Genetic Polymorphism of Enzymes Involved in Xenobiotic Metabolism and the Risk of Lung Cancer

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Abstract

Chronic inhalation of cigarette smoke is a major risk factor for the development of lung cancer. It has been suggested that genetic susceptibility may contribute to the risk, because only a small portion of smokers develops the disease. Several polymorphisms that involve the metabolic activation or detoxification of carcinogens derived from cigarette smoke have been found to be associated with lung cancer risk. Many studies have focused on the relation between the distribution of polymorphic variants of different forms of the metabolic enzymes and lung cancer susceptibility. In this respect two groups of genetic polymorphisms of enzymes involved in xenobiotic metabolism, cytochrome P450 (*CYP*) and glutathione S-transferases (*GSTs*), have been discussed. *CYP* multigene superfamily consists of 10 subfamilies (*CYP1–CYP10*). A positive association between development of lung cancer and the mutant homozygous genotype of *CYP1A1* gene has been reported in several Japanese populations but such an association has not been observed in either Caucasians or African-Americans. The relation between *CYP2D6* and lung cancer remains conflicting and inconclusive. Several polymorphisms have been identified at the *CYP2E1* locus. No definitive link between the polymorphisms of *CYP2E1* and the risk of lung cancer has, however, been identified. The role of other *CYP2* isoforms in lung carcinogenesis has not been sufficiently investigated. *GSTs* form a superfamily of genes consisting of five distinct families, named *GSTA*, *GSTM*, *GSTP*, *GSTT* and *GSTS*. The role of *GSTM*, *GSTT1* or *GSTP1* polymorphism in modifying the lung cancer risk may be more limited than has been so far anticipated.

Although some genetic polymorphisms discussed here have not shown significant increases/decreases in risk, individuals with differing genotypes may have different susceptibilities to lung cancer. Hopefully, in future studies it will be possible to screen for lung cancer using specific biomarkers.

Key words: genetic polymorphism, drug-metabolizing enzymes, lung cancer, ethnic difference, molecular epidemiology

Introduction

Lung cancer mortality has been increasing rapidly in recent years in Japan and has exceeded that of stomach cancer in male Japanese (1). Although chronic inhalation of cigarette smoke is a major risk factor for the development of lung cancer, it appears important to examine genetic susceptibility to the disease.

Cigarette smoke contains several thousand chemicals, of which about 50 compounds are known carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aromatic amines and

N-nitroso compounds. Some of these compounds are reactive carcinogens, but most are procarcinogens, which need to be activated by phase I enzymes such as those encoded by the cytochrome P450 (*CYP*) supergene family, and converted into reactive carcinogens. All these reactive carcinogens can bind to DNA and form DNA adducts capable of inducing mutations and initiating carcinogenesis. *CYPs* are a multigene superfamily of mixed function monooxygenases. Based on sequence homology, the *CYP* superfamily is divided into 10 subfamilies, *CYP1–CYP10*. Subfamilies *CYP1*, *CYP2*, *CYP3* and *CYP4* are primarily involved in drug metabolism (2).

Following phase I reaction, phase II enzymes such as glutathione S-transferases (*GSTs*) are responsible for detoxification of activated forms PAH epoxides. *GSTs* are constitutively found in a wide variety of tissues, with different characteristic patterns of *GST* isozymes. *GSTs* also form a superfamily of genes consisting of five distinct families, named alpha, mu, pi, theta and

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Table 1 Selected human xenobiotic-metabolizing CYP isoforms and examples of human tissue and carcinogens in tobacco smoke activated by them (2)

Feature	CYP1A1	CYP2D6	CYP2E1
Chromosomal location	15q22-qter	22q13.1	10q24.3-qter
Tissue expression	Lung, skin placenta, lymphocyte	Lung, liver intestine,	Lung, liver brain, kidney
Carcinogen substrates in tobacco smoke	BP, other PAHs	NNK	NNK, NNN, N-nitrosodimethylamine

BP: benzo(a)pyrene

NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

NNN: N-nitrososornicotine.

Table 2 Selected human xenobiotic-metabolizing GST isoforms and examples of human tissue and carcinogens in tobacco smoke inactivated by them (5–7)

Feature	GSTM1	GSTT1	GSTP1
Chromosomal location	1p13.3	22q11.23	11q13
Tissue expression*	Lung, prostate, breast, lymphocytes, colon, small intestine, esophagus, stomach, thymus, heart, skeletal muscle, kidney, spleen, testis, uterus, aorta, adrenal gland, brain	Lung, liver, erythrocytes, brain, kidney, small intestine, skeletal muscle, spleen, heart	Lung, placenta, esophagus, bladder, breast, skin, colon, oral cavity, stomach
Carcinogen substrates in tobacco smoke	BPDE	Halomethanes	BPDE, acrolein

BPDE: benzo(a)pyrene diol epoxide.

* GSTs are constitutively found at high levels in a wide variety of tissues with different characteristic patterns of GST isozyme expression.

sigma (3, 4). The major isoforms, which involve the metabolic activation of carcinogens derived from tobacco smoke or detoxification of those activated carcinogens, are listed in Tables 1 (2) and 2 (5–7).

Since advances in molecular biology have allowed many allelic variants of several drug-metabolizing enzymes to be characterized at the molecular level, specific nucleotide changes have been identified as the basis for altered protein structure and/or function. Therefore, the high risk genotype of an individual could be determined easily. The existence of multiple alleles at loci of those enzymes may result in differential susceptibilities of individuals (8). Since genetic polymorphisms have been found for both phase I and phase II enzymes, risk assessment could be increased in sensitivity if polymorphisms in both phases of enzymes are taken into consideration as biomarkers for susceptibility to cancer. It is likely that an individual with the high risk genotype (either a genotype coding for a more active phase I enzyme or a less efficient phase II enzyme, or both of those) might be at higher risk of cancer than that with the opposite genotype (combination).

Current molecular epidemiologic evidence for postulated genetic susceptibility may be determined by the interaction of genetic host factors and cigarette smoking. In this study, we examined the relation between genetic polymorphisms of enzymes involved in xenobiotic metabolism and lung carcinogenesis, with special emphasis on the most studied isoforms *CYP1A1*, *CYP2*, *GSTM1*, *GSTT1* and *GSTP1*.

