Genomics views: Xenobiotic metabolizing enzymes and cancer risks

Kamel Rouissi, MD[1], Bechr Hamrita, MD*[1], Slah Ouerhani, MD[2], Amel Benammar Elgaaied, MD[1]*

(1) Laboratory of Genetic, Immunology and Human Pathology, Faculty of Sciences of Tunis, University of El Manar I. 2092, Tunis, Tunisia
(2) Laboratory of Molecular and Cellular Hematology, Institut Pasteur de Tunis, Tunisia
** Both authors contributed equally to the study.*

✉ *Corresponding Author: Correspondence to: Dr. Bechr Hamrita, MD*
Laboratory of Genetics, Immunology and Human Pathology, Faculty of sciences of Tunis
University of El Manar I, 2092, Tunis, Tunisia.
E-mai: bechrhamrita@yahoo.fr

Keywords: Cytochrome P450, N-acetyltransferase, Glutathione S-transferase, Cancer, Polymorphisms, Xenobiotic enzymes.

ISSN: 2070-254X

Abstract

Epidemiological studies have estimated that approximately 80% of all cancers are related to environmental factors. Individual cancer susceptibility can be the result of several host factors, including differences in metabolism, DNA repair, altered expression of tumor suppressor genes and proto-oncogenes, and nutritional status. In fact, xenobiotic metabolism is the principal mechanism for maintaining homeostasis during the body's exposure to xenobiotics. Most xenobiotics that enter in the body are subjected to metabolism that functions primarily to facilitate their elimination. Metabolism of certain xenobiotics can also result in the production of electrophilic derivatives that can cause cell toxicity and transformation. The balance of xenobiotic absorption and elimination rates in metabolism can be important in the prevention of DNA damage by chemical carcinogens. Thus the ability to metabolize and eliminate xenobiotics can be considered one of the body's first protective mechanisms. However, there are marked species differences in the way mammals respond to xenobiotics, which are due in large part to molecular differences in xenobiotic-metabolizing enzymes and has been related to the enzymatic polymorphisms involved in activation and detoxification of chemical carcinogens and that can impact drug therapy and cancer susceptibility. This paper focus the member of the cytochrome P450 family of enzymes (Cyp2D6), glutathione *S*-transferases (GSTT1 and GSTM1) and *N*-acetyltransferases (NAT1 and NAT2) on genetic polymorphisms involved in the metabolism of endocrine disruptors potentially related to cancer development.

Introduction

Xenobiotics are natural or artificial chemical substances that are alien to the body, such as drugs, industrial products, pollutants, alkaloids, and toxins produced by fungi, plants, and animals, many of them acting as endocrine disruptors. In their natural or bio-transformed state, xenobiotics can affect DNA integrity, leading to cancer if exposure persists (1). Accumulated DNA damage, added to spontaneous replication errors not corrected by the repair system, can cause irreversible mutations which in turn can lead to the development of tumors and/or progression of cancer. Epidemiological studies show that 80 % of all cancers are related to environmental factors like smoking and occupational and dietary exposures (2). Thus, the individual capacity to bio-transform toxic into non-toxic xenobiotics can be considered the first line of defense in the process characterized by successive stages of transformation of potentially toxic chemical substances as a pathway towards their subsequent elimination. The enzymes involved are frequently the ones that determine the intensity and duration of drug action and other xenobiotics, hence their importance in chemical and carcinogenic toxicity. Bio-transformation of xenobiotics involves the modification of their physical properties, generally from lipophilic to hydrophilic, facilitating their excretion. Otherwise, many lipophilic xenobiotics would be excreted so slowly that they would eventually accumulate, destroying the organism by making it biologically nonviable (1). Bio-transformation involves two stages: phase I, mainly involving enzymatic activity of the cytochrome P450 (CYP) family; and phase II, catalyzed by conjugation enzymes like glutathione S-transferase (GST), and N-acetyltransferase (NAT).

Most carcinogenic chemical products are not toxic and require metabolic activation before interacting with cellular macro-molecules. Phase I enzymes promote the activation of drugs and pro-carcinogens for the genotoxic electrophilic intermediaries. Meanwhile, phase II enzymes generally act as inactivating enzymes, that is, they catalyze the binding of intermediary metabolites to cofactors, transforming them into more hydrophilic products, thus facilitating their elimination (3). Therefore, the coordinated expression and regulation of xenobiotic metabolizing enzymes (XMEs) in both phase I and phase II and their metabolic equilibrium in the cells of target organs can be important factors in determining susceptibility to cancer as related to exposure to carcinogens (4). Mutations in these genes can produce partially defective enzymes or ones with altered specificities to the substrates, and thus there may or may not be the production of functional proteins or even enzymes with different levels of activities. The combination of the alleles from these genes can cause an increase or decrease in the susceptibility to certain toxic agents or environmental carcinogens.

