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Genetic Polymorphisms in Xenobiotic Metabolism

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INTRODUCTION

THE MULTI-STEP or multi-hit theory of carcinogenesis states that tumour formation is due to the consequences of multiple mutagenic events. An obvious conclusion to be drawn from this is that predisposition to cancer, due to inherited genetic factors, must have an enormous impact on the development of disease. Indeed, intense study of familial cancer, childhood neoplasms, onco-viruses and animal models have now identified a large number of candidate genes that directly influence the aetiology and pathogenesis of many cancers. Although there is no doubt that these cancer genes have an important role in tumorigenesis, the vast majority of cancers do not appear to have any strong familial link. It is now widely accepted that the initiation and development of tumours are determined by a delicate balance of environmental and host factors.

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ENVIRONMENTAL INFLUENCES IN CANCER

Human cancers can be induced by a plethora of environmental agents. Chemicals, viruses and virtually all forms of radiation have been identified as aetiological agents of specific types of cancer, and epidemiological evidence suggests that exposure profiles and inherited susceptibility to the action of carcinogenic and mutagenic stimuli are both important in the development of tumours [1, 2]. How cells respond to these environmental challenges must be a predominant factor in certain types of cancer, and cells and tissues are able to recruit a number of defence mechanisms to ward off the threat of cancerous lesions [3, 4]. Inter-individual variation in the genes involved must therefore represent genetic susceptibility factors for disease [5]. For instance, it is well documented that mutations in DNA repair enzymes can lead to damaged cells, with point mutations in specific genes, rearrangements and translocations, gene amplifications or deletions, and gross chromosomal aberrations. The activation of cellular oncogenes or the abrogation of tumour suppressor gene function can endow mutated cells with unlimited growth potential, often as a consequence of resistance to endogenous cytotoxic attack and programmed cell death (apoptosis). Failure of the immune system, brought on by the action of drugs or disease, can be a determinant of tumour progression and metastatic potential. Due to the importance of these defence systems in cellular homeostasis and immune surveillance, their association with the cancer process is usually ultimately lethal and, therefore, cannot represent *general* inheritable susceptibility factors. Of course, mutations in many vital genes have been intimately associated with a variety of familial and childhood cancers, but with few exceptions, they tend to act in a recessive manner. This is often shown to be the case by the need for further somatic mutations to occur, or by loss of heterozygosity in the tumour cells. The extant gene function is then modified or absent due to direct mutagenesis or deletion.

CARCINOGEN METABOLISM

It has been estimated that over 80% of human tumours may be due to the action of environmental carcinogens [4]. Animal models of carcinogenesis have shown that even single doses of putative chemical carcinogens are sufficient to cause tumours but, as cancer formation is a polygenic process, the development of these tumours must involve multiple gene mutations and susceptibility factors. It is important here then to first define the parameters of any discussion of an individual's risk of carcinogen-induced disease.

Models of carcinogenesis in animals show that chemical carcinogens are able to induce all types of neoplasia, but this is often a result of the very large quantity of chemicals administered in such studies. In relative terms, it is unlikely that humans would become exposed to such high levels of carcinogen on a routine basis, except through catastrophic industrial/occupational exposure or as a consequence of exposure to toxins such as aflatoxin B1 in specific geographical locations. Under these circumstances, there is little doubt that overt toxicity is the main clinical manifestation of chemical ingestion, as the bodily defence mechanisms would be overwhelmed. However, mild chronic exposure to environmental carcinogens is related to cancer incidence in man, and epidemiological and ecogenetic studies have uncovered major differences in genetic, racial, ethnic, geographical and dietary risk factors for tumour development.

The metabolism of environmental carcinogens that have been absorbed by the body must play a central role in protection against their deleterious effects, and mammals have evolved a host of metabolic enzymes to protect themselves against such compounds. Primarily, these enzymes arose and evolved to combat toxins, carcinogens and mutagens present in the diet [4, 6], but have gained prominence because of inter-individual variations in the metabolism of therapeutic drugs and the effects of environmental pollutants on the body. A wide range of different chemicals have been associated with cancer initiation and progression (Table 1), and a relatively large number of genotoxins, both naturally occurring and synthetic, may be ingested daily in the diet. In addition, the intake of carcinogens can be greatly increased by the choices we make in our lifestyle (e.g. smoking, occupation), which therefore has a profound effect on our ability to inhibit the cancer process.

POLYMORPHISMS IN DRUG METABOLISING ENZYMES

The majority of chemicals associated with cancer are small, lipophilic molecules and, for the body to excrete them efficiently, they need to undergo conversion to more electrophilic derivatives. The disposition of foreign compounds is principally governed by metabolism in the liver, but the level of exposure, site of exposure and the rate of metabolism of carcinogens also have a direct bearing on their tumorigenic potential. Inter-individual variations in the expression of detoxifying enzymes in the liver or other target organs and tissues may, therefore, be a key factor in resistance to chemical insult.

The polymorphic metabolism of drugs led to the principle

Table 1. *Compounds associated with carcinogenesis*

Dietary	Occupational/industrial	Endogeneous	Lifestyle/Drugs
Aflatoxins	Benzo [a] pyrenes and metabolites	Hormones	Cigarette smoke components
Ochratoxins	Benzenes	Growth factors	Alcohol
Amino acid pyrolysates	Phenols	Cholesterol	Food additives
Caffeine	Aromatic amines	Fatty acids	Paracetamol
Phenacitin	Nitrosamines	Leukotrienes	Cyclophosphamide
Benzidines	Halogenated hydrocarbons	Prostaglandins	
Plant toxins	Epoxides		
Gut fauna toxins	Polychlorinated biphenyls		
	Polycyclic aromatic hydrocarbons		
	Anilines		
	Naphthylamines		
	Amino biphenyls		
	Solvents		

that "errors in metabolism" were due to genetically determined enzyme deficiences [7]. Over the past 20–30 years, more and more enzymes have been identified that are responsible for drug and xenobiotic metabolism, and a number of the phenotypic variations in drug responses observed in populations have now been shown to be due to polymorphisms in the expression of these proteins. The clinical relevance of these discoveries to pharmacotherapy, the side-effects of drugs, drug–drug interactions and risk of disease, led to the isolation and characterisation of literally hundreds of these genes, some of which have the sole purpose of metabolic conversion of xenobiotics. The genetic basis of many of these polymorphisms is now understood at the DNA sequence level, and genetic studies in populations have revealed the presence of multiple allelic forms in the gene pool. True genetic polymorphism is defined as the existence of more than one form of a particular gene in any given population. Usually the less common forms have an allele frequency of at least 1%, so the changes cannot be accounted for by somatic mutations alone. Inheritable, functional (informative) polymorphisms of genes are associated with an altered phenotype because the gene products may be absent, expressed at a different level or expressed with an altered specific activity or substrate affinity.