1. *CYP1A1* polymorphisms

Human *CYP1A* gene locus has two distinct members, *CYP1A1*, which is predominantly expressed in extrahepatic tissues, including the lung, lymphocytes and placentas, and the liver specific *CYP1A2*. Substrates for and inducers of *CYP1A1*

include PAHs such as benzo(a)pyrene (BP), whereas *CYP1A2* is active in the metabolism of aromatic amines. Cancer risk associated with PAHs is mediated in part by aryl hydrocarbon hydroxylase (AHH), which is used as an indicator of the phenotype of *CYP1A1*. Since AHH is responsible for the activation of PAHs in cigarette smoke, it may be important for the causation of lung cancer (2).

To date, four genetic polymorphisms have been described in human *CYP1A1* gene (9–12). The polymorphism detected first, which was ascribed to the presence (*CYP1A1**2 allele) or absence (wild-type *CYP1A1**1 allele) of the *Msp* I site, was a thymine to cytosine transition 1197 bp downstream of exon 7 (3801T=>3801C) (9). The second Ile-Val polymorphism occurred in the heme-binding region (Ile462Val, 2455A=>2455G) (10). The third polymorphism results from a single base change adenine to guanine in the 3' noncoding region approximately 300 bp upstream from the polyadenylation site (3205T=>3205C, *CYP1A1**4 allele) (11). This mutation leads to cleavage of the normal 2.3 kb *Msp* I restriction fragment into 1.3 and 1.0 kb fragments. This race-specific polymorphism has been found in African-Americans but not in Caucasians and Asians. A novel mutation (*CYP1A1**5) was 2 bases upstream of the Ile-Val polymorphism (Thr461Asn, 2453C=>2453A) (12).

The *Msp* I polymorphism can be classified into 3 genotypes, predominant homozygous alleles (genotype A), heterozygote (genotype B) and homozygous rare alleles (genotype C) (9). The genotype C is closely related to high inducible *CYP1A1* phenotypic activity (13). An overrepresentation of genotype C was observed in lung cancer patients compared with controls in Asian populations (14–17) but was not observed in non-Asian populations (18–28) (Table 3). This might be sparsity of the allele in Caucasians. For example, the frequency of genotype C allele is 0.33 in Japanese population (14), and less than 0.10 in Caucasian populations (18–20). Individuals with genotype C are most common among Chinese (16, 29) and Japanese (14, 15, 30) (10%) and least common among Caucasians (0–4%) with African-Americans (20, 27, 28) and Koreans (5–6%) (17, 31) intermediate between these groups. Similarly, the Val/Val genotype in Ile-Val polymorphism results in a reduced catalytic enzyme activity (13) and was shown to be associated with lung cancer in a Japanese population (10, 32). The Ile-Val polymorphism was less common than the *Msp* I polymorphism (10, 19, 21, 30). This polymorphism is closely linked to *Msp* I polymorphism not only in a Japanese population (10) but also in Northern Europeans (18).

As for the third polymorphism in the *CYP1A1* gene, no linkage was observed with either the *Msp* I or Ile-Val polymorphism. In African-Americans, the frequency of heterozygotes was 15.2–19.4% in controls and the homozygous variant was rare even in cases (10, 33–35). This race specific polymorphism has been associated with an increased risk of adenocarcinoma (35) whereas

Table 3 Distribution of the *CYP1A1* genotype (*Msp* I polymorphism) in different populations

Author, published year (reference number)	Population	Genotype C (%)	
		Case	Control
Tefre et al., 1991 (18)	Norwegian	221 (0.9)	212 (0.9)
Nakachi et al., 1991 (14)	Japanese	151 (21.2)	375 (10.6)
Hirvonen et al., 1992 (19)	Finnish	87 (0.0)	121 (1.7)
Shields et al., 1993 (20)	African-American	28 (3.6)	23 (8.7)
	European-American	28 (3.6)	25 (4.0)
Alexandire et al., 1994 (21)	Swedish	296 (1.3)	329 (0.1)
Drakoulis et al., 1994 (22)	German	142 (0.7)	171 (0.0)
Sugimura et al., 1994 (26)	Non-blacks (Brazilian)	88 (6.8)	90 (6.7)
	Blacks (Brazilian)	22 (13.6)	22 (9.1)
Kihara et al., 1995 (30)	Japanese	97 (16.5)	185 (17.8)
Jaquet et al., 1996 (23)	Belgian	44 (2.3)	81 (2.5)
Bouchardy et al., 1997 (24)	French	150 (2.0)	171 (0.0)
Garcia-Closas et al., 1997 (25)	American*	416 (1.0)	446 (1.0)
Ishibe et al., 1997 (27)	Mexican-American	62 (16.1)	158 (17.1)
	African-American	109 (1.8)	137 (5.8)
Taioli et al., 1998 (28)	African-American	105 (9.2)	247 (6.2)
Hong et al., 1998 (31)	Korean	85 (7.1)	63 (4.8)
Kiyohara et al., 1998 (15)	Japanese	108 (24.1)	95 (10.5)
Persson et al., 1999 (29)	Chinese	76 (11.8)	90 (11.1)
Kim et al., 1999 (17)	Korean	17 (17.6)	48 (6.3)
Lin et al., 2000 (16)	Chinese	132 (21.2)	259 (13.5)

* Mostly Caucasian-American.

other studies (33, 36) did not confirm the report. As suggested by Talioli et al. (34, 35), the third restriction length fragment polymorphism (RFLP), which might be a useful tool to identify African-Americans at increased risk for lung cancer, does not appear to hold promise. The fourth *Msp* I RFLP may not represent a susceptibility factor for lung cancer (12).

The interindividual difference in genetically determined susceptibility to squamous cell carcinoma of the lung has been investigated in relation to cigarette smoking dose (14). The total amount of cigarettes consumed over ones lifetime was compared among three genotypes in the first *Msp* I polymorphism. The patients with genotype C contracted lung cancer after fewer cigarettes than those with other genotypes. Individuals with genotype C were at significantly higher risk with an odds ratio (OR) of 7.31 (95% confidence interval (CI)=2.13–25.12) at a low dose level of cigarette smoking and the difference in susceptibility between genotypes was reduced at high doses (14).