There are marked inter-individual and inter-ethnic differences in the capacity to metabolize drugs and other xenobiotics. This variation is due to the polymorphisms in the corresponding genes and to physiological, pathological, and environmental factors (5). Inter-individual variability in xenobiotic metabolism has been associated with greater or lesser susceptibility to toxicity or cancer risk in response to the same exposure to a given environmental pollutant. Thus, individuals incapable of adequately detoxifying a metabolic carcinogen

or toxic agent due to reduced enzymatic activity would undergo more DNA and cell damage with the formation of adducts, or chemical elements bound to the DNA and protein macromolecules, genomic instability, and consequently would have a greater risk of developing toxicity or cancer (3). Increasing attention has been focused on our knowledge of variations in susceptibility to diseases within a population and the identification of risk factors so as to orient preventive policies. The literature has shown that variability in the expression of genes for XMEs (xenobiotic metabolizing enzymes) suggests an influence on the biological response to carcinogens. Despite displaying weak indication of risk at the individual level due to association with various other factors, gene polymorphisms (principally those influencing the metabolic activation or detoxification of carcinogenic chemical products) can be important factors for susceptibility at the population level (6). Molecular epidemiology has made great progress in detecting many human gene polymorphisms in XMEs, and some have shown to be correlated to increased risk of cancer. This paper focuses on just a few genetic polymorphisms that have been investigated more extensively due to their association with cancer, such as genes encoding cytochrome P450 (CYP2D6), glutathione S-transferase (GSTM1 and GSTT1) families and the *N*-acetyltransferases (NAT1 and NAT2).

Cytochrome P450 (CYPs)

The human cytochrome P450 superfamily comprises at least ten known and characterized families and numerous sub-families (7). During bio-transformation, cytochrome P450 mediates the phase I reactions in which xenobiotics are detoxified or activated to reactive intermediate substances. The highest concentration of these enzymes has been observed in the liver endoplasmic reticulum, but they are present in all tissues in a tissue-specific manner. In the liver, they determine the intensity and duration of drug action and promote the detoxification of xenobiotics. They also catalyze the activation of xenobiotics to toxic and/or carcinogenic metabolites. The contribution of each P450 enzyme to the activation of carcinogens has been extensively evaluated, and this research has shown that most environmental carcinogens are activated principally by a limited number of them, including the following: CYP1A1, CYP1A2, CYP2E1, CYP2C19, CYP2D6, and CYP3A (8). Many are polymorphic, displaying different metabolic activities, reflected in adverse toxic effects, including carcinogenesis induced by endogenous chemical substances (9).

CYP2D6

The CYP2D6 gene belongs to the CYP2 family and was mapped in human chromosome 22, band 22q13.1 (10). Enzyme CYP2D6 (debrisoquine-4-hydroxylase) metabolizes debrisoquine and many other drugs, like anti-depressants, neuroleptics, many anti-arrhythmics, and lipophilic b-blockers (9). In addition to these substrates, CYP2D6 also acts on the carcinogen nitrosamine NNK (4-methylnitrosamino-1(3-pyridyl)-1-butanone), a component of cigarette smoke (11). The absence of debrisoquine-4-hydroxylase activity can have serious clinical consequences and even lead to death, since usual doses can cause high plasma levels of the drug, leading to side effects (12). Debrisoquine is a drug used for treating hypertension, and a wide variation has been observed in the hypotensive response. A clinical consequence of slow metabolism is the great sensitivity to the anti-hypertensive effects of debrisoquine (13). Although, extensive metabolizers display less risk of the effects of overdoses from debrisoquine and related drugs, they show an increased risk of developing cancer of the liver, gastro-intestinal tract, and lung as compared to slow metabolizers (14).

Most individuals (80-90%) have at least one wild allele (CYP2D6*1) for the CYP2D6 gene and are classified functionally as extensive metabolizers. There are two other groups of individuals: one with intermediate metabolic activity, known as intermediate metabolizers, and the other known as ultra-rapid metabolizers. The first phenotype is attributed to a mutation in the wild allele (CYP2D6*1) and the second to an amplification of either the wild allele or an active mutant allele. Finally, there is a small group (5-10% of Caucasians, 2% of African Americans, and 1% of Orientals) who are poor metabolizers and identified by loss of gene function and absence of protein (15).

An updated review of this complex polymorphism is provided by Sachse et al, (16). According to these authors, different alleles for the CYP2D6 gene consist mainly of point mutations, conversions, gene duplications, and complete gene deletion. Some 15 alleles have been recorded and associated with low activity (CYP2D6*2, *9, *10) and with its absence (CYP2D6*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16). The combination of all these alleles provides a wide range of possible phenotypes in relation to CYP2D6 activity. Given the nature of the substances metabolized by these enzymes, this polymorphism is used principally to identify poor metabolizers with anomalous responses to given drugs. In ultra-rapid metabolizers, the usual doses of given drugs fail to produce the desired pharmacological effect. Determination of CYP2D6 expression serves to detect therapeutic problems due to metabolism and can contribute to individualization of the dose regimen, reaching optimum drug therapeutic levels and reducing both cost and possible adverse effects (17). An association was observed between this gene and lung cancer (18) and oral cancer (19). Increased CYP2D6 activity has been related to some malignant processes. In bladder cancer, Anwar et al, (20) have demonstrated that genetic polymorphism in CYP2D6 could play an important role as host risk factors for development of urinary cancer among Egyptians (20). In north Tunisia, a previous work revealed that CYP2D6*4 allele did not appear to influence bladder cancer susceptibility ($p > 0.05$). A similar result was obtained when he stratified cases group according to tobacco status (21). However, in other work on a cohort from the middle centre of Tunisia a significant association was found between CYP2D6 (G/G) wild type and breast carcinoma risk only in postmenopausal patients ($p = 0.04$) (22). The data suggest that the increased metabolism of one or more agents in the diet or other environmental agents, mediated by CYP2D6, forms reactive intermediaries that influence the initiation or promotion of cancer in various tissues (23). The reduced CYP2D6 activity has been related also to greater risk of oral cancer (17).