The drug- or xenobiotic-metabolising enzymes (XMEs) are classified according to the type of anabolic or catabolic reactions they perform (Table 2). In addition, they can be divided on the basis of their metabolism, with phase I enzyme reactions resulting in the creation of functional groups and "reactive-centres" on substrates (e.g. -OH,-NH_2,-SH,-COOH), and phase II conjugation enzymes acting upon these substrates. Ultimately, the action of phase II metabolic pathways, involving for example, glucuronidation, acetylation or glutathione conjugation, are considered the true detoxification pathway, and are responsible for the bulk of inactivated exogenous and endogenous compounds discharged from the body. As illustrated in Table 2, many XMEs have important endogenous roles in intermediary metabolism, but, in addition, they are active in the detoxification or metabolic activation of a wide variety of structurally diverse xenobiotic compounds. The precise role of these enzymes in cancer susceptibility is, therefore, difficult to establish. Many carcinogens or procarcinogens contain several distinct functional groups and are, therefore, potential substrates for more than one class of XME. The ultimate metabolic fate and, by extrapolation, carcinogenicity, of many of these chemicals is a direct consequence of the relative levels of expression of the XMEs active in their metabolism. There are several reasons why this is so. Firstly, as a consequence of the need for primary oxidative/reductive reactions, the initial rate of metabolism of a carcinogen determines its active exposure level. This is still the case when the metabolism paradoxically transforms a procarcinogen to its ultimately reactive form. Secondly, the influence of the phase II process is also rate-limiting with respect to carcinogen removal, in as much as it determines excretion pathways. Finally, the active/inactive carcinogen may be metabolised by several XMEs at different rates and degrees of specificity. This produces a number of distinct metabolites from a single molecule with varying mutagenic potential, and any of these derivatives may then induce disease. Clearly, the toxicological outcome of exposure, absorption and activation/deactivation of xenobiotics is delicately balanced (Figure 1), and so genetic variations (polymorphisms) in XMEs represent a major risk factor in the tendency to develop tumours. On the basis of our current knowledge, the most important enzymes to consider are the cytochromes P450, the glutathione-S-transferases and the *N*-acetyl-transferases.

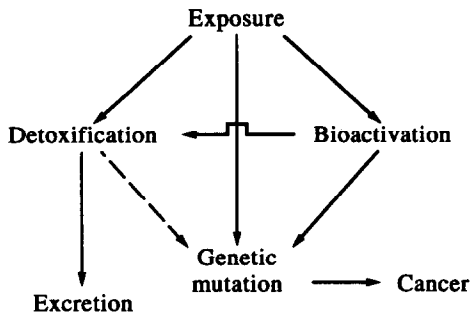

Figure 1. Carcinogen metabolism.

Table 2. Reactions of xenobiotic metabolism

Phase I metabolism		Phase II metabolism	
Oxidation	– ***Cytochromes P450***	Glucuronidation	– ***UDP glucuronyl transferases***
	– ***Alcohol and aldehyde dehydrogenases***	Glucosidation	– UDP glucosyl transferases
		Sulphation	– ***Sulphotransferases***
	– Aromatases	Methylation	– ***O-,N-,S- methyl transferases***
	– ***Oxidases***	Conjugation	– ***Glutathione S-transferases***
Reduction	– P450 reductase		– N-acyl transferases
	– N-reductases		– ***N-acetyl transferases***
Hydrolysis	– ***Epoxide hydrolases***	Esterification	– Fatty acid transferases
	– Esterases	Condensation	– Non-enzymic
	– Amidases		
Isomerisation	– Isomerases		
Miscellaneous			
Peroxidation	– Glutathione peroxidase		
Radical scavengers	– ***Superoxide dismutases***		

For a detailed description of xenobiotic and drug metabolism see [154]. Enzymes in bold italics indicate that a member of that family is subject to known genetic polymorphism.

THE CYTOCHROMES P450

Human cytochromes P450 are a gene superfamily of mixed-function mono-oxygenases. All isoforms are haem-containing monomeric enzymes located in the endoplasmic reticulum or mitochondrial membrane of cells. With the NADPH-P450 reductase as cofactor, they function as the terminal oxidase in an electron transport chain. Evolution and divergence has resulted in the emergence of over 200 known genes in mammals, and all organisms analysed possess P450s or P450-like activity [8]. Current estimates predict that the human genome contains at least 50 different P450 genes [9]. Mammalian P450s are divided into 10 families of proteins (Table 3), based on similarities in nucleotide base sequence and amino acid composition, and many of these gene families are further divided into subfamilies of genes. Overall, human P450s share approximately 40% amino acid and DNA sequence homology [10], mostly due to highly conserved regions, including the N-terminal membrane anchorage region, the cytochrome P450 reductase binding site and the C-terminal haem-binding domain. At the DNA sequence level, several members of the same gene family share almost identical intron/exon structure, and members of the same subfamily invariably cosegregate to a single chromosomal region. In some cases, even different subfamilies within a gene family are clustered [11].

Detailed study of human cytochromes P450 at the microsomal level, as well as experiments with purified protein and isolated cDNAs, have shown that the major tissue of cytochrome P450 activity is the liver, but all tissues express these enzymes in a tissue-specific manner. Of the enzymes identified so far in humans, the CYP1, CYP2 and CYP3 families of genes are all located in the endoplasmic reticulum, and their major role is the metabolism of xenobiotics. The remaining P450 families all have essential roles in intermediary metabolism. The CYP4 family of enzymes are involved in the oxidation of fatty acids and prostaglandins, and all other classes are involved in steroid metabolism. The CYP7 gene product, cholesterol-7-hydroxylase, is the first enzyme in bile acid production from cholesterol [12]. The other microsomal P450s and the two mitochondrially expressed enzyme families, CYP11, the side chain cleavage enzyme, and CYP27—cholesterol 27 hydroxylase, are crucial to steroid hormone biosynthesis and bile acid formation, respectively.

Table 3. Cytochrome P450 families

Gene family	Sub-families*	Substrates†	Regulated by†
CYP1	2	Xenobiotics	Substrates
CYP2	6	Xenobiotics	Substrates, hormones, cytokines
CYP3	1	Xenobiotics	Substrates, hormones
CYP4	2	Fatty acids, prostaglandins Leukotrienes	Peroxisome proliferators, hormones, fatty acids
CYP7	—	Cholesterol	Cholic acid
CYP11	2	Cholesterol/sterols	Hormones
CYP17	—	Sterols	Hormones
CYP19	—	Sterols	Hormones
CYP21	—	Sterols	Hormones
CYP27	—	Cholesterol/bile acids	Hormones

* P450 nomenclature used as in [9]. *CYP* represents cytochrome P450.
† Only general substrates and regulatory examples are given.

A number of the cytochromes P450 involved in xenobiotic metabolism are now known to be polymorphically expressed in humans, and, by extrapolation from animal studies, these genetic variations are thought to be associated with the adverse affects of exposure to environmental chemicals (Table 3).

CYP1 FAMILY

The *CYP1* locus encodes two distinct but highly homologous proteins, CYP1A1 and CYP1A2, which are distinguished by their extra-hepatic and predominantly liver-specific expression, respectively. Both enzymes have been implicated in the activation of procarcinogens by catalysing a variety of reactions e.g. N-hydroxylation and aromatic epoxidation; CYP1A1 is involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs), and CYP1A2 in nitrosamine and arylamine metabolism. The induction of both enzymes is mediated by a cytosolic protein called the arylhydrocarbon or Ah (dioxin) receptor [13], which is activated by PAH and some nitrosamines and arylamines.

