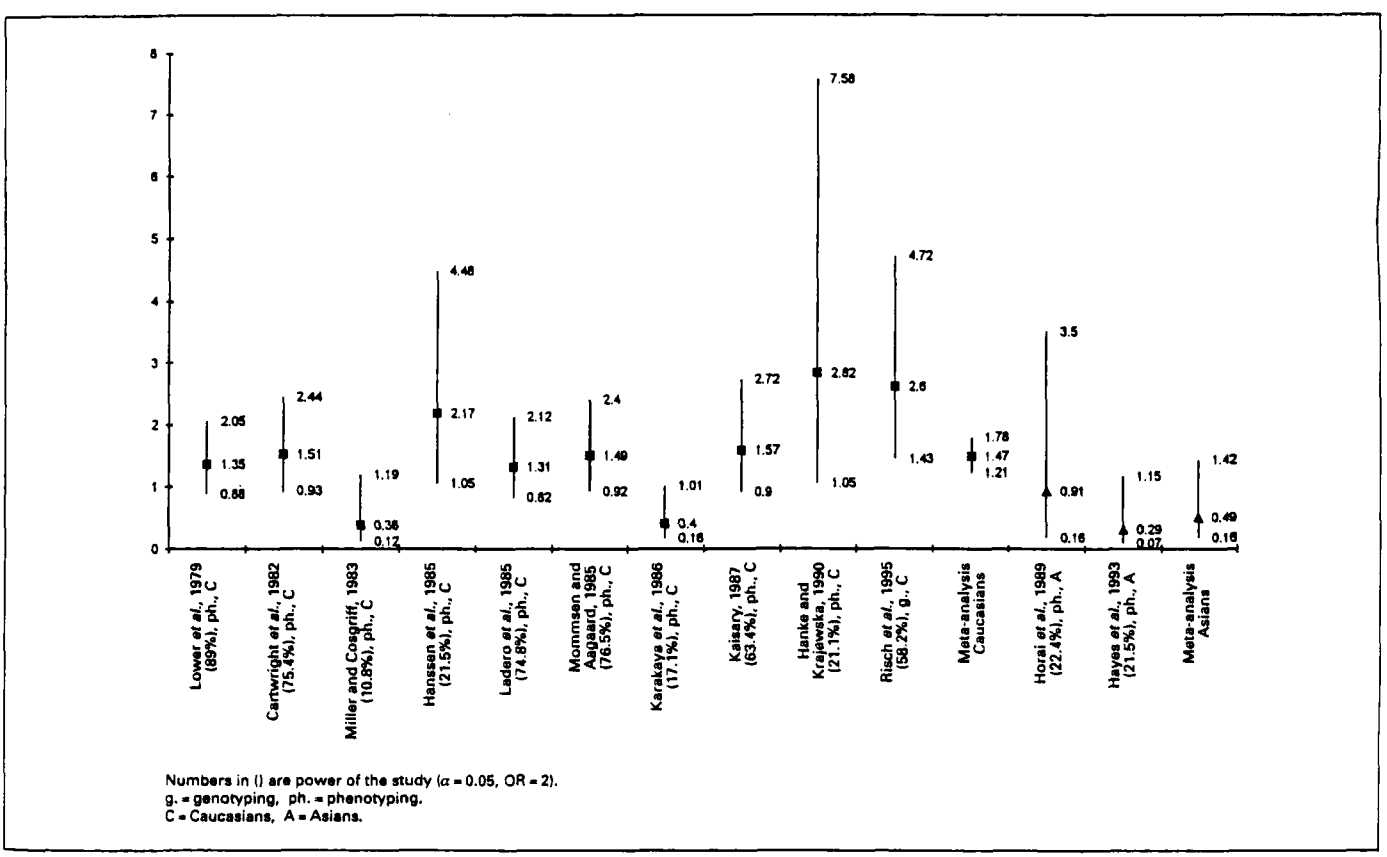

Figure 1. Meta-analysis: *N*-acetyltransferase and bladder cancer.