Other studies demonstrate that patients with lung cancer have shown a greater frequency of extensive metabolizers (EM) genotype. This association is supported by the discovery that CYP2D6 can activate nitrosamine 4-(methylnitrosamino)-1-(3-piridyl)-1-butanone, specific to tobacco for reactive metabolites (11). Kato et al, (24) observed that the levels of DNA adducts in the lung were increased as a function of CYP2D6 activity, a result consistent with activation of tobacco mediated by this enzyme (24).

CYP2E1

Many studies have investigated the association between the CYP2E1 5'-flanking region (RsaI/PstI) polymorphism and head and neck cancer susceptibility, but the results were conflicting. Using the fixed effects model, Lu et al, (25) have found significant association between PstI/RsaI polymorphism and head and neck cancer risk.

Significant results were also found in East Asians and Mix populations when stratified by ethnicity. However, no significant associations were found for Caucasians in all genetic models. Stratified analyses according to source of controls, significant associations were found only in hospital base controls.

In the subgroup analyses by tumor types, significant association was detected only in oral cancer group, while no significant associations among laryngeal- or pharyngeal- cancer subgroup. In Egypt, Anwar et al, (20) have demonstrated any differences in the distribution of CYP2E1 polymorphism between bladder cancer patients and controls as detected by PstI restriction fragment length polymorphism (RFLP) analysis (20). These previous findings are in accordance with other work which demonstrates the absence of any association between CYP2E1 gene polymorphisms and breast cancer (22).

CYP2C19

CYP2C19 is one of the enzymes involved in the metabolism of tamoxifen into active metabolites. In patients on tamoxifen, CYP2C19*2 and *3 variants, known for their lack of enzyme activity, were associated with a significantly longer breast cancer survival rate than patients with the wild-type (26). In Japanese population, a genetic polymorphism of CYP2C19 was associated with susceptibility to biliary tract cancer (27).

Regarding hormone-associated tumors such as ovarian, cervical or prostate cancers, many conflicting data have been found. In this field, numerous studies conducted on different populations and ethnic groups have indicated the absence of a major impact of the CYP2D6, CYP2C19 and CYP2E1 genes in cancer risk (28). These previous findings are in accordance with ours which demonstrate the absence of any association between CYP2C19 and CYP2E1 gene polymorphisms with breast cancer. In addition, when compared with the allele frequencies of the CYP2D6, CYP2C19 and the CYP2E1 genes in Italian, Portuguese and Egyptian populations, we found similar frequencies in healthy Tunisian individuals (29-31) .

Glutathione S-transferase (GST)

The glutathione S-transferases (GSTs) are key phase II metabolic reaction enzymes, and they play critical roles in protection against products of oxidative stress and electrophiles. (32). These conjugation reactions facilitate the excretion of many xenobiotics, including carcinogens, toxins, and drugs in the form of mercapturic acids. Different GST isozymes have been identified in human populations, some with tissue-specific expression (33). In mammals they are expressed at a higher level in the liver, constituting more than 4% of total soluble protein (34). At least five related gene families, mu, alpha, pi, theta and sigma, have been identified, and genetic polymorphisms have been reported for GSTM1 and GSTT1, resulting in either decreased or altered enzyme activity (35).

GSTM1

Five mu class genes (M1-M5) on chromosome 1p13 have been identified (36). Inter-individual differences in GSTM1 are due either to gene deletion (the null genotype) or allelic variation, resulting in production of a catalytic active protein with closely similar catalytic activities which differ by a single base pair in exon 7, introducing a restriction site for Hae II in the gene sequence (37). GSTM1 is expressed at a high level in the liver. Epidemiological studies suggest that individuals, who are homozygous null, have an increased risk for cancer at a number of sites, lung, bladder, colon, breast (38). The risk of lung cancer in association with GSTM1 null genotype is dependent on the extent of tobacco smoke exposure, the frequency of GSTM1 null being significantly higher in the high exposure group. It may suggest that at low exposure level the genotype of the GSTM1 is less important, and that other detoxification pathways could handle low doses. Tobacco smoke is considered a risk factor in larynx cancer, and the GSTM1 null genotype was associated with an increased risk, but only among smokers that smoke 20 g tobacco/day or less (39).

GSTT1

Two genes have been identified in the theta class: GSTT1 and GSTT2, located in chromosome 22, in the same region (22q11.2) (40). The null polymorphism at the GSTT1 shows large variation and occurs at a frequency of 16%-40%. The biological consequences of this polymorphism are fairly difficult to predict, as the enzyme is involved in both detoxification and activation reactions. Lack of GSTT1 enzyme activity was observed among 19% of Caucasians (41). Many studies have reported that the GSTT1 null genotype (GSTT1*0) was associated with increased risk for bladder and lung cancers (42). However, some studies reported that the risk of cancer was increased only among those with the GSTT1 positive (wild-type) genotype (43).

GSTT1, GSTM1 and susceptibility to cancer

Most of the studies on the role of GST polymorphisms in the development of cancer have focused on GSTM1 and GSTT1. In relation to lung cancer, Seidegard et al, (44) showed that smokers deficient in GSTM1, that is, homozygotes for the null allele, showed increased risk for this type of cancer (44). The presence of the whole gene appeared to protect against chemically-induced cytogenetic damage and DNA adducts in the lung (45). In the same way, Ryberg et al, (46) showed that the level of DNA adducts in the lungs of male smokers was influenced more by GSTP1 than by GSTM1 (46).

According to these authors, smokers with at least one mutant allele for GSTP1 showed significantly higher levels of DNA adducts than controls, while the GSTM1 null genotype did not show higher levels. When they combined the two polymorphisms GSTM1 and GSTP1 they observed that patients with the GSTM1 null genotype and the GSTP1 genotype with at least one mutant allele had significantly higher levels of adducts than other combinations (46).