Recently, a meta-analysis of published studies was undertaken by Houlston to clarify the effect of variation within the *CYP1A1* gene defined by the *Msp* I and Ile-Val polymorphisms (37). The ORs for the genotypes C and Val/Val were found to be 1.27 (95% CI=0.91–1.77) and 1.62 (95% CI=0.93–2.82), respectively. His results have provided little support for the role of those polymorphisms in the development of lung cancer. As stated earlier, the frequency of both *Msp* I and Ile-Val polymorphisms varies among ethnic groups. Therefore, the role of the *CYP1A1* gene as a risk factor for lung cancer may vary among ethnic groups.

CYP1A1 polymorphisms are attractive with respect to biologically plausible susceptibility factors. The findings in the Japanese population studies to date reveal high risks of lung cancer for the *CYP1A1-Msp* I polymorphism. However, direct applicability to screening strategies in lung cancer in Japan awaits further studies.

2. *CYP2D6* polymorphism

More than 30 commonly prescribed drugs are substrates for *CYP2D6*, including those that act on the cardiovascular and central nervous system. This major polymorphism that inactivates the *CYP2D6* gene leads to profound changes in drug pharmacokinetics and can therefore have severe clinical consequences, leading to marked drug side effects and even, in extreme cases, death (38).

Phenotyping analysis is carried out by administration of the probe drug debrisoquine and subsequent urine analysis at 8-hour intervals to determine the ratio between the parent compound debrisoquine and the primary metabolite 4-hydroxydebrisoquine. This ratio varies 10,000-fold in the Caucasian population (39).

A number of alleles have now been characterized at the *CYP2D6* locus. The phenotypic consequence of many of these allelic variants remains to be established. The first genetic polymorphism in the metabolism of drugs associated with the expression of *CYP2D6* in humans was 4-hydroxylation of the antihypertensive drug debrisoquine (40). The genetic polymorphism of the *CYP2D6* is an autosomal recessive trait (41) expressed in three major phenotypes, commonly termed ultra-rapid metabolizers (UM), extensive metabolizers (EM) and poor metabolizers (PM) (42, 43). A minority of individuals have been shown to inherit multiple copies of the wild-type *CYP2D6* gene (44). These UM require doses of medication that vastly exceed the normal range in order to achieve a therapeutic effect (45). The interindividual variations in the activity of *CYP2D6* are closely linked to mutant alleles encoding the *CYP2D6* gene (46). Inactivating mutations at the *CYP2D6* gene are *CYP2D6**3 (deletion of A2549 in exon 5), *CYP2D6**4 (1846G=>1846A at splicing site), *CYP2D6**5 (complete deletion of the wild-type allele *CYP2D6**1), *CYP2D6**6A (deletion of T1707 in exon 3) and *CYP2D6**16 (a hybrid of 5'-*CYP2D7*/*CYP2D6*-3' gene with breakpoints between the end of intron 7 and the start of exon 9 for both genes involved) while decreased enzyme activity is seen with *CYP2D6**9 (deletion of Lys281) and *CYP2D6**10A (Pro34Ser, 100C=>100T in exon 1 and Ser486Thr, 4180G=>4180C in exon 9). Carriers with one wild-type allele and one inactivating allele are predicted as HEM (47). The *CYP2D6**3, *CYP2D6**4 and *CYP2D6**5 alleles account for the majority (greater than 90%) of the PM phenotype (46). The phenotype-genotype concordance, which predicts the metabolic phenotype by genetic analyses, was found to be between 93.4% and 100% (48).

The genetically determined capacity of the *CYP2D6* is suspected to be involved in the activation of tobacco-specific nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Interindividual differences in the metabolic capacity of the *CYP2D6* may be expected to be a key factor in susceptibility to develop several types of cancers where environmental chemicals are implicated (49, 50). There have been a series of studies on the association of a genetic polymorphism at the *CYP2D6* gene with lung cancer susceptibility (Table 4). All case-control studies have been performed in Caucasians with the exception of the study of London et al. (51), which was performed in African-Americans. Among controls, Caucasian populations have a PM frequency of between 6–10% (48, 51–62), whereas African-Americans have only a 3% frequency (62) and Asians less than 1% (63, 64).

A meta-analysis of the published findings as of 1992 has demonstrated an overall OR of 0.43 for lung cancer patients with the PM genotype (65). Among the studies in shown in Table 4, the

Table 4 Distribution of the *CYP2D6* genotype in different populations

Author, published year (reference number)	Population	PM (%)	
		Case	Control
Sugimura et al., 1990 (52)	American*	45 (0.0)	38 (10.5)
Roots et al., 1992 (53)	German	109 (3.7)	125 (9.6)
Wolf et al., 1992 (54)	British	361 (3.6)	720 (4.3)
Hirvonen et al., 1993 (55)	Finnish	106 (0.9)	122 (5.7)
Agundez et al., 1994 (56)**	Spanish	89 (0.0)	98 (7.0)
Tefre et al., 1994 (57)	Norwegian	204 (9.8)	117 (5.1)
Rannung et al., 1995 (58)	Swedish	84 (9.5)	68 (5.9)
Dolžan et al., 1995 (59)	Slovene	200 (2.5)	107 (6.5)
Strücker et al., 1995 (60)	French	249 (6.8)	271 (5.5)
Legrand et al., 1996 (51)**	French	249 (6.0)	265 (4.9)
London et al., 1997 (62)	African-American	158 (2.5)	246 (3.3)
	European-American	185 (5.9)	464 (5.8)
Shaw et al., 1998 (61)	Italian	98 (7.1)	110 (2.7)
Laforest et al., 2000 (48)	French	148 (5.4)	171 (6.4)

*: Mixed population: 92.1% of controls and 43.2% of patients are European-American, and the remainder is African-American.

: Determined by the presence of *CYP2D69 allele.