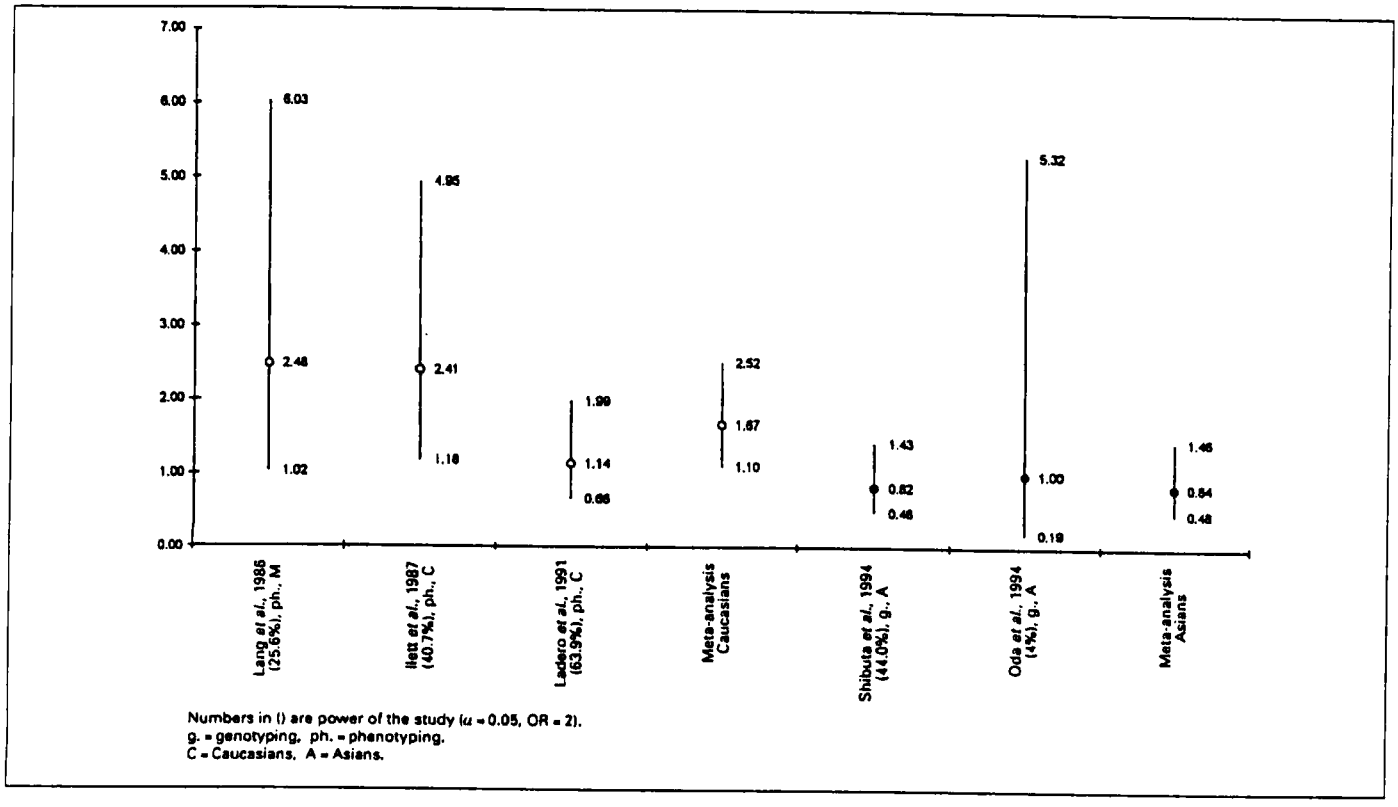

Figure 2. Meta-analysis: *N*-acetyltransferase and colorectal cancer.