To-Figueras et al, (47) did not observe a significantly greater frequency of the GSTM1 null genotype in lung cancer cases in a Caucasian population, which agrees with data from Nyberg et al, (48) (47-48). The frequency of the other genotypes GSTM1 A, GSTM1 B, and GSTM1 A/B did not differ between cases and controls (Nyberg et al, 1998), unlike the results of other studies on bladder tumors, larynx, and skin, in which a protective role was proposed for GSTM1*A (49-50) and GSTM1*A/GSTM1*B (51).

Various others authors have described family clustering in oral cancer and the proposed explanation involves gene polymorphisms for drug-metabolizing enzymes (52). Jourenkova et al, (53) studied the effect of GSTM1 and GSTT1 genotypes on the risk of cancer of the larynx and observed an increased risk related to the GSTM1 null genotype and greater risk for GSTT1 null (53). Individuals lacking both genes GSTM1 and GSTT1 had a two-fold risk, although not significant, as compared to those with at least one of the genes, and a three-fold risk as compared to those with both genes. In addition, a statistically significant interaction was observed between the GSTM1 genotypes and levels of tobacco consumption. However, Park et al, (54) failed to find an association between the GSTM1 null allele and oral cancer (54). Meanwhile, Matthias et al, (55), studying cancer of the upper aero-digestive tract (oral, laryngeal, and pharyngeal squamous cell carcinoma), did not observe differences in the frequency of the GSTT1 null genotype between cases and controls, but did observe that the frequency of genotype GSTM1 was significantly lower in patients with oral, laryngeal, and pharyngeal squamous cell carcinoma, suggesting a protective effect (55).

Recent epidemiological studies on GSTs and breast cancer have been illustrated. Some studies suggest an association between the GSTM1 null genotype and breast cancer in postmenopausal women (56). More recently, Park et al, (54) observed that the GSTM1 null genotype showed a statistically significant association with breast cancer, increasing the risk in premenopausal but not in postmenopausal women, while the GSTT1 null genotype showed similar risk levels in all the groups analyzed (54). When they combined these two genotypes, they observed that the presence of both null alleles significantly increased the risk of this cancer, especially in premenopausal women and those who consumed alcohol, suggesting a gene-environment interaction in individual susceptibility to breast cancer (54). These results disagree with those of Curran et al, (57) who failed to find any association between polymorphisms GSTM1, GSTT1 and breast cancer in an Australian sample (57). Other independent studies on various types of cancer have also shown this positive association between these genes and lung cancer (58), bladder cancer (20), colorectal cancer (59), breast cancer (60), esophageal cancer (61), oral cancer (62), and leukemia (63).

In North Tunisia, we have conducted a case control study to assess the role of smoking, slow NAT2 variants, GSTM1 and GSTT1 null genotypes in bladder cancer development. In all groups of patients, we have shown that GSTM1 and GSTT1 null genotypes did not appear to be a factor affecting bladder cancer susceptibility. Furthermore, we found that NAT2 slow acetylator individuals temporarily carrying wild-type GSTT1 or GSTM1 null genotypes have a strong increased risk of bladder cancer (OR=26 and 22.17, respectively) (64).

N-acetyltransferase (NAT)

The NAT locus on human chromosome 8 encodes three distinct NAT genes, the two active NAT genes, NAT1 and NAT2, separated by an inactive pseudogene. NAT2 and NAT1 genes are polymorphically expressed, and variation at the NAT2 locus is responsible for the classic acetylation polymorphism.

NATs catalyze the acetylation of carcinogens and other xenobiotics. Arylamines, hydrazines and hydrazides are metabolized by NAT-mediated *N*-acetylation. A majority of *N*-acetylation requires acetyl-CoA as a cofactor; however, some NATs can also utilize *N*-arylhydroxamic acid as acetyl donors. NATs are cytosolic enzymes and are found in a large number of tissues. The ubiquitous expression profile of the NATs suggests a fundamental role in protection against reactive metabolites. *N*-acetylation is usually considered a detoxification process because it renders the nitrogen atom less susceptible to oxidation, an oxidative process mediated mainly by CYP1A2. NATs can also catalyze the bioactivation of arylhydroxylamines and arylhydroxamic acids by either an acetyl CoA-dependent *O*-acetylation or by intramolecular *N*, *O*-acetylation producing reactive *N*-acetoxyarylamine intermediates. These metabolites are considered the ultimate carcinogens, because they are able to react with DNA to form covalent adducts. As a consequence, these enzymes play a role in the carcinogenesis process initiated by aromatic amines and in arylamine-induced toxicities. *O*-acetylation is catalyzed by both NAT1 and NAT2.

Early epidemiological studies have shown that slow acetylators have an increased risk of bladder cancer compared with rapid acetylators (38), especially in workers occupationally exposed to aromatic amines. However, the NAT2 phenotype did not influence bladder risk among Chinese workers exposed to benzidine, suggesting that NAT2 *N*-acetylation is not a critical detoxification pathway for benzidine. It has been found that mono-functional acetylation is activation rather than a detoxification pathway for benzidine (65).

NAT2 activity is expressed in human liver and intestinal tissues. The most studied NAT2 polymorphisms are due to point mutations in the coding region. Slow acetylators, who possess NAT2 mutant alleles, produce proteins that are either poorly expressed, unstable, or have partially reduced catalytic activity. There are at least 15 different NAT2 allelic variants, of which the NAT2*4 is the most predominant and confers a fast acetylating phenotype. Of all the NAT2 allelic variants identified, three (NAT2*5, NAT2*6 and NAT2*7) were shown to account for most of the slow acetylators.

