

Investigating the joint effect of GSTM1, CYP1A1 and smoking on modifying the risk of lung cancer.

Protocol for a HuGE association review

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Summary Statement

The aim of this systematic review and meta-analysis is to estimate the joint effects of GSTM1, CYP1A1 and smoking habits on modifying the lung cancer risk.

Background

Genes

GSTM1

The glutathione *S*-transferases (GSTs) are a family of enzymes known to play an important role in the detoxification of several carcinogens found in tobacco smoke (1). The GST enzymes are dimeric proteins that catalyze conjugation reactions between glutathione and tobacco smoke substrates such as aromatic heterocyclic radicals and epoxides. This conjugation reaction also facilitates excretion and thus constitutes a detoxification step. In addition to this role in phase II detoxification, GSTs are also able to modulate the induction of other enzymes and proteins important in cellular functions such as DNA repair. The GST enzymes are therefore important in maintaining cellular genomic integrity and therefore may potentially play an important role in susceptibility to cancer.

GST enzymes are coded for at five loci known as alpha, mu, theta, pi and gamma. The *GSTM1* locus has been mapped to chromosome 1p13.3 and individuals with homozygous deletions of the *GSTM1* locus have no enzymatic functional activity of the enzyme. Phenotype assays have confirmed this lack of function by demonstrating a 94 percent (or greater) concordance between phenotype and genotype (2-4). Three alleles have been identified at the *GSTM1* locus: one deletion allele and two others (*GSTM1a* and *GSTM1b*) that differ by a C to G substitution at base position 534 which results in the Lys to Asn substitution at amino acid 172 (5, 6). This amino acid substitution does not result in any functional change between these two alleles and as a result are categorised together as the positive conjugator phenotype.

A recent meta-analysis conducted by Benhamou et al. (7) of 43 published case-control studies including >18000 individuals found a slight increase of risk of lung cancer for individuals with the *GSTM1* null genotype (OR = 1.17, 95% confidence interval 1.07-1.27). In addition to this Benhamou et al. (7) also conducted a pooled analysis of the original data for 9500 individuals from 21 case-control studies revealed no evidence

of increased risk of lung cancer among carriers of the GSTM1 null genotype (age, gender and centre-adjusted OR = 1.08, 95% confidence interval 0.98-1.18).

CYP1A1

Cytochrome P-450 (CYP) 1A1 is a key enzyme in phase I bioactivation of xenobiotics (8). CYP1A1 contributes to aryl hydrocarbon hydroxylase activity by catalyzing the first step in the metabolism of a number of polycyclic aromatic hydrocarbons such as the tobacco carcinogen benzo[a]pyrene (9). The CYP1A1 gene is located on chromosome 15q22-q24 and comprises seven exons and six introns spanning 5810bp (10). Several mutations in the CYP1A1 gene have been described (see website for details and also for nomenclature: <http://www.imm.ki.se/CYPalleles/cyp1a1.htm>). The four principle sequence variants are described here: a T3801C substitution at the 3' end of the gene (mutation 1) that gives rise to a *MspI* restriction enzyme site (11); a A2455G substitution (mutation 2) resulting in a Ile462Val exchange in the heme binding region of exon 7 (12); a C2453A variant (mutation 4) resulting in a Thr461Asn amino acid change (13); and an African American-specific T3205C substitution (mutation 3) in intron 7 (14). Mutations 1 and 2 have been shown to be in close linkage disequilibrium and to be associated with a more inducible form of CYP1A1 (12, 15-17). The resulting higher levels of the enzyme would result in an increased capacity to activate polycyclic aromatic hydrocarbons and thus, possibly, a greater susceptibility to cancer (18).

A recent pooled analysis conducted by Le Marchand et al. (18) of 11 studies containing a total of 1950 cases and 2617 controls showed that individuals heterozygous and homozygous for the exon 7 variant (mutation 2) had pooled odds ratios of 1.15 (95% confidence interval: 0.95-1.39) and 1.54 (95% confidence interval: 0.97-2.46), respectively. These results are consistent with a previous meta-analysis of CYP1A1 mutation 2 and lung cancer conducted by Houlston (19) showing subjects heterozygous and homozygous for mutation 2 to have odds ratios of 1.16 (95% confidence interval: 0.92-1.48) and 1.62 (95% confidence interval: 0.93-2.82), respectively. The Houlston (19) meta-analysis was based on seven studies of which five were included in the pooled analysis conducted by Le Marchand et al. (18).