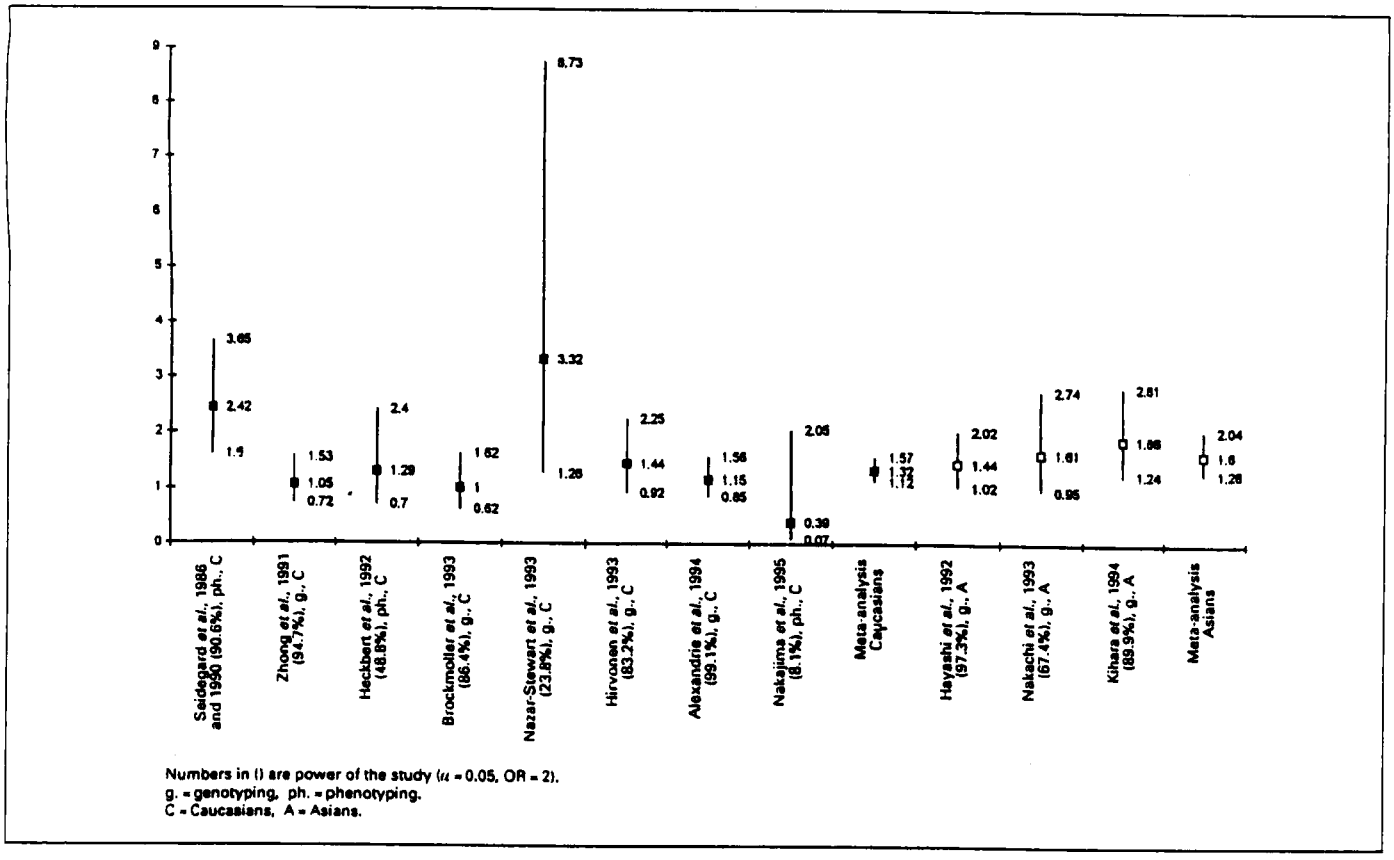

Figure 3. Meta-analysis: GSTM1 and lung cancer.

