Combinations of Susceptible Genotypes and Individual Responses to Toxicants

Ari Hirvonen

Finnish Institute of Occupational Health, Helsinki, Finland

The variation in individual responses to exogenous agents has been shown to be exceptionally wide. It is because of this large diversity of responsiveness that risk factors to environmentally induced diseases have been difficult to pinpoint, particularly at low exposure levels. Opportunities now exist for studies of host factors in environmentally induced cancer or other diseases in which an environmental component can be presumed. Many of the studies have shown an elevated disease proneness for individuals carrying the potential at-risk alleles of metabolic genes, but a number of controversial results have also been reported. One possible explanation for the divergent findings is lack of knowledge of the other potentially relevant genotypes for a given exposure. This paper gives an overview of the published data on combinations of genetic polymorphisms in relation to individual susceptibility to environmental toxicants. — *Environ Health Perspect* 105(Suppl 4):755–758 (1997)

Key words: CYP1A1, CYP2E1, CYP2D6, GSTM1, GSTT1, NAT1, NAT2, genetic polymorphisms

Introduction

People are exposed to agents (chemical, physical, and biological) that may contribute to the prevalence of genetic damage and chronic diseases in the population. Many of the diseases are affected by both host factors and the external environment. For instance, although carcinogenesis in humans is known to be significantly influenced by external factors, the metabolic activation or inactivation of the procarcinogens may modulate the process. This is best exemplified by tobacco smoking: cigarette smoking is the main cause of lung cancer but only a minority of smokers develop pulmonary cancers.

Recent findings suggest that inherited differences in metabolic capacity may in fact play a primary role in susceptibility to environmentally induced diseases (1–4). Genetic polymorphisms exist in a number of phase I (activating) and phase II (inactivating) enzymes. It is conceivable that individuals with genotypes associated with a more efficient activating enzyme and a less efficient inactivating enzyme might be at particularly high risk of adverse health effects, if exposed to toxicants.

Phase I enzymes cytochrome P450 1A1 (CYP1A1) and 2D6 (CYP2D6) are the most studied candidates as modifiers of individual responses to environmental agents (1–4). The rare Val and m2 alleles of CYP1A1 gene (frequencies 1–2% in Caucasians) may increase individual cancer risk by heightening aryl hydrocarbon hydroxylase (AHH) enzyme inducibility. In contrast, the segment of Caucasians (approximately 7%) who possess two deficient CYP2D6 alleles (poor metabolizer [PM] genotype) may be at decreased risk of cancer (1–4), but at increased risk of several central nervous system disorders (5–8).

Among phase II enzymes, N-acetyltransferases (NATs) and glutathione S-transferases (GSTs) have attracted most of the recent interest. The GST M1 (GSTM1) and T1 (GSTT1) genes are polymorphic so that the absence of enzyme activity results from a homozygous deletion of the respective gene, called the null genotype (9,10). About 50% of Caucasians have the GSTM1 null genotype, which may pose an increased risk of various environmentally induced cancers (11–14). The GSTT1 null genotype, which putatively has a frequency of 15 to 25% in Caucasians, has been associated with enhanced susceptibility to primary brain tumors (astrocytoma and meningioma) (15), and to myelodysplastic syndromes, which are clonal proliferative disorders of bone marrow that often progress to acute myeloid leukemia (16).

The human N-acetylation polymorphism is demonstrable by individual variations in metabolism of several substrates, including sulfamethazine and isoniazid (17). The differences in the metabolism of these compounds distinguish phenotypically slow and fast acetylators. The slow acetylators, whose frequency worldwide ranges from about 10% to more than 90% (18), may be at increased risk of arylamine-induced cancers (19–22).

Previous studies on acetylation polymorphisms have focused on the NAT2 gene locus, and allelic variants of NAT2 gene correlate with differences in acetylation capacity (23–26). However, recent evidence shows structural heterogeneity in the NAT1 gene locus also, correlating with N-acetylation activity distinct from that typified by isoniazid and sulfamethazine (27,28). It has been suggested that NAT1 is primarily responsible for NAT activity in human uroepithelium (29), and correlation between NAT1 polyadenylation signal polymorphism and risk of colorectal cancer has been reported (30).

Given the number and variability in expression of carcinogen-metabolizing enzymes and the complexity of chemical exposures, assessment of a single polymorphic genotype cannot be expected to be sufficient for evaluating individual susceptibility to environmental agents. Establishment of a broader risk profile for each individual or subgroup is required. The relatively scarce data presently available on the combinations of susceptible genotypes in individual responses to toxicants is reviewed below.