PM genotype determined by the presence of the inactivating allele was not significantly associated with decreased (48, 52–55, 62) or increased (57, 58, 60, 61) risk of lung cancer. Many of these studies have been contradictory either because of the small numbers of patients studied or because of the limitations and controversy surrounding the pharmacogenetic assay used to identify the affected individuals. For the *CYP2D6**9 allele, Agundez et al. (56) reported that the *CYP2D6**9 mutant allele was 6-fold more frequent among lung cancer patients than controls. However, Legrand et al. (62) reported that the *CYP2D6**9 allele was present in 4.9% of controls and 6% of lung cancer patients and that this mutant allele might not be a risk factor for lung cancer in a French population.

The effect of tobacco smoking on lung cancer risk might rise with increasing *CYP2D6* activity, and the effect of *CYP2D6* activity might rise with increasing tobacco consumption. The significant interaction between *CYP2D6* activity and smoking dose was observed (66). Among individuals with high *CYP2D6* activity, heavy smokers showed a 5-fold increased risk compared with light smokers.

The relation between the *CYP2D6* polymorphism and lung cancer remains conflicting and inconclusive. There is some biological plausibility to more efficient activation of N-nitroso compounds such as NNK in tobacco smoke by the EM genotype, but the absence of any reports of that activity in the lung as the target organ is problematic for the *CYP2D6*-lung cancer hypothesis. While there is a possible molecular epidemiologic association between the prevalence of the EM genotype and lung cancer, the association, if it exists, may be weak.

3. *CYP2E1* polymorphism

CYP2E1 is involved in metabolic activation of carcinogenic tobacco-specific N-nitrosamines (including N-nitrosoethylmethyldamine (NDMA), N-nitropyrrrolidine), aniline, benzene and other low-molecular-weight compounds. The *CYP2E1* is constitutively expressed in liver and can also be induced by chemicals such as ethanol and acetone in humans (67). Protein levels and catalytic activities of *CYP2E1* have been shown to vary among individuals (68, 69). Although the genetic basis of such differences has not

been fully clarified, RFLPs in *CYP2E1* gene by *Dra* I (intron 6) (70, 71), *Rsa* I (intron 5) (71) and *Rsa* I/*Pst* I (5'-flanking region) (72, 73), and *Taq* I (intron 7) (74) have been reported.

The relation between the *CYP2E1* *Dra* I polymorphism and lung cancer was first reported in a Japanese population by Uematsu et al. (71). The *Dra* I RFLP is caused by a base pair change (*CYP2E1**6 allele, 7632T=>7632A) in intron 6 of the *CYP2E1* gene. They divided the *Dra* I RFLP polymorphism into three genotypes; heterozygotes (CD) and two forms of homozygotes (CC and DD). Since this polymorphism is caused by a base pair change of the *CYP2E1* gene, it is unlikely that this change directly affects the variability of the gene expression. Although it could be a marker in linkage disequilibrium with an active site, its effect on the *CYP2E1* phenotype is still unclear (72). Racial variation in the frequency of this polymorphism has been reported (Table 5). A rare allele (C) frequency for Asians (29, 75) was somewhat higher than that for Caucasians. In a Japanese population, the distribution of *Dra* I genotypes in lung cancer patients was significantly different from that of healthy controls (71). The frequency of genotype CD in lung cancer patients was higher than in controls, while the genotypes CC and DD were lower. In Finnish (76) and Swedish (77) populations, there is a much lower allele frequency for C in controls (0.09) and no association between lung cancer risk and *Dra* I polymorphism was seen. There is no clear evidence that this polymorphism is related to lung cancer risk.

The polymorphism in the 5' flanking region at -1053 bp (C=>T) is found to be in linkage with another point mutation at -1293 bp (G=>C) (72, 73, 77, 78). The *Rsa* I/*Pst* I RFLP has two alleles c1 (wild-type *CYP2E1**1 allele) and c2 (*CYP2E1**3 allele). The racial differences in allelic frequency have been reported by Kato et al. (78). Kato et al. (78) reported that the allelic frequencies of this polymorphism were markedly different in Japanese, African-Americans, and Caucasians. The *Rsa* I rare allele c2 was present at a frequency of 0.02 in Caucasians, 0.02 in African-Americans and 0.27 in Japanese (P<0.05). Stephens et al. (79) also reported the ethnic-differences in allelic frequency of *Rsa* I RFLP. A rare c2 allele frequency for Taiwanese, African-Americans and European-Americans was 0.28, 0.01 and 0.04, respectively. High frequencies of the c2 and C alleles in the *CYP2E1* gene have consistently been found in several Asian populations (29, 75, 80, 81), in contrast to other ethnic groups (77, 82). This polymor-

Table 5 Distribution of the *CYP2E1* genotype in different populations

Author, published year	Population	Mutant genotype (%)	
(reference number)		Case	Controls
<i>Dra</i> I polymorphism			
Uematsu et al., 1991 (71)	Japanese	47 (0.0)	56 (10.7)
Hirvonen et al., 1993 (76)	Finnish	101 (1.9)	121 (0.8)
Persson et al., 1993 (77)	Swedish	193 (0.0)	152 (0.06)
Wu et al., 1998 (85)	Mexican-American	45 (2.2)	92 (3.4)
	African-American	92 (0.0)	114 (0.9)
Persson et al., 1999 (29)	Chinese	76 (6.6)	112 (5.4)
Wang et al., 1999 (75)	Taiwanese	119 (5.9)	231 (8.6)
<i>Rsa</i> I polymorphism			
Persson et al., 1993 (77)	Swedish	184 (0.0)	148 (0.07)
Watanabe et al., 1995 (80)	Japanese	316 (4.1)	503 (3.2)
Oyama et al., 1997 (81)	Japanese	126 (5.6)*	612 (4.1)
Persson et al., 1999 (29)	Chinese	76 (2.6)	113 (5.3)
Wang et al., 1999 (75)	Taiwanese	119 (0.8)	231 (6.9)

* Non-small cell lung carcinoma.