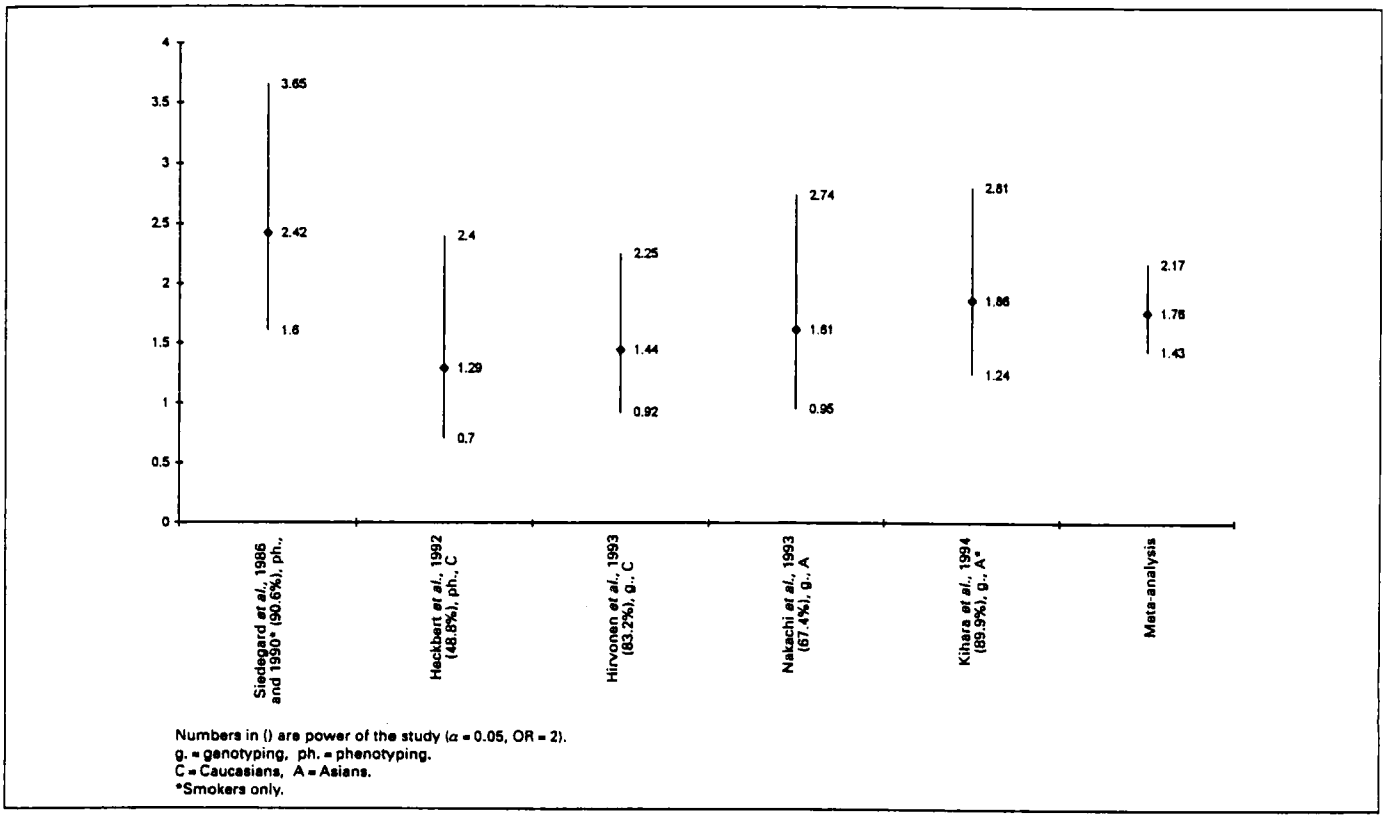

Figure 4. Meta-analysis: GSTM1 and lung cancer—studies considering smoking (incident cases and healthy controls only).

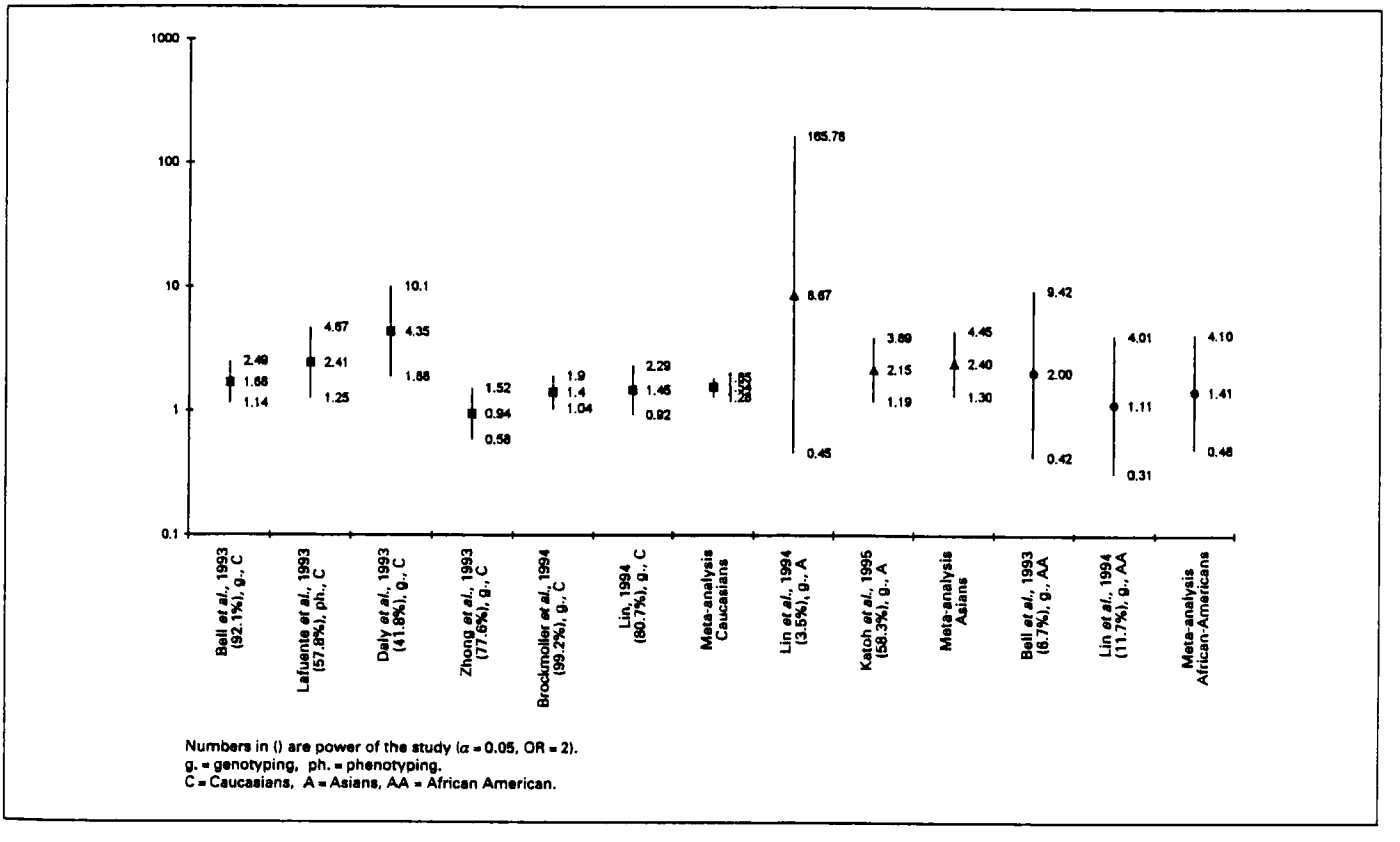

Figure 5. Meta-analysis: GSTM1 and bladder cancer.