Combined Genotypes and Disease Susceptibility

The first observation of the combined effect of metabolic genes in disease proneness was by Hayashi and co-workers (31) who described a 5.8-fold relative risk (95% CI 2.3–13.3) for all lung cancer types and a 9.1-fold relative risk (95% CI 3.4–24.4) for squamous cell carcinoma of the lung in Japanese individuals who were homozygous for the CYP1A1 Val allele and concurrently lacked the GSTM1 gene. A similar, although less pronounced risk (OR 3.0, 95% CI 1.2–7.2) for developing this histological
type of lung cancer was attributed to Caucasians carrying the CYPIAI allele m2, and having the GSTM1 null genotype (32). Subsequent evaluation of the effect of smoking in the Japanese study revealed that this genotype composition may pose an especially remarkable risk for squamous cell carcinoma (OR 41.0, 95% CI 8.7–193.6) at low-dose cigarette smoking (33). These findings are consistent with the notion that some procarcinogens in cigarette smoke are activated by CYPIAI and inactivated by GSTM1.

The major importance of the high AHH inducibility in combination with homozygous GSTM1 null genotype in environmentally induced lung cancer is further underlined by the observation that the expressing GSTM1 gene appears to have a protective effect against bronchial cancer among individuals with inducible CYPIAI enzyme (34). In contrast, no multiplicative effect was found for CYPIAI and GSTM1 genotypes in a study which found a somewhat higher breast cancer risk (OR 1.6, 95% CI 1.2–23.4) for postmenopausal, light smokers with the CYPIAI Val allele compared to those without the allele (35). Interestingly, the presence of the GSTM1 gene was recently correlated with induction of only low levels of CYPIAI mRNA (36).

The protective role of GSTM1 gene in environmental exposures was supported by a study among Finnish asbestos workers, where the GSTM1 null genotype was associated with a 2-fold risk (OR 1.8, 95% CI 1.0–3.5) of asbestos-associated malignant mesothelioma (37). Rather surprisingly, the NAT2 slow acetylator genotype appeared as a similarly important modifier of the mesothelioma risk (OR 2.1, 95% CI 1.1–4.1). Moreover, a striking interaction was observed between these genotypes; highly asbestos-exposed workers without the GSTM1 gene and with the NAT2 slow acetylator genotype were 7.4-fold (95% CI 1.6–34.0) more prone to develop the neoplasm than fast acetylators with GSTM1 gene.

N-acetylation may be a comparably important detoxification step in environmental exposures, as is glutathione conjugation. Thus, the combination of NAT1 and NAT2 susceptible genotypes may be a particularly unfavorable genotype composition in arylamine exposures. The recently observed association between increased risk (OR 1.9, 95% CI 1.2–3.6) of colorectal cancer and the fast NAT1 acetylator allele (NAT1*10) was most apparent (OR 2.8, 95% CI 1.4–5.7) among the fast NAT2 acetylators (30).

Given the substrate specificities of the GSTs, individuals with null genotypes at both GSTM1 and GSTT1 may be at particular risk of environmentally induced cancers. To date, only a few studies have addressed this issue comprehensively. Warwick and co-workers (38) studied the role of combinations of CYP2D6, GSTM1, and GSTT1 genotypes in susceptibility to cervical intraepithelial neoplasia and squamous cell carcinoma of the lung. In this study, no combined effect was detected for the two GST genes. However, the CYP2D6 extensive metabolizer (EM) genotype and the GSTT1 null genotype were shown to be important risk factors for these pulmonary disorders, both individually and in combination. The GSTT1 null and CYP2D6 PM genotypes were shown recently to also increase the risk for two common pituitary brain tumors, astrocytoma and meningioma, but no interactive effects between the genotypes were identified (39). Moreover, in another study, no association between the CYP2D6 or GSTM1 genotypes and susceptibility to the pituitary tumors was detected (39).

Since the recently observed association between the GSTT1 null genotype and total ulcerative colitis was also shown to be uninfluenced by the GSTM1 genotype (40), the yet published data does not indicate concurrent deficiency of the GSTM1 and GSTT1 genes playing an important role in individual disease proneness. However, our preliminary findings that this genotype composition poses about a 2.5-fold risk of lung cancer compared to the presence of both of the GST genes (Saarikoski et al., unpublished data) warrant more thorough evaluation of this issue. Finally, the combined effect of CYP2E1 and GSTM1 genotypes was examined in a study suggesting a 2.5-fold risk of hepatocellular carcinoma for individuals with wild-type CYP2E1 gene compared to those with at least one variant allele in the transcription regulatory area of the gene (41). Again, no modulating effect was found for the GSTM1 genotype.

**Exposure Markers and Combined Susceptibility Genotypes**

Attempts to relate metabolic phenotype or genotype to risk of environmentally induced diseases are now extending to studies on various markers of exposure such as DNA adduct formation and indicators of cytogenetic damage, e.g., sister chromatid exchanges and micronuclei.