Considerable inter-individual variations in *CYP1A2*-mediated caffeine metabolism are known to exist [14], but a genetic polymorphism has not yet been identified in the *CYP1A2* gene. However, as CYP1A2 substrates are amongst the most potent procarcinogens known, this gene merits further study in relation to colon, bladder and liver cancers and, indeed, many other cancer types [15]. In particular, *CYP1A2*-mediated metabolism has been implicated in the activation of dietary heterocyclic amines [16] and aflatoxin B_1 [17].

CYP1A1 enzyme is predominantly expressed at a low level in human lung and leucocytes, and is known to be induced in the bronchial airways of over 80% of lung cancer patients who smoke [18]. Consequently, CYP1A1 has been closely associated with an increased risk of tobacco smoke-induced peripheral pulmonary carcinoma [19]. Tobacco smoke is known to contain several classes of powerful carcinogens, such as PAHs, aromatic amines, and nitrosamines. The metabolism of many of these compounds is mediated by CYP1A1, producing the reactive intermediates involved in DNA-adduct formation [13, 20]. Approximately 10% of the human population exhibits a CYP1A1 high-inducibility phenotype (in response to inducers, such as benzo(a)pyrene and 3- methylcholanthrene), and may be at an increased risk of lung cancer due to increased activation of these procarcinogens. A further polymorphism associated directly with the *CYP1A1* gene locus has been linked to an MspI RFLP, produced by a C→T mutation in the 3′ non-coding region of the *CYP1A1* gene, 250 bp downstream of the polyadenylation signal [21, 22]. In a Japanese population of lung cancer sufferers, 21.2% were found to be homozygous for this RFLP, compared to only 10.6% of healthy controls [21]. Further study showed that the MspI polymorphism cosegregates with an A → G transition at nucleotide position 4889 in the *CYP1A1* gene. This point mutation maps to the haem-binding region of the protein and produces an amino acid change of isoleucine to valine [23]. The valine substituted CYP1A1 has a higher enzyme activity than the parent gene, and individuals who are homozygous for the MspI/Val mutations were reported to have an approximate 7-fold increased risk of developing Kryberg type I lung cancer, even at low levels of cigarette smoking [23, 24]. However, a similar study in Caucasians did not reproduce these findings [25]. This is probably due to the lower prevalence of the higher activity *CYP1A1* genotype in Europeans, which is estimated to be 1% or less. Use of polymerase chain reaction (PCR)-based assays, developed to detect the Ile → Val substitution in genomic DNA samples in case-controlled epidemiological studies

of different races and lung cancer types, should shed further light on the involvement of CYP1A1 inducibility in cancer susceptibility.

CYP2 FAMILY

The CYP2 family of XMEs is by far the largest P450 family, and consists of at least six subfamilies of proteins in humans. Considerable heterogeneity in the expression of the CYP2 enzymes exists in individuals. Several *CYP2* genes have similar patterns of regulation and appear to exhibit some level of coordinate regulation. Many genes e.g. *CYP2E1* and *CYP2A6* also have overlapping substrate specificities [26]. In the case of the CYP2A and CYP2B subfamilies, similarities in their regulation may be a result of the proximity of the *CYP2A* and *CYP2B* genes on chromosome 19 (Table 4), and the fact that their divergence was relatively recent. Although *CYP2F1* is also found at the same chromosomal location, it is not expressed in the liver, unlike *CYP2A6*, *CYP2A7* and *CYP2B6*. Expression of *CYP2F1* is only detectable in the lungs of smokers [27], and the gene has not yet been characterised to any great extent.

CYP2A6 and *CYP2A7* are expressed in human liver, and *in vitro* expression studies have shown that the CYP2A6 enzyme (coumarin 7-hydroxylase) activates the tobacco nitrosamine carcinogen, NNK [28]. A mutant form of *CYP2A6* has been identified, termed the CYP2A6v-inactivating allele, which may, at least in part, explain the skewed excretion profile of 7-hydroxycoumarin seen in Caucasian populations [29, 30]. Recent data from this laboratory have shown that a genetic polymorphism exists in the *CYP2A7* gene, as a result of an alternative splicing mechanism (Ding & Wolf, unpublished observations). To date, however, substrates for CYP2A7 have not been identified. Further study of these putative genetic polymorphisms and the tissue distribution of CYP2A6 and CYP2A7 expression may further define the relationship between nitrosamine metabolism and cancer susceptibility in a variety of epithelia.

CYP2B SUBFAMILY

Two human *CYP2B* genes have been characterised to date, *CYP2B6*, and an inactive pseudogene *CYP2B7P*. No discernible genetic polymorphism exists in the *CYP2B6* gene. However, two aberrant mRNAs have been shown to be expressed in human liver which are produced from alternative splice sites within the gene [31–33]. CYP2B6 expression is hypervariable in different individuals, and is also subject to a very potent induction by phenobarbital in the liver. As mentioned above, CYP2B6 and CYP2A6 appear to be similarly regulated and livers that contain abundant CYP2B6 also express large quantities of CYP2A6. Whether any of these inter-individual variations in expression are associated with particular forms of hepatic or other cancers, such as leukaemia, is as yet unclear. CYP2B6 has, however, been implicated in the activation of aflatoxin B_1 and cyclophosphamide.

CYP2C SUBFAMILY

The *CYP2C* subfamily of genes is the most complex of all of the human P450s so far studied. At least five different but highly homologous gene sequences have been isolated *CYP2C8*, *CYP2C9*, *CYP2C18*, *CYP2C19* and *CYP2C21*. All of these genes are found clustered on chromosome 10. The *CYP2C* genes are constitutively expressed in human liver and are generally less responsive to classical xenobiotic enzyme-inducers [26]. There is scant information on any relationship between CYP2C-mediated metabolic reactions and cancer susceptibility, but there is a marked inter-phenotypic variation in CYP2C drug metabolising activity, which is best characterised by the

Table 4.

CYP gene subfamily	No. of genes*	Chromosome location [Ref.]	Principle tissue†	Suspect carcinogens‡	RFLPs§
1A	2	15q 22 -q 24 [155]	L, Lu, (E)	Benzo(a)pyrene Arylamines Nitrosamines	*BglII*, *EcoRI*, *MspI*, *N*
2A	2+	19q 13.1 -q 13.2 [156]	L	Aflatoxin B_1 Nitrosamines	*SstI*
2B	2+	19q 13.1 -q 13.2 [156]	L, Lu, G	Aflatoxin B_1 Cyclophosphamides	*BamHI*, *MspI*
2C	5+	10q 24.1 -q 24.3 [157]	L, G	Benzo(a)pyrenes	*XmaI*, *SmaI*
2D	3/4	22q 13.1 [63]	L, B, G?	NNK	*XbaI*, *BstNI*, *HpaII*, *N*
2E	1	10 [158]	L, B, Le	Nitrosamines Ethanol Benzene	*XmnI*, *PstI*, *RsaI*, *DraI*
2F	1	19q 13.1 -q 13.2 9 [11]	Lu	?	None known
3A	4	7q 21.3 -q 22.1 [159]	L, G	Benzo(a)pyrenes Aflatoxin B_1 Cyclosporin	*StuI*
4A	2	1	L, Br	(Fatty acids)	None known
4B	1	1p 12 -q 34 [27]	Lu	Aflatoxin B_1 Aromatic amines	None known