Environmental exposure

Smoking

Exposure to tobacco smoke is a known risk factor for lung cancer and has been established as the most important etiological factor of lung cancer for both men and women (20-22). Tobacco smoke is known to contain at least 55 carcinogens that can be classed into three classes: polycyclic aromatic hydrocarbons, *N*-nitrosamines, and Asz-arenes (20, 23). Of the polycyclic aromatic hydrocarbons, benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide (benzo[a]pyrene) is the most studied. Activation of benzo[a]pyrene results in its transformation into 7,8-diol-9,10-epoxide (BPDE), a known substrate for the GSTM1 enzyme (24).

The metabolism of carcinogens such as benzo[a]pyrene involve activation steps which produce reactive intermediates and detoxification steps that produce water-soluble, excretable compounds. These activation steps are often mediated by the CYPs pathway and can result in the formation of compounds that can covalently bind to DNA, forming products known as adducts. The accumulation of these DNA adducts at critical loci such as oncogenes or tumour suppressor genes can lead to somatic mutations and disruption of the cell cycle (23). Individuals without the ability to produce the GSTM1 enzyme may potentially accumulate more DNA adducts through their inefficiency at excreting activated carcinogens such as 7,8-diol-9,10-epoxide (25).

Disease

Lung cancer

Although the incidence has peaked in the US and most of Europe, lung cancer is showing increasing incidence and mortality in many countries around the world. An estimate of 1.2 million new cases of lung cancer were diagnosed worldwide in 2000 accounting for approximately 12% of all new cancer cases, and 1.1 million died from the disease accounting for approximately 18% of all deaths from cancer (26). Lung

cancer has a worldwide incidence rate in men that is 2.7 times greater than in women and is also the number one cancer killer in men and the second largest in women (second to breast cancer). Lung cancer mortality rates among men are now abating in several countries whereas in women the mortality rate in most countries continues to climb, as predicted by later onset tobacco abuse (27). The principal histological types of lung cancer are squamous cell carcinoma, large cell carcinoma, small cell carcinoma, and adenocarcinoma. The former three types are strongly associated with smoking and in recent decades the frequency of adenocarcinoma has risen whilst that of squamous cell carcinoma has declined in several developed countries (28-34). This increase in adenocarcinoma incidence could partly be explained by the increase in filtered cigarette smoking.

Interaction

Polymorphisms of the genes encoding phase I (e.g. CYP1A1) and phase II (e.g. GSTM1) xenobiotic metabolising enzymes have been shown to be associated with susceptibility to lung cancer in a number of epidemiological studies (35). The CYP1A1 genotype and the GSTM1 null genotype gene-gene interactions are proposed to result in a risk greater than the additive risk for DNA damage and cancer. The combined variants have been associated with a significantly increased risk for lung cancer (36-38). In human cells, the deletion of GSTM1 is associated with strong inducibility of CYP1A1 gene transcription by 2,4,7,8-tetrachlorodibenzo-para-dioxin (39). When BPDE-DNA adduct levels were measured in lung tissue of smokers, a significant interaction between deficiency of the GSTM1 phenotype and high CYP1A1 inducibility or CYP1A1 allelic variants was observed (40, 41), leading to very high adduct levels in Caucasians with the CYP1A1 *MspI/MspI* – GSTM1 null genotype suggesting that this combination predisposes to an increased risk for tobacco-assisted DNA damage and lung cancer.

Hung et al. (42) recently conducted a pooled analysis of CYP1A1, GSTM1 and lung cancer in Caucasian non-smokers. The analysis included a total 302 cases and 1631 controls showing a combined effect of the CYP1A1 mutation 2 polymorphism and the GSTM1 null genotype with an odds ratio of 4.67 (95% confidence interval: 2.00-10.9)

compared with the concurrent presence of the CYP1A1 wild-type and GSTM1 non-null genotype.