phism might be functionally important because it is located at a putative binding site for the transcription factor HNF-1 and has been associated with higher levels of *CYP2E1* transcription. Recently, an increase in transcriptional activity, mRNA and protein expression *in vitro* was observed in the c2/c2 genotype (83). It is conceivable that the *CYP2E1* *Rsa* I/*Pst* I polymorphism might contribute to differences in susceptibility to lung cancer. The c2 rare allele has been demonstrated to be less common among Swedish lung cancer patients (77) and this difference is more pronounced when adenocarcinoma patients are excluded from the cancer group. In the Finnish study, Hirvonen et al. (76) failed to show any association of this polymorphism with lung cancer. London et al. (84) suggested that the rare c2 allele was not associated with an increased risk of lung cancer. This polymorphism did not also affect lung cancer risk in one Japanese population (80) whereas the c2/c2 genotype was associated with an increased risk of squamous cell carcinoma (OR=2.45, 95% CI=0.92–6.48) in another Japanese population (81). Wu et al. (85) reported that individuals who lack a c2 allele might be at higher risk for developing lung cancer in Mexican-Americans, but not in African-Americans. Using the combined genotypes c1/c2 and c2/c2 as the reference, the OR for c1/c1 genotype was 14.0 (95% CI=1.9–101.5) among Mexican-Americans. Moreover, they suggested a greater than multiplicative interaction between cigarette smoking and the *CYP2E1* c1/c1 genotype by stratified analysis. The OR for the c1/c1 genotype and pack-years of smoking (=30 vs. 0, dichotomized categories) was 45.8 (95% CI=5.7–368.7) in the overall population. In that case, the interaction term was not statistically significant probably due to the small sample size that also resulted in the wide CI. A major reason for this disagreement might be an ethnic difference in allelic frequency of the polymorphism and the small sample size. Larger studies might be necessary to rule out a modest association between the *CYP2E1* *Rsa* I polymorphism and lung cancer risk.

Given the low gene frequency in the European- and African-American population studies to date and the relatively modest ORs associated with these polymorphisms, demonstration of a *CYP2E1*-lung cancer relation would require case and control groups of greater than 1,000 subjects each. These study magnitudes have not been reported in the molecular epidemiology literature as it relates to lung cancer. The low estimates of attributable risk imply that the *CYP2E1* polymorphisms at this time must be considered unlikely as strong determinants of lung cancer risk in the Caucasian and African-American populations. In addition, since all polymorphisms are located in non-coding regions of *CYP2E1*, a novel polymorphism(s) in *CYP2E1* would be a promising tool for the detection of the genetic susceptibility to lung cancer.

4. GST polymorphisms

Following the phase I reaction, phase II enzymes such as glutathione S-transferases are responsible for detoxification of activated forms of PAH epoxides. The GSTs activities are inherited in an autosomal dominant fashion (86) and a 100- to 200-fold difference in cytosolic GSTs in lymphocytes from healthy subjects (87, 88). GST genes form a superfamily of at least 13 genes consisting of five distinct families, named alpha (*GSTA*), sigma (*GSTS*), mu (*GSTM*), pi (*GSTP*) and theta (*GSTT*). Certain genes within the *GSTM*, *GSTT* and *GSTP* subfamilies (*GSTM1*, *GSTT1* and *GSTP1*) are polymorphic in humans and the levels of individual

enzymes expressed can be influenced by induction and by genetic polymorphism. Since these polymorphisms are considered in terms of risk from certain potentially carcinogenic chemicals, they are currently being investigated as possible cancer risk modifiers. The phenotypic absence of *GSTM1* and *GSTT1* activity is due to homozygosity for deletion of these genes, termed the null genotype (6, 86). Genetic polymorphism at the *GSTP1* locus results from a single base pair substitution in exon 5 (Ile104Val, 313A=>313G) and exon 6 (Ala113Val, 341C=>341T) (89). *In vitro* cDNA expression study suggests that these amino acid substitutions reduce enzyme activity (90). For *GSTA* genes, one study has reported an RFLP in *GSTA2* (91) but whether there is a genetic basis for this variation and its implications is unclear. Less is known about the association between *GSTA2* and newly found *GSTS* genes, and lung cancer risk. Most interest in the possible consequences of GSTs polymorphisms has therefore focused on the polymorphisms at the *GSTM1*, *GSTT1* and *GSTP1* gene loci. Table 6 summarizes the frequencies of homozygous deletions in *GSTM* and *GSTT1*, and homozygotes for the mutant allele of *GSTP1*. The homozygous deletion of the *GSTM1* gene was shown to occur in approximately 50% of the population of various ethnic origins (8) while the homozygous deletion of the *GSTT1* gene is distributed at between 10% and 64% of various ethnic groups (8). The frequency of the *GSTT1* null genotype in Caucasian populations is 30% or less but the null type genotype frequency in Asian populations may be similar to that of *GSTM1*. Individuals homozygous for the 105 valine allele (the mutant allele) are most common among African-Americans (19%) and least common

Table 6 Distributions of the *GSTM1*, *GSTT1* and *GSTP1* genotypes in different populations

Author, published year (reference number)	Population	Mutant genotype (%)	
		Case	Control
<i>GSTM1</i> deletion polymorphism			
McWilliams et al., 1995 (93)	12 case-control studies		
Houlston, 1999 (94)	19 case-control studies (the studies based on genotyping methods)		
Lan et al., 2000 (101)	Chinese	122 (67.2)	122 (50.8)
Kiyohara et al., 2000 (102)	Japanese	86 (61.6)	88 (55.7)
Spitz et al., 2000 (103)	European-American	498 (49.4)	463 (48.8)
London et al., 2000 (104)	Chinese	232 (52.6)	710 (60.1)
<i>GSTT1</i> deletion polymorphism			
Deakin et al., 1996 (105)	British	108 (15.7)	509 (18.5)*
Kelsey et al., 1997 (106)	Mexican-American	60 (16.7)	146 (11.6)
	African-American	108 (25.0)	132 (22.0)
To-Figueras et al., 1997 (107)	Spanish	160 (24.4)	192 (19.3)
Jourenkova et al., 1997 (108)	French	150 (18.0)	172 (15.7)
Saarikoski et al., 1998 (109)	Finnish	208 (12.7)	294 (13.3)
Lan et al., 2000 (101)	Chinese	122 (59.8)	122 (52.5)
Kiyohara et al., 2000 (102)	Japanese	86 (54.6)	88 (44.3)
Spitz et al., 2000 (103)	European-American	484 (27.3)	458 (22.7)
London et al., 2000 (104)	Chinese	232 (57.8)	710 (60.0)
<i>GSTP1</i> Ile-Val polymorphism (exon 5)			
Harris et al., 1998 (111)	Australian	178 (14.6)	199 (9.0)
Saarikoski et al., 1998 (109)	Finnish	206 (8.3)	294 (9.2)
To-Figueras et al., 1999 (112)	Spanish	164 (10.4)	200 (10.0)
Kato et al., 1999 (113)	Japanese	47 (0.0)	122 (4.1)
Kihara et al., 1999 (114)	Japanese	382 (4.7)	257 (3.1)
Kiyohara et al., 2000 (102)	Japanese	86 (2.3)	88 (0.0)