* Some subfamilies are thought to be more complex than originally thought and more genes may exist in humans (+). † Only tissues with significant levels of detectable expression are given (for review see [160]). L, liver; Lu, lung; G, gastrointestinal tract; B, brain; Br, breast; Le, leucocytes; E, extrahepatic tissues. ‡Only main types of suspect carcinogens are given as examples. Compounds in parenthesis are implied carcinogens or tumour promoters. NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. § RFLP, restriction fragment length polymorphism in DNA. Those restriction enzymes that are underlined are informative for an aberrant gene. See [160]. *N* = No restriction site associated with a known, informative DNA sequence polymorphism.

polymorphism in S-mephenytoin 4-hydroxylation (Figure 2) [34]. Recently, *CYP2C19* was identified as the gene responsible for this polymorphism, and was found to be expressed in over 90% of human liver samples, with the level of expression of the enzyme in microsomes correlating with the relative level of 4-hydroxylation activity [35]. Epidemiological studies have shown that individuals can be characterised as either extensive (EM) or poor (PM) metabolisers of mephenytoin. The PM phenotype is recessive and autosomally inherited, and is due to a complete absence of enzyme. Both the homozygous normal and heterozygote genotypes are classified as extensive metabolisers. The PM phenotype occurs in approximately 5% of Caucasians, but occurs at much higher frequencies (18–23%) in Oriental populations [34, 36]. The phenotype arises due to a 40 bp deletion at the beginning of Exon 5 of the *CYP2C19* mRNA (bp 643–682). This deletion leads to premature termination of protein synthesis and an inactive, truncated protein, lacking the haem-binding region [37]. The only difference in the gene structure of the PM allelic variation is a G→A transition mutation at position 681 in Exon 5, which produces a cryptic splice acceptor site for Exon 4. Now that the genetic basis for the polymorphism has been uncovered, use of a simple PCR-based genetic assay across Exon 4/Intron 4/Exon 5 should enable detailed investigation of whether this polymorphism is associated with specific types of disease.

CYP2D SUBFAMILY

The P450 *CYP2D* locus has merited particular attention as a genetic susceptibility factor for cancer, since the discovery of a functional polymorphism of debrisoquine 4-hydroxylase activity in man [38]. This enzyme deficiency is characterised, like the CYP2C polymorphism, by the inability of certain individuals, termed poor metabolisers (PMs), to metabolise a wide range of drugs such as debrisoquine, codeine and bufuralol (for review see [39, 40]). The *CYP2D* gene cluster usually contains three genes: *CYP2D6*, which encodes the active P450 debrisoquine 4-hydroxylase, an inactive pseudogene *CYP2D8p*, and an inactive gene *CYP2D7* [41, 42]. A number of mutations in the *CYP2D6* genes have been identified which explain the lack of enzyme in the liver of affected individuals (Table 5, Figure 2). Still further mutations have been discovered which may alter the activity of the CYP2D6 protein (Table 5), and recently ultra-rapid metabolisers have been identified who carry inherited amplification of an active form of the gene [43]. The metabolic deficit

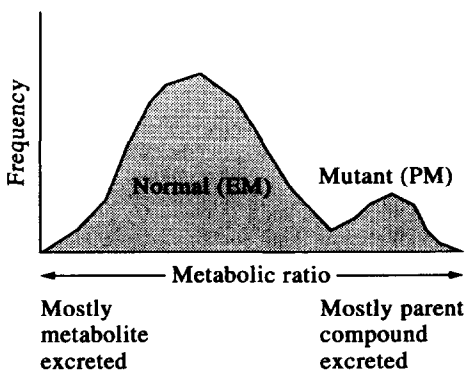

Figure 2. Metabolic polymorphism. Measurement of drug metabolic ratios in populations showed that inborn errors of metabolism were due to specific metabolic enzymes. The human *CYP2C19* and *CYP2D6* genes are polymorphic, and population studies show a bimodal distribution of phenotypes.

Table 5. CYP2D6 mutant alleles

CYP2D6 allele	Mutation	Functional outcome	Allele frequency in PMs	Ref.
A	A_{2637} deletion (Exon 5)	No enzyme	<5%	161
B	$G\text{-}A_{1933}$ (+ others)	No enzyme	>50%	58,161,162
C	Lys_{281} deletion (bp 2703–2705)	Reduced activity	—	163
D	Whole gene deletion	No enzyme	10%	164–166
E	44kb *XbaI* RFLP (Extra gene in locus)	No enzyme	30%*	57
J	C_{188} - T (Exon 1) G_{4268} - C (Exon 9)	Reduced activity	<1% (Japan)	167
L	Amplified 2D6	Ultra high metabolism	—	43

* The *CYP2D6E* allele occurs only with *CYP2D6B*-like mutations. A 44-kb *XbaI* DNA fragment contains an extra copy of an inactive *CYP2D7* gene (see [57]). From PCR analysis of *CYP2D6* mutations [57–59] it is detected as a *CYP2B6*-type mutation and is inactive because of the G to A transition at the junction of Intron 3 and Exon 4.

giving rise to poor metabolisers affects 5–10% Caucasians [44], approximately 1% of Orientals and Asians [45, 46] and 1% of black Africans [47], and has been variously associated with a number of types of cancer, where carcinogen metabolism may be involved. The majority of these studies have been carried out using pharmacokinetic assays using marker drugs to compare the debrisoquine hydroxylation phenotype of cancer patients and case-matched controls. Using this methodology, strong associations between the extensive metaboliser phenotype (normal) have been found with the incidence of lung cancer [30, 48–51], but this association has often proved variable [52–55]. The CYP2D6 polymorphism has also been linked to the incidence of bladder [55] and breast cancer [56]. With the availability of DNA based assays which give an unequivocal genotype [57–59], the above associations remain equivocal and the current indications are that a genetically based association does not exist [54, 60–62]. The reason for the dichotomy between phenotype and genotype studies is unclear, but may be due to the small numbers of individuals assessed in many pharmacokinetic studies, the vagaries of drug–drug interactions, and the effect of the disease state on *in vivo* hydroxylation rates. In genotyping studies, there is also a wide variation in the proportion of PMs in the control Caucasian population, from under 5% to over 16% [61]. Clearly these difficulties need to be resolved, particularly in the study of smoking-related diseases, such as lung and bladder cancer. The carcinogen, NNK, a component of cigarette smoke, has been reported to be a substrate for CYP2D6 [28]. Relative to other CY2D6 substrates, however, its affinity for the enzyme would appear to be very low. Such observations indicate that CYP2D6 metaboliser status may only be a factor at specific cigarette doses and may be related to the level of tobacco consumption. However, extreme care must be taken when breaking down statistical data in this manner and it also runs the risk of leaving population sizes which are too small for accurate statistical analysis to be performed.

Some interesting associations have also been observed between

the CYP2D6 polymorphism and other cancer types [54]. In a large study, the incidence of the PM genotype in leukaemia cohorts was found to be increased compared with a normal control population. This indicates that CYP2D6 is involved in the detoxification of certain leukaemia-related carcinogens or tumour promoters. Although many solvents such as benzene and anticancer drugs (e.g. cyclophosphamide) can induce leukaemia, these are not CYP2D6 substrates. Alternatively, the apparent association could be related to allele loss in the tumour cells, or that the *CYP2D* locus is in linkage disequilibrium with an oncogene on the same region of chromosome 22. Recent mapping of the locus [63] places *CYP2D* close to that of the platelet-derived growth factor B chain (*PDGFB*) gene, although the significance of these findings needs to be further investigated.