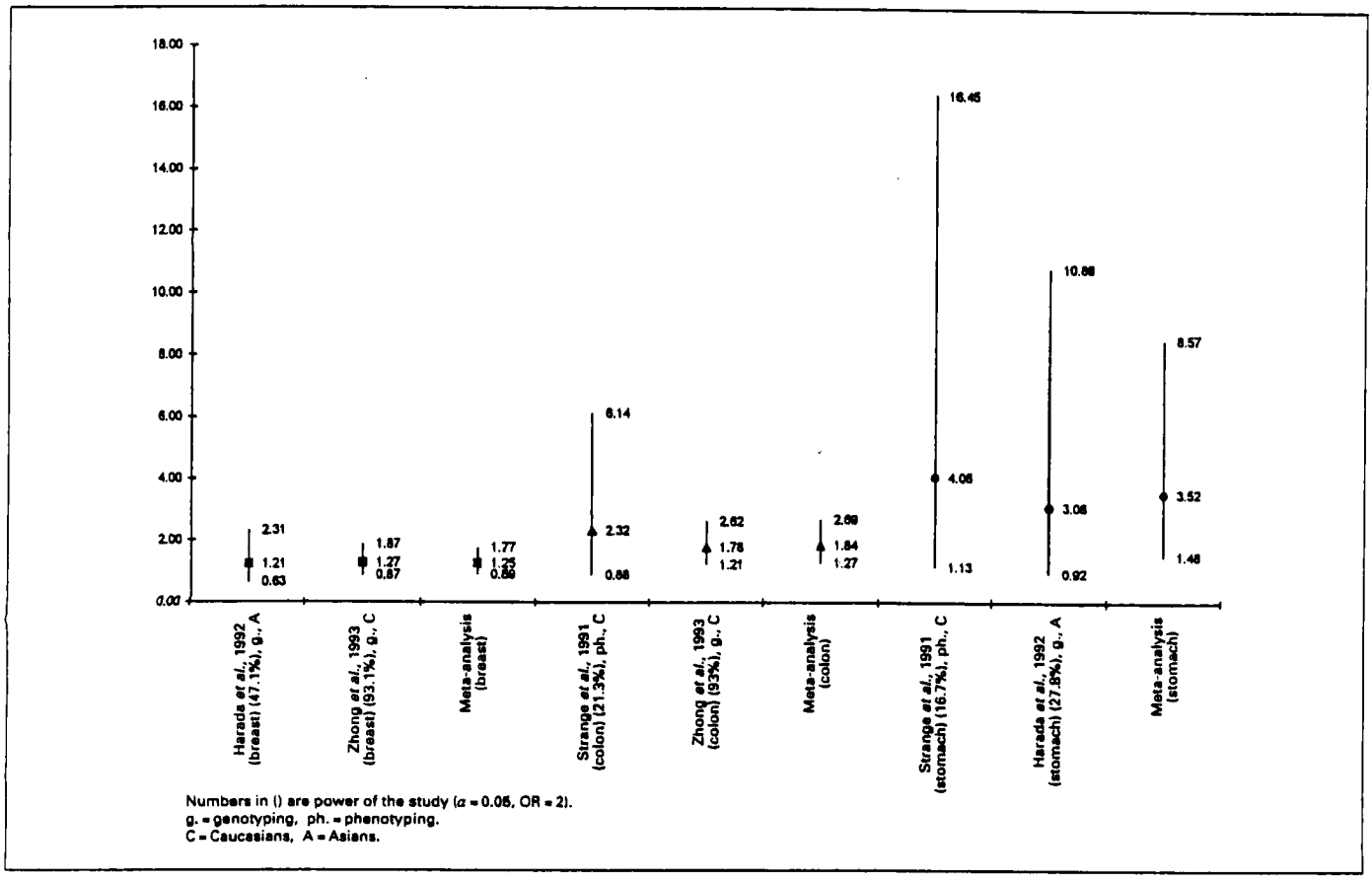

Figure 6. Meta-analysis: GSTM1 and breast, colon and stomach cancer.