Methodology

Criteria for considering studies

Types of studies

We will identify and includes reports that study the association of GSTM1 or/and CYP1A1 with lung cancer. Case-control or cohort designs will be considered and studies that restrict participants to patients with lung cancer only will be excluded.

Types of participants

Cases diagnosed with lung cancer will be considered. Controls must be healthy individuals.

Types of exposure

Participant GSTM1 (null genotype) and CYP1A1 (mutant 1 or 2) status will be considered as homozygous wild-type, heterozygous or homozygous mutant according to the genotype information. The GSTM1 genotype may be presented as the recessive model (null genotype – homozygous mutant vs non-null genotype – heterozygote and homozygous wild-type).

Types of outcome measured

Diagnosed lung cancer (squamous cell carcinoma, small cell carcinoma, large cell carcinoma and adenocarcinoma).

Search methods

The search terms were identified through searching of keywords from previously identified articles and reviews, search terms from previous meta-analyses, thesaurus terms, and the MEDLINE MeSH database. The results of these searches will be complemented with hand searching the reference lists of retrieved articles and searching conference proceedings. Our search strategy will be implemented in two stages in order to evaluate the inclusion of cancer terms. The first search will be conducted without the cancer terms and then second search with the cancer terms. The two searches will then be compared and the more comprehensive of the two searches will be utilised.

Search strategy (MEDLINE)

Search 1: Gene and lung cancer/neoplasm (without cancer terms)

(1...OR...11) AND (12...OR...14 OR 30 OR 31)

Search 2: Complete

(1...OR...11) AND (((12...OR...14) AND (15...OR...29)) OR 30 OR 31)

1. glutathione S-transferase*
2. glutathione S transferase*
3. glutathione transferase[MESH]
4. GSTM1
5. cytochrome p-450*
6. cytochrome p450*
7. cytochrome p-450 CYP1A1
8. cytochrome p450 CYP1A1
9. CYP1A1
10. P4501A1
11. aryl hydrocarbon hydroxylases[MESH]
12. lung
13. respiratory tract
14. lung[MESH]
15. cancer*
16. neoplasm*

17. neoplasms[MESH]
18. carcino*
19. carcinoma[MESH]
20. tumour*
21. tumor*
22. tumour[MESH]
23. dna adduct*
24. dna adducts[MESH]
25. squamous cell carcinoma*
26. large cell carcinoma*
27. small cell carcinoma*
28. adenocarcinoma*
29. non-small cell carcinoma*
30. lung neoplasms[MESH]
31. respiratory tract neoplasms[MESH]

Selection of studies

Titles and abstracts of studies identified on search of electronic databases (GSS) will be assessed (AF and GSS) to determine whether they meet the inclusion criteria. The original full-text articles of those possibly meeting these criteria will be obtained and the reference lists will be checked to identify any further relevant studies. Previous meta-analyses for CYP1A1 and lung cancer, GSTM1 and lung cancer, and CYP1A1, GSTM1 and lung cancer will provide a good starting point for key references in this field.

Data extraction

We will use standardised data collection forms devised to accommodate the information required from these studies. Descriptive data such as study design, outcome measures, the number and smoking habits of participants will be extracted. In addition to the descriptive data collected, information allowing a quality assessment of individual studies will also be collected and considered, based on the

criteria proposed by Little et al. (43). This will include information on study population selection method, the genotyping method used and whether lab workers were blinded to disease status, whether the effects of confounders on smoking association were assessed, conformity to HWE, and the risk of population stratification. All data will be extracted in duplicate (AF and GSS) with any disagreements will be adjudicated by a third reviewer (JH).

Data synthesis

This project will allow the framework for modelling gene-gene-environmental interaction from a meta-analysis perspective, as described in Salanti and Higgins (submitted), to be assessed with the additional development of any extensions to this methodology arising from this project (GAS and JH).

Potential conflict of interest

None.

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