CYP2E SUBFAMILY

Cytochrome P450 CYP2E1 catalyses the activation and formation of DNA adduct by human carcinogens such as the N-nitrosamines, benzene and many other low molecular weight chemicals and solvents [64]. Such compounds are found in the environment, and are also significant components of cigarette smoke. CYP2E1 is characterised by its inducibility by alcohol (ethanol), and by its action on acetone and other ketones and alcohols. Certain studies have shown genetic and racial variations in the P450 *2E1* gene, identified by *PstI*, *DraI* and *RsaI* RFLPs in genomic DNA of lung cancer patients [65–67]. Two of the changes associated with these RFLPs involve the 5′ flanking (promoter) region of the gene [65] and are reported to affect the transcriptional activity of the gene (increased inducibility). The other RFLP is associated with a mutation in Intron 6 of the gene [68], but any positive relationship between CYP2E1 activity and lung cancer incidence requires further study.

CYP3 FAMILY

The human P450 3A subfamily of enzymes was originally characterised by its involvement in the hepatic metabolism of testosterone to its 6-hydroxy metabolite [69]. Subsequently, the enzyme has been shown to play a central role in the metabolism of drugs and carcinogens such as nifedipine, lidocaine, and the immunosuppressive drug, cyclosporine [70]. Four human *CYP3A* genes are clustered on chromosome 7q, namely *CYP3A3*, *CYP3A4*, *CYP3A5* and *CYP3A7*. All four genes exhibit a high degree of sequence homology, especially CYP3A3 and CYP3A4 proteins which differ only in 11 of 503 amino acid residues [71]. The catalytic activities of these two enzymes, as measured by nifedipine oxidase activity, cannot be differentiated [72]. Similarly, CYP3A5 activity is almost indistinguishable from the 3A3/3A4 catalytic spectrum, except for minor differences in affinity for certain substrates such as in cyclosporine hydroxylation [73]. The CYP3A7 gene product was originally identified in fetal liver, but has now also been identified in adult liver. This enzyme has been proposed to be involved in steroid metabolism [26].

All the members of the CYP3A family of enzymes catalyse the activation of aflatoxins and other carcinogens, and considerable variations in the respective hepatic expression of these P450s may contribute significantly to carcinogenesis. For instance, the CYP3A4 gene product has been implicated in protection against aflatoxin B_1-induced liver cancer in smokers in China [74]. The mechanism of this effect is complex, however, as CYP1A2 and CYP2A6 also play a role in aflatoxin metabolism. The major detoxification product of aflatoxin B1, aflatoxin Q1 is, however, predominantly produced by CYP3A4. It has been estimated that in certain individuals, up to 60% of the total hepatic P450 content is CYP3A4. In others, however, it contributes less than 10%. This large (up to 60-fold) variation in relative levels of expression of the CYP3A isoforms implies that in certain individuals metabolism by CYP3A proteins provides the major carcinogen activation pathway, whereas in others it is not so important. Recent studies on CYP3A mRNA, isolated from adult and embryonic liver samples, showed a 10-fold inter-individual variation in CYP3A4 expression in the adult liver samples [75]. In addition, 23% of the adult livers contained CYP3A5-specific mRNA and, interestingly, in over 50% of adult livers, CYP3A7 transcripts were detected. This hypervariation in the expression of different CYP3A proteins must be studied further in order to determine their relative role and importance in xenobiotic-induced disease. Current evidence, however, indicates that this variation has a hormonal or environmental rather than a genetic basis.

The preceding discussion of the cytochrome P450 superfamily of XMEs underlines the importance of the influence that metabolism has on the cancer process. The overlapping substrate spectra of the cytochromes P450, and the relative levels of expression and activity of the different enzymes are all important biological criteria in the rate of carcinogen activation and detoxification. For example, with the aflatoxin B_1 model, at least seven different P450 isoforms are known to metabolise this potent mutagen. All of the CYP3A family, CYP1A2, CYP2B6 and CYP4B1 are able to metabolise aflatoxin, producing metabolites of varying tumorigenic potency. Therefore, the relative content of each P450 isoform in any given tissue, but especially in the liver, predetermines an individual's response to environmental chemicals, and methods to assess relative risk for cancer incidence must recognise this. It is also particularly difficult to determine the genetic mechanism for phenotypic variance. Where clear differences in expression and response exist, as in the cases of the genetically determined CYP1A1, CYP2C19, CYP2D6 and CYP2E1 polymorphisms, convenient PCR-based assays can aid in the assessment of multiple risk factors for cancer susceptibility. For enzymes where variability is due to apparent epigenetic or inducer/repressor modulation events, however, non-invasive pharmacokinetic assessment is the only screening method we can use, in the absence of molecular evidence for inherited defects.

THE GLUTATHIONE S-TRANSFERASES

The glutathione S-transferases (GSTs) also play a central role in the detoxification of carcinogens (phase II metabolism), and consist of a superfamily of enzymes involved in the conjugation of a wide range of electrophilic substrates with glutathione (GSH). This reaction facilitates the direct excretion of compounds into urine or bile, or more often through further metabolism by transpeptidases, N-acetylases and then excretion. This detoxification pathway helps protect cellular components from the toxic effects of many exogenous and endogenous electrophilic compounds. Often these are the primary metabolites produced by the action of the cytochromes P450 (phase I metabolism). The multiple forms of GST, with overlapping substrate specificities, exhibit GSH-peroxidase activity toward lipids and DNA, and also transport or remove hormones and hydrophobic molecules through non-catalytic binding [76, 77]. Like the cytochromes P450, GSTs are an essential part of an organism's adaptive response system to chemical insults and are regulated by endogenous (hormones) and exogenous factors (xenobiotics). Many GSTs are transcriptionally induced by

glucocorticoids through response elements (GREs) in the 5′ flanking regions of the genes, and also by response elements which are dependent on xenobiotic (XRE) and antioxidant substrates (AREs) [78]. This mechanism of regulation of GST expression emphasises their productive role in cells and tissues. GST induction by xenobiotic substrates and anti-oxidant chemoprotectors (carcinogenesis inhibitors), such as butylated hydroxyanisole and naturally occurring flavones, has been shown to enhance the rate of carcinogen detoxification [79,80].

Four classes of cytosolic GST and one microsomal form have so far been characterised in humans (Table 6). Because of their identification in a wide range of organisms, from mammals to insects and bacteria, their emergence and evolution is thought to have been controlled by the same factors that produced the divergence of the cytochrome P450 family of XMEs. Unlike P450s, however, the GSTs are expressed in virtually all cells and tissues (for review, see [81], with the exact complement and level of expression of GST isozymes dictated by cell-specific, environmental, hormonal and genetic factors [2]. The particular GST expression spectrum of a cell is then an important factor in determining an individual's sensitivity to carcinogens, in the same manner as P450 isoforms. Relatively high levels of expression of the GSTs are found in the gonads [81, 82], liver [83, 84] and colon [85] which emphasises their protective role against genotoxic insult in vital organs, germ cells or differentiation tissues which experience constant exposure to external factors.