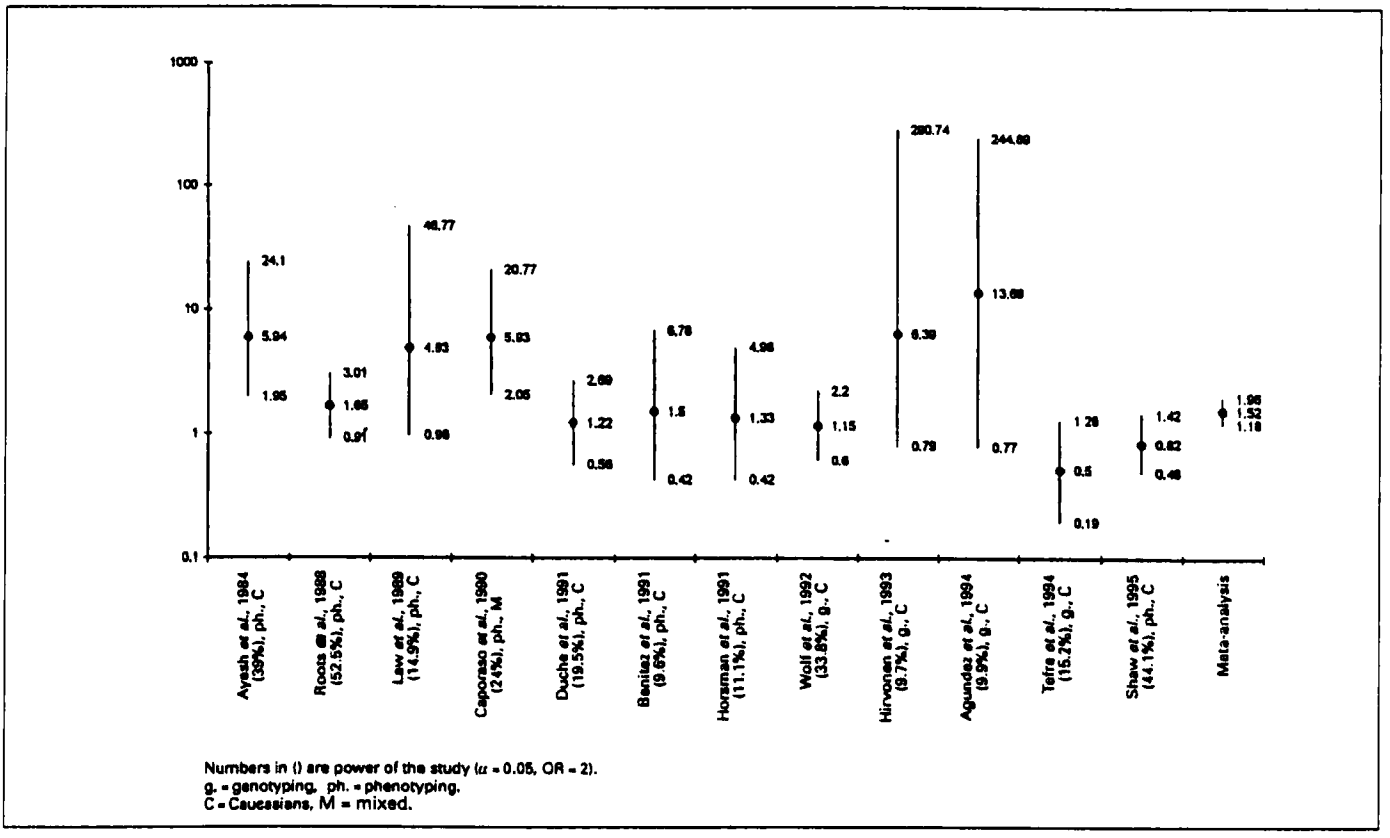

Figure 7. Meta-analysis: CYP2D6 and lung cancer.