Genotoxic agents can form DNA adducts and cytogenetic changes via a complex metabolic pathway that includes CYPIA1; intermediates can be detoxified by conjugation through pathways including GSTs and NATs. A recent study failed to support the importance of this metabolic pathway by reporting lack of any significant association between DNA adduct levels and CYPIA1 and GSTM1 genotypes among nonsmoking fire fighters (42). No relation was found between these genes and the B- and T-micronuclei formation in PAH-exposed chimney sweeps (43,44); the only statistically significant deviation was a 60% increase in aromatic DNA-adduct levels in GSTM1 null chimney sweeps without any CYPIA1 m2 alleles compared to controls with the same genotype composition (44).

However, the GSTM1 null genotype has been associated with significantly higher aromatic DNA adduct levels in bus maintenance workers with the NAT2 slow acetylator genotype compared to those with the GSTM1 gene (45). In a similar study, nonsmoking bus drivers with NAT2 slow acetylator genotype and GSTM1 null genotype had the highest levels of both DNA adducts and cytogenetic damage (46). Moreover, aminobiphenyl-hemoglobin adduct levels are most elevated in smokers possessing this combination of genotypes compared to smokers with other combinations (47). Carcinogenic DNA adduct levels in the mucosa of the urinary bladder were highest in arylamine-exposed individuals who had inherited both the slow NAT2 acetylator genotype and the rapid NAT1 acetylation-associated allele NAT1*10 (48), further addressing the potential importance of individual acetylation capacity.