The cytosolic GST, A, M, P and T (Table 6) are dimeric enzymes with a subunit Mr value of 22–27 kDa, whereas the GST Mic subunit, found in the outer mitochondrial membrane and endoplasmic reticulum, has a molecular weight of approximately 17 kDa. Within families, a high degree of amino acid sequence homology exists (55–95%), but between classes, identity falls to less than 25% in some cases. The alpha, mu and theta class genes are the most complex, and contain several genes in each family (Table 6), whereas the pi and microsomal species are a single gene with the exception of the mouse which has two pi class genes [86]. The cytosolic GSTs exist as homo or heterodimers between peptides of the same gene family and all tissues have a unique spectrum of GST enzymes and, as a consequence, a unique spectrum of activities. The microsomal GST appears to be a trimeric enzyme and has a substrate specificity and mode of regulation which is clearly distinguishable from its cytosolic counterparts. On the basis of the diversity of the GSTs, there is clearly significant potential for variation in GST expression between individuals. A wide variety of human carcinogens are metabolised by GSTs, including aflatoxin B_1, *trans*-stilbene oxide, nitrosources, benzo(a)pyrene and halogenated alkanes and alkenes. In the latter case, alkane and alkene procarcinogens are activated as a consequence of microsomal GST metabolism [90].

Table 6. Human glutathione S-transferases

GST family*	No. of genes	Chromosome	Reference
Alpha class A	>2	6	168
Mu class M	5	1p 13†	169
Pi class P	1	11	170
Theta class T	>2	?	171
Microsomal Mic	1	12	172

* Nomenclature system is that of [175]. † Several reports suggest that sequences highly homologous to GSTM genes are located on human chromosomes 3, 6 and 13 [173, 174]. Although there is no concensus on what these sequences represent [169], the M Class GSTs may be more complex and dispersed throughout the genome.

In addition to their role in chemical carcinogens and drug resistance, GSTs are also implicated in the mechanism of drug resistance in tumour cells, and a number of anticancer drugs have been shown to be substrates for these enzymes [92]. Overexpression of GSTs in many tumours coupled with an increased expression of P-glycoprotein (the protein product of the multi-drug resistance gene), is often observed in tumour cells made resistant to anticancer drugs. For example, high GSTA and GSTP expression, coupled with increased expression of the multiple drug resistance gene, may confer breast tumours with increased resistance to chemotherapeutic drugs [93]. However, there remain disparities in the experimental data which do not clearly define the role of the GSTs in this phenomenon.

The GSTs, like the cytochrome P450 enzymes, are subject to complex patterns of regulation. It is expected that individuality in the levels of expression of specific isozymes may lead to individuality in susceptibility to external toxins and carcinogens. One GST which has been extensively studied, because of a well defined phenotypic polymorphism is *GSTM1*. This polymorphism was first associated with the metabolism of *trans*-stilbene oxide in peripheral blood leucocytes [94]. Subsequent experiments have shown that *GSTM1* is homozygous nulled in 40–50% of the Caucasian population due to a gene deletion [95, 96]. This polymorphism has now been studied in relation to many cancer types and in one of the first studies on the null allele (*GSTM*(Θ)), only 35% of lung cancer patients who smoked were *GSTM1* positive, compared with 59% of control smokers [96]. Other reports, using pharmacokinetic assays assessing *GSTM1* activity in leucocytes or tissue biopsies, support the suggestion of an association with lung cancer, especially in smoking-related lung carcinomas [97, 98]. In subsequent genotyping studies, however, this association could not be fully confirmed as only a weak positive correlation with the null genotype was seen in squamous lung carcinoma, and an apparently negative correlation was observed with lung adenocarcinoma [99]. Also, other phenotyping studies failed to show any relationship between lung cancer and the *GSTM1* null phenotype [100]. Again these differences may be due to the limitations of pharmacokinetic assays, and inadequate numbers of individuals studied. The situation, therefore, remains unclear.

Several PCR-based genotyping assays have now been developed, which have allowed more detailed study of the role of the *GSTM1* polymorphism in cancer susceptibility [100, 102–104]. Most of the available PCR assays are also able to differentiate between two common allele variants which occur in *GSTM1* positive individuals. These alleles encode GSTM1-a and GSTM1-b, which are functionally identical but contain lysine or asparagine at amino acid position 172 of the protein, respectively. Using these and other assay methods, the GSTM1(θ) phenotype/genotype has been shown to be significantly over-represented in carcinoma of the stomach [105], colon [105, 106] and skin [107]. In studies of lung cancer in Japanese populations, 66% of patients with squamous cell carcinomas carried the null genotype compared to only 47% of healthy controls [108]. However, no apparent association between

human bladder and breast cancer was observed [106, 108]. The association between the *GSTM1* null genotype and the incidence of colon cancer is of particular interest. Overall, 61% of colorectal cancer patients had the null genotype compared to only 42% of controls. When these data were subdivided into tumour site, over 70% of individuals with tumours located in the proximal colon were *GSTM1* null compared to 54% of the distal colon group [106]. This represents a significantly higher risk of colon cancer in the proximal colon cancer subjects. All of these associations imply that GSTM1 (and GSTs in general) functions directly to inactivate environmental agents which produce tumours, particularly those associated with tobacco use, skin exposure and sporadic gastrointestinal disease.

Up to 60% of the human population has been shown to be unable to conjugate and hence detoxify halomethanes [109]. In human erythrocytes, this detoxification reaction is dependent on conjugation with glutathione, a reaction catalysed by a specific GST isozyme, GSTT1. The genetic basis for this phenotypic variation in monohalomethane metabolism has recently been reported [110], and has been shown to result from a deletion at the *GSTT1* gene locus. PCR-based genotyping assays for the *GSTT1(0)* genotype should, therefore, provide unequivocal evidence for any association with lack of expression of *GSTT1* and cancer susceptibility. It will also be of interest to determine whether an individual who is homozygous nulled for both *GSTM1* and *GSTT1* has an increased cancer risk.

THE N-ACETYL TRANSFERASES

The acetylation of the amino, hydroxyl and sulphydryl groups of many compounds is ubiquitous in animals. The metabolism of amino groups by N-acetylation, using acetyl CoA as cofactor, is by far the most common reaction to occur. This represents the major route of arylamine/hydrazine drug and xenobiotic metabolism in mammals [111]. By extrapolation from animal studies, the human N-acetyl transferases (NATs) have the capacity to activate or detoxify a large number of arylamine carcinogens [2, 112], and primarily function as phase II conjugation enzymes. For example, they have been linked to the detoxification of dietary heterocyclic amine carcinogens and/or occupational exposure to amino biphenyls and naphthylamines [111]. Like the GSTs, the NATs are cytosolic enzymes and are found in a large number of tissues, including liver, lung, colon and both red and white blood cells. The ubiquitous expression profile of the NATs suggests a fundamental role in protection against reactive molecules, not only in the liver but in nearly all target tissues exposed to environmental insult.