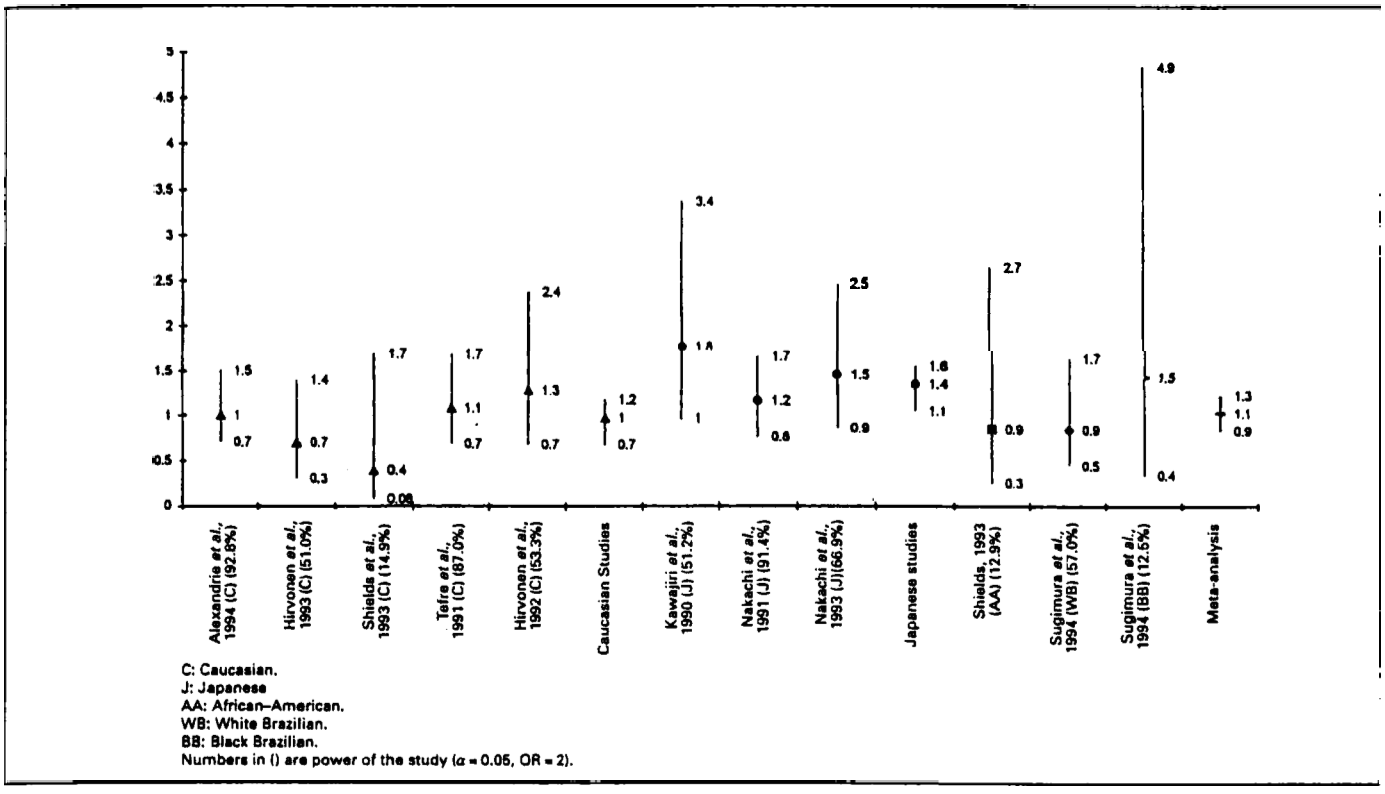

Figure 8. Meta-analysis: combined MspI variant genotypes and lung cancer.

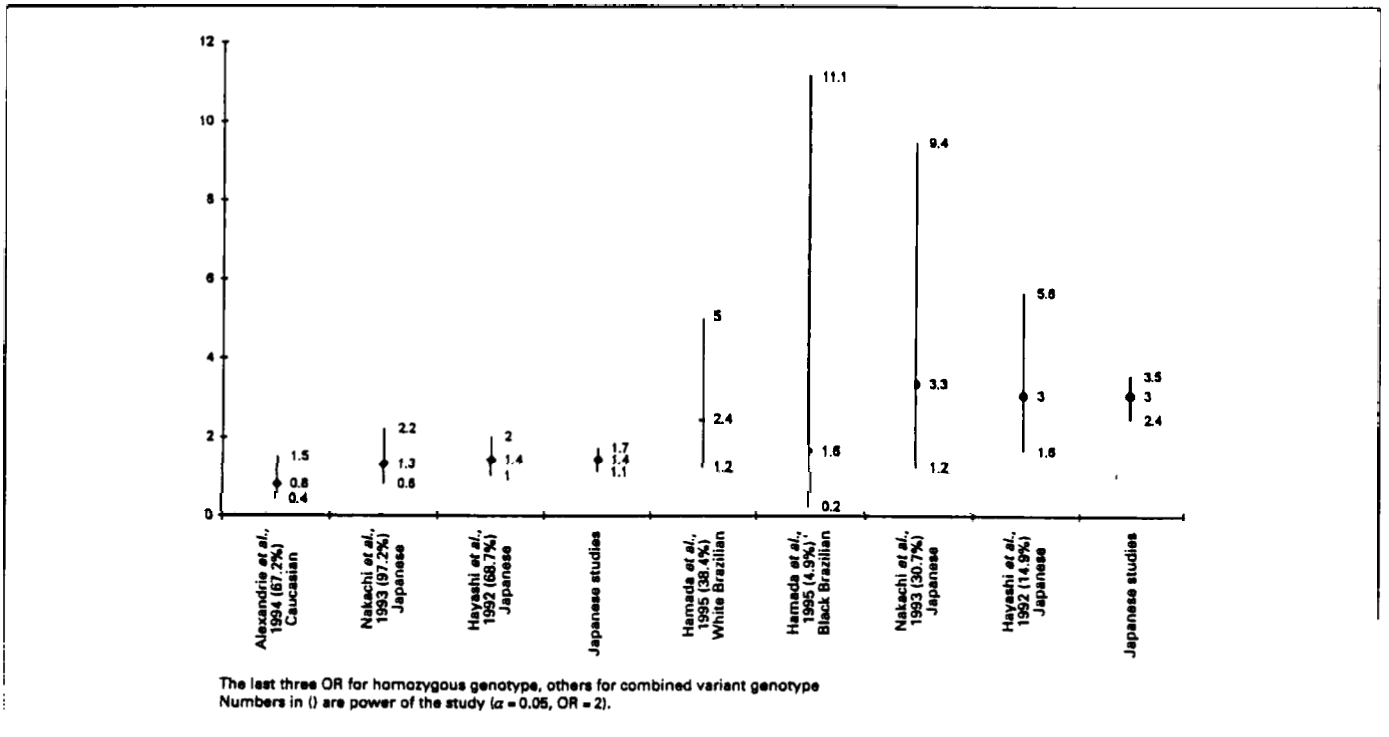

Figure 9. Meta-analysis: Exon7 polymorphism and lung cancer.

overall estimate was 1.74 (1.1–2.76). When the histotype is considered, estimates are 1.9 (1.2–2.9) for squamous cell; 1.08 (0.6–1.8) for small-cell carcinoma; and 1.1 (0.7–1.8) for adenocarcinoma.