**Conclusion**

Knowledge of the genetic basis for variations in human metabolic capacity has opened new possibilities for studies focusing on the role of host factors in susceptibility to environmentally induced diseases. Rapid advances in methodology that determines potential metabolic at-risk genotypes, in combination with exposure markers such as DNA adduct levels in target (surrogate) tissues, may soon allow us to identify susceptible individuals and subgroups in environmentally exposed populations. However, the establishment of combined impact of all relevant genes for a given exposure is anticipated to be a prerequisite for this.
1. Idle JR. Is environmental carcinogenesis modified by host polymorphism? Mutat Res 24:239–266 (1991).
2. Nebert DW. Role of genetics and drug metabolism in human cancer risk. Mutat Res 247:267–281 (1991).
3. Harris CC. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res Suppl 51:5023s–5044s (1991).
4. Hirvonen A. Genetic factors in individual responses to environmental exposures. J Occup Environ Med 37:37–43 (1995).
5. Smith CAD. Debrisquino hydroxylase polymorphism and susceptibility to Parkinson’s disease. Lancet 339:1375–1377 (1992).
6. Armstrong M, Daly AK, Chorlerton S, Bateman DN, Idle JR. Mutant debrisoquino hydroxylase genes in Parkinson’s disease. Lancet 339:1017–1018 (1992).
7. Boris J, Harsany V, Schneble H, Haegele KD. pNAT and CYP2D6 gene polymorphism in epileptic patients. Biochem Pharmacol 48:1717–1720 (1994).
8. Iwahashi K. CYP2D6 genotype and possible susceptibility to the neuroleptic malignant syndrome. Biol Psychiatry 36:780–782 (1994).
9. Seidengr J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA 85:7293–7297 (1988).
10. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. Human glutathione S-transferase (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276 (1994).
11. Zhong S, Wyllie H, Barnes D, Wolf CR, Spurr NK. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 14:1821–1824 (1993).
12. Hirvonen A, Hugafvel-Pursiainen K, Anttila S, Vainio H. The GSTM1 null genotype as a potential risk modifier for squamous cell carcinoma of the lung. Carcinogenesis 14:1479–1481 (1993).
13. Bell DA, Stephens DA, Castrano T, Umbach DM, Watson M, Deakin M, Elder J, Hendrickse C, Duncan H, Strange RC. Polydénylation polymorphism in the acetyltransferase I gene (NAT) increases risk of colorectal cancer. Cancer Res 55:3537–3542 (1995).
14. Hayashi SI, Watanabe J, Nakachi K, Kawajiri K. Genetic linkage of lung cancer-associated Msp I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450A1 gene. J Biochem 110:407–411 (1991).
15. Alexandrie A-K, Sundberg MI, Seidegård J, Seidegård J, Törnling G, Rannug A. Genetic susceptibility to lung cancer with special emphasis on CYP1A1 and GSTM1: a study on host factors in relation to age at onset, gender and histological cancer types. Carcinogenesis 15:1785–1790 (1994).
16. Nakachi K, Imai K, Hayashi S, Kawaji K. Polymorphisms of the CYP1A1 and glutathione S-transferase α genes associated with susceptibility to lung cancer in relation to cigarette dose in Japanese population. Cancer Res 53:2994–2999 (1993).
17. Anttila S, Hirvonen A, Hugafvel-Pursiainen K, Karjalainen J, Nurminen T, Vainio H. Combined effect of CYP1A1 inducibility and GSTM1 polymorphism on histological type of lung cancer. Carcinogenesis 15:1133–1135 (1994).
18. Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JR, Brasure JR, Laughlin R, Nemoto T, Michalek AM, Harrington A et al. Cytochrome P450 1A1 and glutathione S-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res 55:3483–3485 (1995).
19. Vauy C, Laine R, Nouguez P, de Cotten P, Jaulin C, Praz F, Popond P, Amor-Gueret M. Human glutathione S-transferase M1 null genotype is associated with a high inducibility of cytochrome P450 1A1 gene transcription. Cancer Res 55:5520–5523 (1995).
20. Hirvonen A, Pelin K, Tammilehto L, Karjalainen A, Mattson J, Persson M, Fürst T, Gisselsson D, Franson J, Sandler M, et al. Combined metabolic genotypes and environmental exposures. Environmental Health Perspectives • Vol 105, Supplement 4 • June 1997 757
K, Linnainmaa K. Inherited GSTM1 and NAT2 defects as concurrent risk modifiers for asbestos-associated human malignant mesothelioma. Cancer Res 55:2981–2983 (1995).
38. Warwick A, Sarhanis P, Redman C, Pemble S, Taylor JB, Ketterer B, Jones P, Allderdie J, Gilford J, Yenig L et al. Theta class glutathione S-transferase GSTT1 genotypes and susceptibility to cervical neoplasia: interactions with GSTM1, CYP2D6 and smoking. Carcinogenesis 15:2841–2845 (1994).
39. Perreir CW, Clayton RN, Pistorello M, Boscaro M, Scanari M, Bates AS, Buckley N, Jones P, Fryer A, Gilford J et al. GSTM1 and CYP2D6 genotype frequencies in patients with pituitary tumours: effects on p53, ras and gsp. Carcinogenesis 16:1643–1645 (1995).
40. Duncan H, Swan C, Green J, Jones P, Brannigan K, Alldersea J, Fryer A, Strange RC. Susceptibility to ulcerative colitis and Crohn’s disease: interactions between glutathione S-transferase GSTM1 and GSTT1 genotypes. Clin Chim Acta 240:53–61 (1995).
41. Yu M-W, Gladek-Yarborough A, Chiampraset S, Santella RM, Liaw Y-F, Chen C-J. Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. Gastroenterology 109:1266–1273 (1995).
42. Rothman N, Shields PG, Poirier MC, Harrington AM, Ford DP, Strickland PT. The impact of glutathione S-transferase M1 and cytochrome P450 1A1 genotypes on white-blood-cell polycyclic aromatic hydrocarbon-DNA adduct levels in humans. Mol Carcinog 14:63–68 (1995).
43. Carsten U, Alexandrie A-K, Högstedt B, Rannug A, Bratt J, Hagmar L. B- and T-lymphocyte micronuclei in chimney sweeps with respect to genetic polymorphisms for CYPIA1 and GSTT1 (class Mu). Mutat Res 289:187–195 (1993).
44. Ichiba M, Hagmar L, Rannug A, Högstedt B, Alexandrie A-K, Carstensen U, Hemminki K. Aromatic DNA adducts, micronuclei and genetic polymorphisms for CYPIA1 and GSTT1 in chimney sweeps. Carcinogenesis 15:1347–1352 (1994).
45. Hou S-M, Lambert B, Hemminki K. Relationship between hprt mutant frequency, aromatic DNA adducts and genotypes for GSTM1 and NAT2 in bus maintenance workers. Carcinogenesis 16:1913–1917 (1995).
46. Norppa H, Pelin K, Järventaus H, Ollikainen T, Knudsen L, Okkels H, Scarpato R, Migliore L, Hirvonen A. Polymorphisms of xenobiotic-metabolizing enzymes: influence on cytogenetic parameters in vitro and in vivo. Abstract. Nordtox/NordEMS-96: The 4th Nordic Toxicology Meeting, 27–31 March 1996, Storlien, Sweden.
47. Yu MC, Ross RK, Chan K, Henderson BE, Skipper PL, Tannenbaum SR, Goetzee GA. Glutathione S-transferase M1 genotype affects aminobiphenyl-hemoglobin adduct levels in white, black, and asian smokers and nonsmokers. Cancer Epidemiol Biomarkers Prev 4:861–864 (1995).
48. Badavi A, Hirvonen A, Bell DA, Lang N, Kadlubar FF. Role of aromatic amine acetyltransferases NAT1 and NAT2, in carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res 55:5230–5237 (1995).