NAT1 protein is found in many human tissues, including the liver and bladder. NAT1 seems to be primarily responsible for the NAT and *O*-acetyltransferase activities in bladder and colon tissues. NAT1 activity in urinary bladder mucosa represents a major bioactivation step that converts urinary *N*-hydroxy-arylamines to reactive *N*-acetoxy esters to form DNA adducts. The activity of NAT1 is significantly correlated with putative aromatic amine adducts. Individuals classified as slow NAT2 and fast NAT1 would be at the highest risk. These individuals do also have a higher level of carcinogen–DNA adducts in bladder DNA than people carrying other genotype combinations (66-67). There are at least 8 identified allelic variants of human NAT1. Certain NAT1 variants, NAT1*10 and NAT1*17, have an elevated *N*- or *O*-acetylation compared with the more common NAT1*4 variant.

A significant association between the NAT1 genotype and lung cancer risk was observed in smokers, with the slow acetylators having a significantly higher risk, whereas no association between lung cancer risk and NAT2 was observed (Bouchardy et al, 1998). However, in a Swedish study, an increased risk for lung cancer was observed, but only in never-smokers (48).

A slightly increased risk of breast cancer was observed in smoking women classified as NAT2 slow, and this increased risk correlated with a higher mammary gland DNA adduct level. In contrast, NAT1 did not have any influence on the adduct level (68).

In case of bladder cancer, smokers with the NAT1*10 allele had an increased risk. There was evidence of a gene-dosage effect, as individuals homozygous for the NAT1*10 alleles had the highest risk. The NAT2 genotype alone did not significantly influence the bladder cancer risk, but the cancer risk from smoking exposure was particularly high in those with NAT2 slow alleles in combination with one or two copies of the NAT1*10 allele (69). In Tunisia, For the NAT2 slow acetylator genotype, the NAT2*5/*7 diplotype was found to have a 7-fold increased risk of bladder cancer (OR=7.14) (64). Mechanistically it is assumed that if an individual is NAT2 slow, arylamines may be rapidly detoxified in the liver, so that the activated hydroxylated arylamine may never reach the bladder epithelium, where NAT1 could act upon it.

Occurrence or specific types of mutations in oncogenes or tumor suppressor genes may partially be determined by the NAT2 activity. In bladder cancer, 7 out of 25 acquired mutations in the p53 cancer suppressor gene were transversions, and 6 out of these 7 mutations were in individuals having two slow NAT2 alleles (70). Differences in metabolic activity play a role in the mutational pattern and hence the pathobiology of the disease. In contrast, no significant association between p53 gene mutations and NAT2 polymorphisms was observed in patients with non-small-cell lung cancer, although slow acetylators had an increased risk compared to fast acetylators (71). Information about the role of NAT enzymes as a risk factor in colorectal cancer is ambiguous (72). In the support of NAT as a risk factor, a Japanese study suggests that people classified as rapid acetylators

are more likely to have a point mutation in the K-ras than people classified as low or intermediate. This suggests that aromatic amines do play a role in the etiology of colon cancer.

Conclusion

Many cancers are caused by various forms of environmental and viral exposure suggests that such causes could be avoided through preventive measures. In addition, metabolism of carcinogens under genetic control as an important factor in modulating individual susceptibility to cancer is a plausible hypothesis. Information on susceptibility to cancer is valuable for identifying high-risk individuals, allowing for early diagnosis and reduction of risk exposure to carcinogens, some of them possibly acting as endocrine disruptors. Recent knowledge on the basic genetics of metabolic variation has provided new possibilities for the study of individual susceptibility to cancer induced by the environmental factors. With the advent of techniques based on the polymerase chain reaction (PCR), it is now possible to identify the genotype of an individual with a series of enzymatic polymorphisms involved in the metabolism of xenobiotics, some of which are potent carcinogens. New molecular biology techniques have allowed for much more direct correlations between a particular genotype and the incidence of cancer and other chemically-induced diseases. Given the number of polymorphisms, the variability in the expression of XMEs, and the complexity of chemical exposure, determination of a single polymorphic enzyme may not be sufficient, and it appears to be necessary to establish a risk profile for each individual or sub-group. The conflictive results observed in the literature show the still present difficulty to evaluate this complex phenomenon. Cohorts used for major studies and the number of genes responsible for determining risk are still insufficient and not clear. Some individuals are more sensitive towards xenobiotics than others, and this may in part be due to differences in metabolic capacity. A genetic high risk profile cannot be made at the present time, as the profile will depend not only on the compound under investigation but also on the adverse health effect. Studies combining various XME genotypes from phases I and II of metabolism may be provide more information than the analysis of individual genes, since if genetic susceptibility is partially mediated by polymorphic variation, the risk associated with only one locus is probably small, due to the multiplicative interaction model probably at play. One of the futture challenges in molecular epidemiology may be resides in the ability to evaluate different scenarios in which interactions among several genetic polymorphisms, and among gene/s and environmental carcinogens yield different susceptibility levels on cancer etiology.