Finally, in the case of CYP1A1 (combined MspI variant genotype) and lung cancer, the overall meta-OR is 1.1 (0.9–1.3), with estimates of 1.0 (0.7–1.2) in Caucasians and 1.4 (1.1–1.6) in Japanese studies (Figure 8). If only homozygous subjects are considered, the meta-OR for Caucasians is 0.9 (0.2–4.5), while in the Japanese it is 2.6 (2.2–3.0). For the Exon 7 polymorphism the meta-OR for the Japanese, considering both heterozygous and homozygous subjects, was 3.0 (2.4–3.5), while for homozygous it was 1.4 (1.1–1.7) (Figure 9). Apparently, therefore, Japanese subjects show an increased risk if they are homozygous for the *MspI variant* or if they are heterozygous for the Exon 7 variant. A study conducted in Brazilians has been reported separately, since their ethnic background is partly Caucasian, partly African, partly Indian, and therefore they constitute a separate category. African–Americans have not been extensively studied. Only one study analysed the association between lung cancer and the MspI polymorphism, no study looked at the Exon 7 polymorphism, and three studies looked at the AA polymorphism. The meta-OR for the heterozygous variant of the AA polymorphism was 0.92 (95% c.i. 0.52–1.32).

The role of the CYP1A1 polymorphisms on breast cancer risk has been analysed in four papers, three of them considering Exon 7, and one considering all three polymorphisms. The meta-OR for breast cancer with the Exon 7 polymorphism (heterozygous variant) in Caucasians was 1.31 (95% c.i. 0.8%–1.77).

Discussion

The most evident result of the present review is the high level of heterogeneity in the epidemiologic studies concerning four metabolic polymorphisms and the risk of cancer. Such heterogeneity concerns both technical aspects (design and conduction) of the investigations and their results.

Design aspects that may have influenced the outcome include a high proportion of studies making use of prevalent cases, the frequent use of hospital controls, a low response rate in a few investigations, the variable metabolic ratios which were used in phenotype-based investigations, and the lack of adequate adjustments for covariates (potential confounders). However, when the impact of such methodological aspects was considered by restricting attention to better designed studies, the association was reinforced. In fact, in the case of GSTM1 and lung cancer restriction to better designed studies increased both the estimates and the homogeneity of ORs.

A matter of concern is the difference found between phenotype-based and genotype-based studies. For example, in the case of CYP2D6 the genotype-based investigations tended to yield quite variable results, in contrast with the more stable estimates provided by phenotyping. While phenotyping is affected by methodological problems related to the administration of the parent compound, compliance of volunteers and measurement of metabolites, the determination of the genotype depends on the knowledge of relevant mutations. One can expect limited sensitivity of genotyping due to incomplete knowledge of the relevant mutations. The apparent discrepancy between phenotype-based and genotype-based studies is a challenge for future research.

A further element of variability is related to inter-ethnic comparisons. For example, the relationship between GSTM1 and lung cancer seems to be stronger in Asians than in Caucasians. An extreme case is represented by CYP1A1, where Caucasians do not show an association while Asians show associations with lung cancer limited to the homozygous recessive MspI genotype or the heterozygous Exon 7 genotype.

The assessment of exposure to carcinogens which are believed to be metabolized by polymorphic enzymes is a particularly important issue. In the absence of exposure to carcinogens, subjects carrying different alleles of polymorphic genes should show the same risk of developing cancer. An aspect which has not been touched upon in the present review is the combined use of markers of internal dose and markers of metabolic susceptibility. The measurement of markers of internal dose such as DNA or haemoglobin adducts allows the assessment of the role, played by metabolic polymorphisms, as modifiers at the molecular level of exposure to carcinogens. A detailed knowledge of exposure to carcinogens, their metabolic transformation and their effective dose at molecular level will allow better understanding of the role played by each factor in cancer development.

We believe that a critical analysis of the literature, including epidemiological considerations on the study design and interpretability, is crucial for an assessment of the scientific evidence concerning metabolic polymorphisms. Future studies should include sound methodological validation, in particular systematic comparisons between phenotype and genotype, and studies of the sources of variation of both measurements. Improvement of study design should take the different aspects we have considered into account.

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