Published after 1999.

* Hospital controls.

among Japanese (0–3.1%) with Caucasians (6.5–11.7%) intermediate between these groups (92).

The association between *GSTM1* polymorphism and lung cancer risk has been controversial in the published literatures. The reason for the discrepancies in the *GSTM1* findings is unclear. One problem may be the relatively weak influence of any one genotype on disease risk and the unequal distribution of confounding factors such as polymorphisms at other relevant loci in cases and controls. Differences in exposure to carcinogens such as cigarette smoke are also likely to be important with the effects of such variables exaggerated in studies of small numbers of subjects.

McWilliams et al. (93) combined 12 case-control studies and determined lung cancer risk. They concluded that *GSTM1* deficiency is a moderate risk factor for lung cancer development with an OR of 1.41 (95% CI=1.23–1.61) using Mantel-Haenszel methods for stratified data. The association appeared stronger for Japanese (OR=1.6, 95% CI=1.2–2.1) than for Caucasians (OR=1.2, 95% CI=1.0–1.4). The studies, which determined *GSTM1* status by phenotyping (3 studies) or genotyping (8 studies) yielded a summary OR of 1.8 (95% CI=1.3–2.5) or 1.3 (95% CI=1.2–1.6), respectively. A recent meta-analysis based on genotyping studies (19 studies) showed that *GSTM1* deficiency conferred a 1.13-fold increase in the risk of lung cancer (95% CI=1.04–1.25) (94). Pooling the 4 studies that were based on phenotyping methods, the OR was higher (OR=2.12, 95% CI=1.43–3.13) than those based on genotyping methods. The studies published before 1998 are not shown in Table 6 because of the existence of these two meta-analyses.

Individuals deficient in the *GSTM1* have been reported to be at high risk for smoking related lung cancer (86, 95–98). Smokers with the *GSTM1* enzyme have approximately one-third of the risk for lung carcinoma of smokers without the enzyme (95, 99, 100). A recent study also suggested that the effect of the nulled *GSTM1* gene on susceptibility to lung cancer may be more evident in heavy smoker patients (101) whereas others found nonsignificant association of the *GSTM1* null genotype with lung cancer (102–104).

Individuals with the *GSTT1* null genotype have been reported to have a nonsignificant risk for lung cancer (101, 102, 104–109) while a statistically significant OR of 1.41 for the *GSTT1* null genotype was reported by Spitz et al. (103).

There is less information on the role of the *GSTP1* gene as a cancer risk modifier. Given that *GSTP1* is the most abundant isoform in the lungs (110), it is anticipated to be of particular importance in the detoxification of inhaled carcinogens. Six published studies have reported that *GSTP1* polymorphism in exon 5 did not increase the risk of lung cancer (102, 109, 111–114).

Since the *GSTM1* and *GSTT1* genes are involved in the detoxification of tobacco-smoke-derived carcinogens, individuals with concurrent lack of these genes are expected to be at particular risk of developing lung cancer. A significant association was also observed for concurrent lack of the *GSTM1* and *GSTT1* genes and susceptibility to squamous cell carcinoma (109). For that cell type, the risk was 2.3-fold (95% CI=1.0–5.3) when compared with that of individuals having other genotype combinations. In contrast, that genotype combination did not affect the risk for other histological types of lung cancer. Kelsey et al. (106) and Kiyohara et al. (102) also showed significant increased ORs for the association of lung cancer and the presence of both null polymorphisms.

However, these findings are in contrast to these of previous studies, in which no association between the concurrent lack of these genes and susceptibility to lung cancer was observed (105, 107–109). The significant increased risk for lung cancer has appeared for individuals with concurrently high levels of smoking and the combined *GSTM1* and *GSTT1* null genotypes compared with those with at least one null and low levels of smoking (108).

Epidemiological studies examining the protective association of cruciferous vegetables within smoking strata have had mixed results. Isothiocyanates (ITCs) are nonnutrient compounds in cruciferous vegetables with anticarcinogenic properties. ITCs are effective inhibitors of tumorigenesis in animal model systems (115). One proposed mechanism for their protective action is through direct inhibition of their catalytic activities, with induction of phase II enzymes. ITCs are potent inhibitors of NNK metabolism (116). The protective effect of ITCs was seen primarily among individuals with homozygous deletion of *GSTM1* (OR=0.36, 95% CI=0.20–0.63) and particularly with deletion of both *GSTM1* and *GSTT1* (OR=0.28, 95% CI=0.13–0.57) (117). A large joint effect of both null genotypes and low ITC intake (OR=0.18, 95% CI=0.06–0.58) was observed by Spitz et al. (103).

The biological plausibility of the protective effect of GST through its conjugating activity is compelling. However, for a polymorphism as prevalent as the *GSTM1* null genotype, any overriding influence on the process of carcinogenesis should have been easily detectable in molecular epidemiologic studies, but the findings described above are less than compelling. The findings do suggest that the *GSTM1* null genotype and the concurrent lack of *GSTM1* and *GSTT1* are modestly associated with susceptibility to lung cancer. Although some of the inconsistencies reported in the role of *GST* genotypes in lung cancer risk could be due to unexpected confounding from dietary factors such as ITCs (diet-gene interaction), the overall role of *GST* polymorphisms in modifying the lung cancer risk may therefore be more limited than has been so far anticipated.