References

1. Parkinson A (1996). Biotransformation of xenobiotics in: casarett and doull's toxicology. *The Basic Science of Poisons*, 6: 113-186.
2. Doll R and Peto R (1981). The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *Journal of the National Cancer Institute*, 66: 1191-1308.
3. Raunio H, Husgafvel-pursiainen K, Anttila S, Hietanen E, Hirvönen A and Pelkonen O (1995). Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility. *A review gene*, 159: 113-121.
4. Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinoda N and Watanabe J (1990). Identification of genetically high risk individuals to lung cancer by dna polymorphisms of the cytochrome gene. *Febs Letters*, 263: 131-133.
5. Ingelman-sundberg M (1998). Functional consequences of polymorphism of xenobiotic metabolising enzymes. *Toxicology Letters*, 102-103:155-160.
6. Perera FP (1997). Environment and cancer: who are susceptible? *Science*, 278: 1068-1073.
7. Nebert DW (1991). Role of genetics and drug metabolism in human cancer risk. *Mutation Research*, 247: 267-281.
8. Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi S (1993). The cyp1a1 gene and cancer susceptibility. *Critical Reviews in hematologic oncology*, 14: 77-87.
9. Autrup H (2000). Genetic polymorphisms in human xenobiotic metabolizing enzymes as susceptibility factors in toxic response. *Mutation research*, 464: 65-76.
10. Gough AC, Smith D, Howell, Wolf R, Bryant P and Spurr K (1993). Localization of the cyp2d gene locus to human chromosome 22q131 by polymerase chain reaction in situ hybridization and linkage. *Analysis Genomics*, 15: 430-432.
11. Crespi L, Penman B, Gelboin V, and Gonzalez J (1991). A tobacco smoke-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is activated by multiple human cytochrome p-450s including the polymorphic human cytochrome p-4502d6. *Carcinogenesis*, 12: 1197-1201.
12. Bertilsson L (1995). Geographical/interracial differences in polymorphic drug oxidation current state of knowledge of cytochromes p450 (cyp) 2d6 and 2c19. *Clinical Pharmacokinet*, 29: 192-209.
13. Idle R, Mahgoub A, Lancaster R and Smith L (1978). Hypotensive response to debrisoquine and hydroxylation phenotype. Life Sciences, 22: 979-984.
14. Nebert W and Gonzalez J (1987). P450 genes: structure evolution and regulation. *Annual Review of Biochemistry*, 56: 945-993.
15. Pavanello S and Clonfero E (2000). Biological indicators of genotoxic risk and metabolic polymorphisms. *Mutation research/reviews in mutation research*, 463: 285-308.
16. Sachse C, Brockmöller J, Bauer S and Roots I (1997). Cytochrome p450 2d6 variants in a caucasian population: allele frequencies and phenotypic consequences. *American Journal of Human Genetics*, 60: 284-295.
17. Chen H, Sandler P, Taylor A, Shore L, Liu E, Bloomfield D and Bell A (1996). Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (gstt1). *Gene Defect Lancet*, 347: 295-297.
18. Roots I, Drakoulis N, Ploch M, Heinmeyer G, Loddenkemper R, Minks T, Nitz M, Otte f and Koch M (1988). Debrisoquine hydroxylation phenotype acetylation phenotype and abo blood groups as genetic host factors of lung cancer risk. *Klinische wochenschrift*, 66: 87-97.
19. Worrall S, Corrigan M, High A, Starr D, Matthias C, Wolf R, Jones W, Hand P, Gilford J, Farrell W, Hoban P, Fryer A and strange R (1998). Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome p450 cyp2d6. *Pharmacogenetics*, 8: 433-439.
20. Anwar W, Abdel-rahman S, El-zein A, Mostafa M, and Au W (1996). Genetic polymorphism of gstm1 cyp2e1 and cyp2d6 in Egyptian bladder cancer patients. *Carcinogenesis*, 17: 1923-1929.
21. Ouerhani S, Marrakchi R, Bouhaha R, Ben Slama M, Sfaxi M, Ayed M, Chebil M and Elgaaied A (2008). The role of cyp2d6*4 variant in bladder cancer susceptibility in tunisian patients. *Bull Cancer*, 95: 1-4.
22. Khedhaier A, Hassen E, Bouaouina N, Gabbouj S, Ahmed S, Chouchane L (2008). Implication of Xenobiotic Metabolizing Enzyme gene (CYP2E1, CYP2C19, CYP2D6, mEH and NAT2) polymorphisms in breast carcinoma. *BMC Cancer*, 8: 109.
23. Nebert W (1997). Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? *American Journal of Human*

Genetics, 60: 265-271.

24. Kato S, Bowman ED, Harrington AM, Blomeke B and Shields PG (1995). Human lung carcinogen-DNA adduct levels mediated by genetic polymorphisms in vivo. *Journal of the National Cancer Institute*, 87: 902-907.

25. Lu D, Yu X and Du Y (2010). Meta-analyses of the effect of cytochrome P450 2E1 gene polymorphism on the risk of head and neck cancer. *Mol Biol Rep* (In press).

26. Ruiter R, Bijl MJ, Van Schaik RH, Berns EM, Hofman A, Coebergh JW, Van Noord C, Visser LE and Stricker BH (2010). CYP2C19*2 polymorphism is associated with increased survival in breast cancer patients using tamoxifen. *Pharmacogenomics*, 11: 1367-1375.

27. Isomura Y, Yamaji Y, Ohta M, Seto M, Asaoka Y, Tanaka Y, Sasaki T, Nakai Y, Sasahira, N, Isayama H, Tada M, Yoshida H, Kawabe T (2010). A genetic polymorphism of CYP2C19 is associated with susceptibility to biliary tract cancer. *J Gastroenterol*, 45: 1045-1052.