Like the microsomal GST, the NAT enzymes are not subject to the same pathways of gene regulation as many cytochrome P450s or other GSTs. However, individuality in the expression of these enzymes is the result of genetic polymorphism. The polymorphic acetylation of isoniazid, sulphamethazine and procainamide is another classic example of a genetic defect in the biotransformation of xenobiotics. High, inter-individual variation in the acetylation of isoniazid (an antitubercular drug) is exemplified by a trimodal distribution in its rate of elimination. On this basis, patients can be classified as "rapid" or "slow" acetylators [112–114]. The defect is known to be determined by a single gene locus and is inherited as an autosomal recessive trait, affecting 40–70% of European and North American Caucasians [114]. Considerable racial variation has been reported, however, with up to 90% of North Africans being phenotyped as slow acetylators [115] and 18–30% of Black Africans [116], 10–30% of Japanese and Korean Orientals [117–119], and as few as 5% of Eskimos [116]. Interestingly, Chinese populations appear to be similar to Caucasian populations, with more than 50% of individuals of the slow acetylator type [119]. Epidemiological studies suggest an association between acetylator status and cancer susceptibility and imply that arylamine metabolism is a major factor in the aetiology of these diseases [123]. The majority of these studies employed pharmacokinetic assays for NAT activity, using caffeine, isomazid or sulphamethazine as marker drugs in volunteer subjects, or using arylamine chemicals in assays in liver biopsy samples. The use of marker substrates and chromatographic isolation of NAT proteins also indicated that two functional enzymes exist, the polymorphic NAT-2 and a monomorphic enzyme NAT-1, which can both be distinguished in human liver [123, 124]. It was later shown that the genetic polymorphism was related to the expression of NAT2, although recent studies suggest that the expression of NAT1 may also be genetically polymorphic. The genetic defect in the *NAT-2* gene has been associated with a susceptibility to bladder and colon cancer, with slow acetylators having an increased risk of bladder cancer, but a decreased risk of developing colon carcinomas [2, 119, 123]. Many arylamine carcinogens, such as 4-aminobiphenyl and β-naphthylamine, are present as environmental pollutants or as tobacco smoke components, and are implicated in the higher incidence of urinary bladder cancers in chemical workers and cigarette smokers [119, 123]. Both NAT1 and NAT2 proteins catalyse the detoxification of bicyclic aromatic amines, compounds which are known to be potent bladder carcinogens. NAT1 "slow acetylators" have been shown to have an increased risk of developing bladder cancer, presumably as a consequence of their compromised ability to detoxify these compounds. In contrast, however, NAT proteins have been shown to metabolically activate aromatic amines to reactive electrophiles. These compounds have been implicated in the aetiology of colon cancer. "Slow acetylators" are, therefore, afforded some degree of protection from the deleterious effects of these compounds, while there is a statistically significant increase in the incidence of colon cancer among fast acetylators [126].

The recent molecular genetic characterisation of the *NAT* locus has allowed more definitive studies to be carried out on some of these cancer associations. The *NAT* locus on human chromosome 8p 21.2–23.1 [127, 128] encodes three distinct *NAT* genes. The two active genes, *NAT-1* and *NAT-2*, are separated by an inactive pseudogene, *NATP*. The *NAT-1* and *NAT-2* genes contain no introns and have been shown to correspond to previously isolated liver cDNAs [128]. Two rapid and six slow acetylator alleles of the *NAT-2* gene have now been identified, and these mutations in the slow alleles appear to affect the activity of the protein rather than their absolute level of expression [112]. The mutant forms of the gene can be determined at the genetic level by allele-specific PCR assays and PCR-RFLP analysis using Fok I, Kpn I, TaqI, DdeI and BamH I restriction enzymes [129–131]. A combination of discrete point mutations in the slow acetylator *NAT-2* alleles give rise to either the loss or gain of these restriction enzyme sites. These assays are currently being used to define the relationship between acetylator status and cancer incidence, but few studies have yet been published which give a clear indication of a genetic association with cancer susceptibility. However, one recent study linked a decreased clearance of a low-dose (chronic) of carcinogen to the slow acetylator genotype, and an increase in DNA adduct formation in exfoliated bladder cells in smokers [132]. This directly supports the previous studies on the protec-

tive role of the wild-type NAT-2 enzyme against bladder cancer. In direct contrast, however, *NAT-2* genotyping of colon cancer patients and a comparison of N-acetyltransferase activity in cytosolic preparations from human colon suggest that the *NAT-1* monomorphic gene, and not the polymorphic *NAT-2*, is the predominant enzyme activity [133].

It is interesting to note that, in both bladder and colon tumours, a significant number of partial chromosomal deletions occur which result in loss of heterozygosity (LOH) of a number of genetic markers on virtually every human chromosome. In relation to the *NAT* locus, 40% of colon tumours [134–137] and 25% of bladder cancers [138] contain deletions that map to the same region of chromosome 8p. In addition, LOH at this site has been reported for lung, prostate, ovarian, stomach and kidney malignancies. From this, it is evident that the loss of one or more genes on chromosome 8p may be responsible for the development of numerous cancers, or the existence of a putative tumour suppressor gene on this arm could be an alternative explanation [136]. Whatever the reason, LOH of the *NAT* locus would be a primary mechanism for alterations in acetylator capacity in tumour cells, as both the NAT-2 and NAT-1 isozymes would be lost. The subsequent alteration in the NAT phenotype in these tumour cells could have far-reaching consequences in their resistance to cytotoxic attack, and may affect the activity of drugs used in anticancer therapy. Investigations into LOH of the *NAT* locus are presently anticipated.

OTHER POLYMORPHISMS IN XMEs

Although a number of other xenobiotic-metabolising enzymes exhibit some characteristics of a genetically determined polymorphism, their effect on carcinogenesis has not been studied in great detail (Table 2). However, polymorphic expression of the alcohol (ADH) and aldehyde dehydrogenases (ALDH) is well documented. As we "voluntarily" expose ourselves to alcohol (ethanol), these defects are an important consideration because both ethanol and acetaldehyde have been implicated in liver cancer and tumours of the mouth and throat [139]. In man, five ADH isozymes have been characterised, two of which (ADH2 and ADH3) are known to be polymorphic [140, 141]. ALDH activity in the liver and other tissues is also governed by five isozymes, with the mitochondrial *ALDH2* gene subject to a well defined, dominant genetic defect in approximately 50% of Orientals [142, 143, 144]. This latter genetic variation is rarely seen in other racial and ethnic groups, but is responsible for the flushing syndrome brought on by acetaldehyde accumulation after drinking. As ethanol is known to be a potent inducer of P450s (CYP2E1) a more detailed study of these gene polymorphisms and chemically induced cancer is warranted.

Recently, two aberrant forms of the xenobiotic metabolising enzyme, microsomal epoxide hydrolase (mEH), have been defined at the DNA level [145]. Both mutant alleles arise because of discrete point mutations which lead to amino acid changes, $Tyr_{113} \rightarrow$ His and $His_{139} \rightarrow$ Arg, which decrease and increase mEH activity by 25 and 40%, respectively. The mEH is expressed at varying levels in most cell types and tissues [146–148], and substrates for this enzyme include procarcinogenic epoxide metabolites of polycyclic aromatic hydrocarbons. mEH has been implicated in both protection against and potentiation of the effects of chemical carcinogens. With the identification of the mutant mEH alleles, inter-individual differences in microsomal epoxide hydrolase activity can now be studied as a genetic susceptibility factor for cancer incidence.