5. Combination of susceptibility genotypes

PAHs require metabolic activation by phase I enzymes to their ultimate forms (reactive intermediates) and these intermediates are then subjected to detoxification by phase II enzymes. Thus, genetically determined susceptibility to lung cancer may depend on the metabolic balance between phase I and phase II enzymes. It is important to identify individuals genetically who are at high risk of lung cancer in terms of polymorphisms of genes for encoding phase I enzymes and phase II enzymes. Nakachi et al. (117) reported that individuals with genotype C combined with nulled *GSTM1* were at significantly high risk with an OR of 16.00 (95% CI=3.76–68.02) for squamous cell carcinoma, at a low dose level of cigarette smoking. In a similar manner, the role of the combined genotypes *CYP1A1* and *GSTM1* as a possible modulator of smoking related lung cancers was studied in relation to the tobacco smoke level in Japanese patients aged below 70 years with squamous or small cell carcinomas of the lung (30). Among male smoking patients, the overall proportion of the *GSTM1* null genotype was slightly higher than among healthy male smoker controls (56.7% versus 48.1%, $P=0.17$). Little difference was observed between smoker patients and corresponding controls in the overall frequencies of genotype C (16–18%). However, when subjects were categorized by both *Msp* I polymorphism in the *CYP1A1* gene and the *GSTM1* polymorphism, the *GSTM1* null

genotype became markedly more expressed in patients with genotype C when compared with the corresponding smoker controls (81.3% versus 39.4%, $P < 0.01$). When ORs were estimated using nonsmoking patients and healthy controls as a reference, the relative risk for developing lung cancer was found to increase in a cigarette dose-dependent manner across all combinations of genotypes. Furthermore, a 7- to 8-fold variation in risk was found among the various combinations; 3.2 in individuals with combined *GSTM1* non-null and genotype C and 21.9 in those with combined *GSTM1* null and genotype C when the smoking dose was high. These results suggest that individuals with genotype C are relatively resistant to tobacco-related lung cancers when combined with the *GSTM1* non-null genotype, but are highly susceptible when combined with the *GSTM1* null genotype.

In Caucasian populations, a combined risk of squamous cell carcinoma was indicated for patients, diagnosed before 66 years of age, carrying both the *GSTM1* null genotype and a rare *CYP1A1* *Msp* I homozygous allele (OR=3.0, 95% CI=1.2–7.2) (21). However, there were few patients with that combined genotype to analyze. The infrequency of this particular combined genotype suggests that it will not likely be applicable to screening European populations. In patients with inducible *CYP1A1*, the expressing *GSTM1* gene appeared to have a protective effect against contracting bronchial lung cancer, since 88% of the lung tumors in this patient group were peripheral, whereas almost equal numbers of peripheral and bronchial tumors were observed in those patients lacking the gene (118). Neither the *CYP1A1* *Msp* I heterozygous genotype alone nor the *GSTM1* null genotype alone were associated with a significant increase in lung cancer risk, although having both genetic traits was associated with a 2-fold increase in risk (95% CI=1.0–3.4) (25). The presence of at least one copy of the *CYP1A1* *Msp* I variant allele was found to be associated with a 2.4-fold (95% CI=1.2–4.7) increase in the risk of squamous cell carcinoma when this gene was considered singly and a 3.1-fold (95% CI=1.2–7.9) increase when combined with a *GSTM1* deletion (119). The effect of the *GSTM1* null genotype on lung cancer has not been modified by the *CYP1A1* genotype in a French population (108).

The findings on combination phases I and II carcinogen bioactivation polymorphisms are controversial. Combination bioactivation polymorphisms are attractive with respect to biologically plausible susceptibility factors. The findings in Japanese population studies to date reveal high ORs for lung cancer. However, only 10 to 24% of Japanese lung cancer patients display these combination polymorphisms. For non-Asian populations, the relevance of the *CYP1A1* *Msp* I and *GSTM1* polymorphisms to lung cancer is questionable, given their low prevalence in both lung cancer and general populations. Combined *CYP1A1* and *GSTM1* genotypes is thus a potential predictor of genetic susceptibility to smoking-related lung cancers in populations where *CYP1A1* alleles are common.

6. Other isoforms of CYP

CYP2A6 mediates 7-hydroxylation of coumarin, a component of cigarette smoke and activates several nitrosamines in tobacco smoke, including NNK (120). The catalytic selectivity of *CYP2A6* appears to overlap with that of *CYP2E1*. The location of this isoform is in extrahepatic tissues such as lung, nasal and pharyngeal. Four variants have been identified, namely *CYP2A6**2 (Ile160His, 479T=>479A in exon 3), *CYP2A6**3 (gene conversion

between wild-type *CYP2A6**1A allele and the neighboring *CYP2A7*), *CYP2A6**4A-4D (the four deletion alleles *CYP2A6* gene) and *CYP2A6**5 (Gyl479Val, 1436G=>1436T in exon 9). The *CYP2A6**2 allele detected by *XcmI* may be associated with reduced activity (121). Originally, the *CYP2A6**3 allele identified by *Dde* I was expected to reduce activity because of its sequence similarity to *CYP2A7*, which codes for the inactive enzyme (122). Very recently, improvements have been made to the original genotyping methods (123) and *CYP2A6**3 was shown to be lacking in different populations (124–126). One reason for the original misclassification turned out to be due to the very common *CYP2A6**1B allele (normal enzymatic activity) exhibiting a gene conversion between the 3'-flanking regions of the *CYP2A6* and *CYP2A7* genes (123). Four *CYP2A6**4 alleles cause a lack of enzymatic activity (123). The *CYP2A6**5 allele also abolishes the catalytic activity of the encoded enzyme due to a point mutation. The frequency of the major *CYP2A6* alleles in healthy controls has been observed among various populations. The allelic frequencies of *CYP2A6**2 were rather low in both Caucasian (2.3–3.0%) and Chinese (0–0.7%) populations, whereas the *CYP2A6**4 alleles were fairly common in Chinese (8.6–15.1%) and rare in Caucasian (0.5–1.0%) (127, 128). A small study showed that a decreased risk of lung cancer (OR=0.5, 95% CI=0.1–2.1) was observed among individuals with both the *CYP2A6**2 and *CYP2A6**3 alleles (129). The whole gene deletion was associated with a decreased risk of lung cancer among a Japanese population (130). Likewise, individuals with at least one *CYP2A6**4 allele were at a 2-fold (95% CI=1.2–3.2) increased risk of lung cancer compared with those without the *CYP2A6**4 allele (128).