28. Agundez JA (2004). Cytochrome P450 gene polymorphism and cancer. *Curr Drug Metab*, 5: 211-24.

29. Lemos, MC, Carrilho F, Rodrigues F., Coutinho E, Gomes L, Carvalheiro M, Regateiro FJ (2007). Genetic polymorphism of CYP2D6 influences susceptibility to papillary thyroid cancer. *Clin Endocrinol*, 67: 180-3.

30. Ruas JL, Lechner MC (1997). Allele frequency of CYP2C19 in a Portuguese population. *Pharmacogenetics*, 7: 333-5.

31. Hamdy SI, Hiratsuka M, Narahara K, El-Enany M., Moursi N, Ahmed MS (2002). Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9 CYP2C19 CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol*, 53: 596-603.

32. Pickett B and Lu H (1989). Glutathione s-transferases: gene structure regulation and biological function. *Annual Review of Biochemistry*, 58: 743-764.

33. Suzuki T, Coggan M, Shaw D and Board G (1987). Electrophoretic and immunological analysis of human glutathione s-transferase isozymes. *Annals of Human Genetics*, 51: 95-106.

34. Eaton L and Bammler K (1999). Concise review of the glutathione s-transferase and their significance to toxicology. *Toxicology Science*, 49: 156-164.

35. Landi, S (2000). Mammalian class theta gst and differential susceptibility to carcinogens: a review. *Mutation Research*, 463: 247-283.

36. Pearson W, Vorachek W, Xu S, Berger R, Hart I, Vannais D and Patterson D (1993). Identification of class-mu glutathione transferase genes gstm1-gstm5 on human chromosome 1p13. *American Journal of Human Genetics*, 53: 220-223.

37. Fryer A, Zhao L, Alldersea J, Pearson R and Strange R (1993). Use of site-directed mutagenesis of allele-specific pcr primers to identify the gstm1 a gstm1 b gstm1 ab and gstm1 null polymorphisms at the glutathione s-transferase gstm1 locus. *Biochemistry Journal*, 295: 313-315.

38. D'Errico A, Taioli X, Chen X, and Vineis P (1996). Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers*, 1: 149–173.

39. Jourenkova-mironova N, Wikman H, Bouchardy C, Yoho A, Dayer P (1998). Role of glutathione s-transferase gstm1 gstm3 gstp1 and gstt1 genotypes in modulating susceptibility to smoking-related lung cancer. *Pharmacogenetics*, 8: 495-502.

40. Webb G, Vaska V, Coggan M and Board P (1996). Chromosomal localization of the gene for the human theta class glutathione transferase (gstt1). *Genomics*, 33: 121-123.

41. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H., Bathum L, Benhamou S, Boffetta P (2001). Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev*, 10: 1239-48.

42. Abdel-Rahman SZ, Anwar WA, Abdel-Ala WE, Mostafa HM and Au WW (1998). GSTM1 and GSTT1 genes are potential risk modifiers for bladder cancer. *Cancer Detect Prev*, 22: 129-38.

43. Hengstler JG, Kett A, Arand M, Oesch-Bartlomowicz B, Oesch F, Pilch H, Tanner B (1998). Glutathione S-transferase T1 and M1 gene defects in ovarian carcinoma. *Cancer Lett*, 130: 43-8.

44. Seidegard J, Vorachek R, Pero W and Pearson W (1988). Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. *Proceedings of the National Academy of Sciences of the USA*, 85: 7293-7297.

45. Scarpato R, Hirvönen A, Migliore L, Flack G and Norppa H (1997). Influence of gstm1 and gstt1 polymorphisms on the frequency of chromosome aberrations in smokers and pesticide-exposed greenhouse workers. *Mutation Research*, 389: 227-235.

46. Ryberg D, Skaug V, Hewer A, Phillips H, Harries W, Wolf R (1997). Genotypes of glutathione transferase m1 and p1 and significance for lung DNA adduct levels and cancer risk. *Carcinogenesis*, 18: 1285-1289.

47. To-figueras J, Gene M, Gomez-catalan J, Galan C, Fuentes M, Ramon M, Rrodamilans M, Huguet E and Corbella J (1997). Glutathione s-transferase m1 (gstm1) and t1 (gstt1) polymorphisms and lung cancer risk among northwestern mediterraneans. *Carcinogenesis*, 18: 1529-1533.

48. Nyberg SM, Hou K, Hemminki B, and Pershagen G (1998). Glutathione S-transferase M1 and N-acetyltransferase 2 genetic polymorphisms and exposure to tobacco smoke in nonsmoking and smoking lung cancer patients and population controls. *Cancer Epidemiol Biomarkers Prev*, 7: 875–883.

49. Brockmöller J, Cascorbi I, Kerb R, & Roots I (1996). Combined analysis of inherited polymorphism in arylamine n-acetyltransferase 2 glutathione s-transferases m1 and t1 microsomal epoxide hydrolase and cytochrome p450 enzymes as modulators of bladder cancer risk. *Cancer research*, 56: 3915-3925.

50. Brockmoller J, Kaiser R, Kerb I, Cascorbi V, Jaeger V, and Roots I (1996). Polymorphic enzymes of xenobiotic metabolism as modulators of acquired p53 mutations in bladder cancer. *Pharmacogenetics*, 6: 535–545.

51. Jahnke V, Strange R, Matthias C and Fryer A (1996). Glutathione s-transferase and cytochrome-p-450 polymorphism as risk factors for squamous cell carcinoma of the larynx. *American journal of surgery*, 172: 671-673.

52. Foulkes W, Brunet S, Kowalski P, Narod and Franco L (1995). Family history of cancer is a risk factor for squamous cell carcinoma of the head and neck in Brazil: a case-control study. *International Journal of Cancer*, 63: 769-773.

53. Jourenkova N, Reinikainen M, Bouchardy C, Dayer P, Benhamou S and Hirvönen A (1998). Larynx cancer risk in relation to glutathione s-transferase m1 and t1 genotypes and tobacco smoking. *Cancer Epidemiology Biomarkers and Prevention*, 7: 19-23.

54. Park J, Muscat E, Ren Q, Schantz S, Harwick D, Stern C, Pike V, Richie P and Lazarus P (1997). Cyp1a1 and gstm1 polymorphisms and oral cancer risk. *Cancer Epidemiology Biomarkers and prevention*,6: 791-797.

55. Matthias C, Bockmühl U, Jahnke V, Petersen I, Dietel M, Fryer A, Strange RC.(1998).Effect of gene polymorphism on detoxifying glutathione-S-transferase enzymes on chromosomal stability of squamous epithelial carcinomas in the area of the head-neck. *Laryngorhinootologie*, 77:201-6.

56. Charrier J, Maugard C, Mevel B and Bignon J (1999). Allelotype influence at glutathione s-transferase m1 locus on breast cancer susceptibility. *British Journal of cancer* ,79: 346-353.
57. Curran J, Weinstein R and Griffiths R (2000). Polymorphism of glutathione s-transferase (gstm1 gstp1 and gstt1) and breast cancer susceptibility. *Cancer Letters*, 153: 113-120.
58. Lan Q, He X, Costa J, Tian L, Rothman H, Mumford J (2000). Indoor coal combustion emissions gstm1 and gstt1 genotypes and lung cancer risk: a case-control study in xuan wei china. *Cancer Epidemiology Biomarkers and Prevention*, 9: 605-608.
59. Deakin M, Elder J, Hendrickse C, Peckham D, Baldwin D, Pantin C, Fryer A and Strange R (1996). Glutathione s-transferase gstt1 genotypes and susceptibility to cancer: studies of interactions with gstm1 in lung oral gastric and colorectal cancers. *Carcinogenesis*, 17: 881-884.
60. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma M, Benhamou S, Vainio H, Uusitupa M (2001). Glutathione s-transferase m1 m3 p1 and t1 genetic polymorphisms and susceptibility to breast cancer. *Cancer Epidemiology Biomarkers and Prevention*, 10: 229-236.
61. Van lieshout E, Roelofs M, Dekker S, Mulder J, Wobbes T, Jansen B and Peters H (1999). Polymorphic expression of the glutathione s-transferase p1 gene and its susceptibility to barrett's esophagus and esophageal carcinoma. *Cancer Res*, 59: 586-589.
62. Nair U, Matthew B and Bartsch H (1999). Glutathione s-transferase m1 and t1 null genotypes as risk factors for oral leukoplakia in ethnic indian betel quid/tobacco chewers. Carcinogenesis, 20: 743-748.
63. Chen C, Liu G, Pui C, Rivera K, Sandlund T, Ribeiro R (1997). Higher frequency of glutathione s-transferase deletions in black children with acute lymphoblastic. *Leukemia blood*, 89: 1701-1707.
64. Kamel R, Slah O, Raja M, Mohamed R, Mohamed S, Mohsen A, Mohamed C, Amel G (2009). Combined effect of smoking and inherited polymorphisms in arylamine N-acetyltransferase 2 glutathione S-transferases M1 and T1 on bladder cancer in a Tunisian population. *Cancer Genetics and Cytogenetics*, 190: 101-107.
65. Rothman N, Bhatnagar VK, Hayes R, Zenser TV (1996). The impact of interindividual variation in NAT2 activity on benzidine urinary metabolites and urothelial DNA adducts in exposed workers. *Proc Natl Acad Sci USA*, 93: 5084–5089.
66. Badawi AF, Hirvonen A, Bell DA, Lang NP, and Kadlubar FF (1995). Role of aromatic amine acetyltransferases NAT1 and NAT2 in carcinogen–DNA adduct formation in the human urinary bladder. *Cancer Res*, 55: 5230–5237.
67. Badawi AF, Stern SJ, Lang NP, and Kadlubar FF (1996). Cytochrome P-450 and acetyltransferase expression as biomarkers of carcinogen–DNA adduct levels and human cancer susceptibility. *Prog Clin Biol Res*, 395: 109–140.
68. Pfau EM, Stone U, Brockstedt PL, Carmichael H, Phillips DH (1998). DNA adducts in human breast tissue: association with N-acetyltransferase-2 (NAT2) and NAT1 genotypes. *Cancer Epidemiol Biomarkers Prev*, 7: 1019–1025.
69. Taylor JA, Umbach DM, Stephens E, Castranio T (1998). The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res*, 58: 3603–3610.
70. Brockmöller J, Kerb R, Drakoulis N, Staffeldt B (1994). Glutathione s-transferase m1 and its variants a and b as host factors of bladder cancer susceptibility: a case-control study. *Cancer research*, 54: 4103-4111.
71. Oyama T, Kawamoto T, Mizoue T, Yasumoto K, Kodama Y and Mitsudomi T (1997). N-acetylation polymorphism in patients with lung cancer and its association with p53 gene mutation. *Anticancer Res*, 17: 577–581.
72. Oda Y, Tanaka M and Nakanishi I (1994). Relation between the occurrence of K-ras gene point mutations and genotypes of polymorphic N-acetyltransferase in human colorectal carcinoma. *Carcinogenesis*, 15:1365–1369.