Members of *CYP2C* gene subfamily are constitutively expressed. *CYP2C9* metabolizes PAH-type tobacco carcinogens such as BP (2) and catalyzes the hydroxylation of several medications, including warfarin, tolbutamide and phenytoin (131). Two mutant alleles, *CYP2C9**2 (Arg144Cys, 430C=>430T) and *CYP2C9**3 (Ile359Leu, 1075A=>1075C) have been described (132) and appear to decrease activity (133). Thus, these allelic variants may decrease the risk of lung cancer although individuals homozygous for either *CYP2C9**2 or *CYP2C9**3 are relatively uncommon (134). A slightly increased risk of lung cancer has been observed with the presence of the *CYP2C9**2 allele among Caucasians (135, 136), whereas the *CYP2C9**3 allele was not significantly associated with a decreased lung cancer risk (132, 136). Although the functional significance *in vivo* of both allelic variants has been demonstrated for drug metabolism, *CYP2C9**2 for warfarin (137) and *CYP2C9**3 for tolbutamide (131), there is no evidence to address whether either allele influences *CYP2C9* activity for BP metabolism. It is possible that they might differentially influence activity towards BP and thus lung cancer risk. There is some biological plausibility to less efficient activation of PAHs such as BP in tobacco smoke by the mutant genotypes, but any difference of in the patterns of association observed for *CYP2C9**2 and *CYP2C9**3 alleles in relation to lung cancer risk are compatible to chance.

CYP2C19 activities have been exemplified by mephenytoin hydroxylation (138, 139) and the PM phenotype is explained to a great extent by two mutant alleles (140), *CYP2C19**2 (681G=>681A, a mutation in exon 5 causing an aberrant splice site) and *CYP2C19**3 (636G=>636A, a point mutation in exon 4 introducing a premature stop codon). The *CYP2C19**2 is mainly found in Asians (140). The combined *CYP2C19* genotypes

(*CYP2C19*2/*2*, *CYP2C19*2/*3* or *CYP2C19*3/*3*) correspond to that predicted for the *CYP2C19* PM phenotype. The *CYP2C19* PM phenotype comprises 3–5% of Caucasians, but 18–23% of Asians (141). A very small study conducted among Japanese showed that the PM phenotype was associated with an increased risk of squamous cell carcinoma of the lung. There is a possibility that the role of *CYP2C9* and *CYP2C19* is in the detoxification of tobacco-derived carcinogens.

Some studies have suggested a relation between lung cancer and the occurrence of a rare allele, although future research is needed to establish a significant relation.

Conclusions

The molecular epidemiology of lung cancer is a highly dynamic field of study at the interface of environment and inherited susceptibility. In an area where the disease is very clearly linked with the exposure, this approach has the potential of tremendous insight into carcinogenesis and to lead to the very real, tangible benefits of disease prevention and early detection.

While the literature depicts a field in its infancy, several recent trends and future directions are likely to bear fruit. The precision of exposure histories requires improvement, as has been embraced by several recent studies, to be further refined by validated biomarkers of carcinogen exposure. The validation of procarcinogen metabolizing polymorphisms by comparing them with the development of intermediate markers of carcinogenesis is a powerful approach already coming into practice. The search for polymorphisms in biologically plausible steps in carcinogenesis will continue to progress as the understanding of biological plausibility itself advances. Surrogate tissues such as blood leukocytes require validation against target lung tissue and the search for other surrogate tissues, such as sputum, nasal epithelium, warrant considerable attention. The rigor and size of study designs will need to increase, as multiple comparison and power issues dictate. Prospectively collected data of cohort studies over time for development of disease are irreplaceable in both understanding the molecular steps in carcinogenesis and validating screening and prevention trials. Race- and population-specific batteries of risk factors assessment are required to deal with different gene pools. Finally, the disease-susceptibility findings gathered on groups and individuals must be handled confidentially and with discretion in a manner consistent with the highest ethical standards.

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Lung cancer is the leading cause of cancer death in the developed countries. It has been hypothesized that an individual's susceptibility to cigarette smoking-related lung cancer is, in part, conferred by the balance between the capacity to activate inhaled procarcinogens to ultimate carcinogens by phase I enzymes, CYPs, and to detoxify them by phase II enzymes, GSTs. Most show polymorphism and have been suggested to contribute to individual cancer susceptibility as genetic modifiers of cancer risk. Altered phenotypes and genotypes in *CYP1A1*, *CYP2D6* and *CYP2E1* and in defective *GSTM1* have been discussed with increased risk of developing lung cancer. Although some studies suggested a relation between lung cancer and the occurrence of a rare allele in these polymorphisms, at present, none of the polymorphic sites determined in the *CYP* genes can yet be used as markers for increased risk of lung cancer. Lung cancer was weakly associated with the *GSTM1* polymorphism in a meta-analysis. The lung cancer risk was markedly increased only in Japanese populations who carried simultaneously susceptible genotypes in *CYP1A1* and *GSTM1*.

Future research in this field, where we should consider referring to cancer development as a truly complex phenomenon and consider the possibility that the statistical methods for referring genetic variants to cancer risk may need thorough re-thinking, might focus on the establishment of new polymorphic sites in genes coding for xenobiotic metabolizing enzymes *CYP1A2*, *CYP2A* and *CYP3A* subfamilies, several of which are capable of converting a number of procarcinogens to reactive metabolites. Furthermore, by combining genotypes from different genes of interest (e.g., tumor suppressor genes, genes coding for DNA repair enzymes), the identification of high risk groups can be made more specific and reveal factors of importance in cancer development.

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