Several potential DNA polymorphisms have been found in the human cholesterol 7-hydroxylase gene (cytochrome P450 CYP7), which catalyses the first and rate-limiting step of bile acid synthesis in the liver [149]. A single point mutation in the steroid-binding region of this essential gene, if found as a variant allele in the general population, may have important implications as a dietary host factor in the development of a large number of diseases. High circulating levels of cholesterol are linked to the incidence of heart disease, stroke and gastro-intestinal cancer. These associations may be due to defects in the synthesis of bile acids and steroid hormones, and in the case of intestinal cancer, the action of these compounds as tumour promoters.

COMBINATION STUDIES

As more and more phenotypic and genotypic polymorphisms are discovered, the effect that these have on the metabolism of xenobiotics and individual risk of carcinogen-induced disease becomes increasingly of interest. When it is considered that the overall defence of cells from genotoxic attack is dependent on not only the level of expression of a particular protective pathway, but also the interaction of various activating and deactivating components of the xenobiotic metabolism system, then the study of susceptibility due to polymorphic loci is essential in understanding individual genetic risk of cancer. Since chemical compounds may be subject to several metabolic reactions, combinations of polymorphisms in these enzyme systems need to be assessed in populations to ascertain whether specific cohorts of cancer sufferers, with multiple genetic defects in carcinogen metabolism, can be specifically related to exposure. A small number of such studies have already been published, and of particular interest is a study of lung cancer incidence in Japanese smokers which showed that individuals null at the *GSTM1* locus, who also carried the *CYP1A1* polymorphism, were nearly 30 times more likely to develop squamous cell carcinoma of the lung [108]. Individuals possessing the "high inducibility" CYP1A1 phenotype who were also homozygous nulled for *GSTM1* would, therefore, theoretically be at greatest risk of developing smoking-related disease — their *CYP1A1* genotype leading to an increase in the production of reactive electrophiles from the aromatic hydrocarbon procarcinogens present in tobacco smoke, accompanied by a deficiency in the GSTM1 catalysed detoxification pathway. This increase in cancer risk has been related to low dose cigarette consumption and the association is reduced as exposure increases [150]. These results clearly demonstrate that individuals with a strong genetic susceptibility to smoking-induced lung cancer have a considerably increased risk of developing the disease, even when their tobacco consumption is relatively low. A more recent study on a Caucasian population showed that GSTM1 expression in the lung and liver may have a protective effect against the development of bronchial carcinoma in individuals of the high CYP1A1 inducible phenotype [151]. These differences may well be attributable to inter-ethnic variation in allele frequency at these polymorphic loci. A further development in combined risk factors for lung cancer susceptibility is the identification of a germ line polymorphism in the tumour suppressor gene, *TP53* ($Arg_{72} \rightarrow$ Pro). This variation has been shown to be a significant determinant of individual risk of lung cancer which is separate from the CYP1A1 Pro/Val and GSTM1/Mθ combination susceptibility. The inherited variation in *TP53* appeared to be more important in individuals who had never smoked [152].

Combination studies such as these can only increase our knowledge of the initiation events surrounding all types of cancer, and in the definition of environmentally induced neo-

Table 7. Potential targets for mutation

Target	Effect of debilitating mutations
Chromosomes	Genetic instability, translocations, deletions, etc.
Growth factors/receptors	Increased/inappropriate growth stimuli
Oncogenes	Erratic differentiation and growth
Tumour suppressor genes	Resistance to cytotoxins and apoptosis
Transcription factors	Aberrant/inappropriate gene expression
Cell surface proteins	Cell mobility changes; invasiveness, metastasis
Immune system	Decreased immune-surveillance; opportunistic disease
Stress response genes	Increased mutation rates
DNA repair genes	Faulty repair, increased mutation rates
Metabolism	Increased mutagen/tumour promoter exposure

plasia, by providing a molecular mechanism for tumour induction and progression. Any relationship, either negative or positive, between inborn errors of metabolism and the genetic variation or mutagenesis of important cellular genes, such as oncogenes, growth factors and tumour suppressor genes, will help unravel the complex network of physiological reactions involved in cell transformation. Mutations in vital cellular genes, resulting from the action of direct mutagens and DNA adducts, can produce major changes in the control of cell homeostasis and a cell's response to growth modulation signals. Obviously, the inability to inactivate specific genotoxic compounds, or conversely an increased rate of procarcinogen activation, must have a direct bearing on the types of mutations that can be detected in genes involved in necessary cellular and bodily control processes and cancer (Table 7). For instance, a study of primary lung tumours, biopsied from untreated patients, has produced an apparent association between the GSTM1(θ) genotype and the frequency of mutations in the *TP53* gene. In patients with a history of heavy smoking, 30% of the mutations occurring in *TP53* were G to T transversion mutations at GC base pairs [153]. Over 90% of the tumours of these patients were also *GSTM1* null. These types of transversion mutations have been associated with the action of PAH DNA adducts, such as benzo(a) pyrene derivatives, which are deactivated by the action of GSTM1. Heavy smokers with the null genotype therefore appear to have an increased risk of somatic *TP53* mutation and consequently lung cancer, due to exposure to at least some of the carcinogens present in cigarette smoke. These data indicate that polymorphisms in the XMEs may directly affect the mutation rates of "cancer genes", and that inherited variability in carcinogen metabolism is extremely important in the initial stages of neoplastic transformation. Further study of the mutation patterns observed in vital genes in specific cancer types and their relationship to polymorphisms in XMEs is anticipated with great interest.

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Li–Fraumeni Syndrome

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In 1969, F.P. Li and J.F.Fraumeni described four families in which childhood soft tissue sarcoma was associated with early onset breast cancer and other early onset cancers in their close relatives. Three of these families were identified through pairs of siblings with rhabdomyosarcoma occurring among a series of nearly 650 children with rhabdomyosarcoma in the U.S.A. By making assumptions about family size, Li and Fraumeni estimated that considerably less than one pair of affected siblings would have been expected by chance among this series of children. A further family in which a pair of cousins were affected was also identified. On investigation of the family histories of these four pairs of children, Li and Fraumeni found an unusually high incidence of breast cancer occurring premenopausally in close female relatives, sarcomas also occurring at an early age, and other unusually early onset cancers, including acute leukaemia in other close family members. It was of particular interest that three of the mothers of the index children with soft tissue sarcoma had developed breast cancer under the age of 30 years. Li and Fraumeni proposed that the observed clustering of cancers in these families was due to inherited predisposition [1].

In a subsequent more detailed report, a second pair of cousins with soft tissue sarcoma occurring in childhood was identified, and adrenocortical carcinoma and brain tumours were observed in first degree relatives of other children with soft tissue sarcoma included in the series. These latter observations suggested that these cancers may also be components of the proposed cancer predisposition syndrome [2].

A family showing a similar pattern of cancers had been previously reported [3], and following Li and Fraumeni's reports, other families with patterns of cancer also consistent with their findings were described [4–6]. In a report describing a series of families based on breast cancer probands, in which other unusual clustering of cancers consistent with Li and Fraumeni's findings was observed, Lynch and colleagues coined the term "SBLA syndrome". These letters representing what these authors regarded as the component syndrome cancers, that is, sarcoma (S), breast and brain (B), leukaemia lung and larynx (L) and adrenocortical carcinoma (A) [7]. This term has been used by others, but the syndrome was also commonly known as the Li–Fraumeni syndrome [6], and Li–Fraumeni syndrome (LFS) is now the accepted term.

It was, however, uncertain whether these familial clusters were due to a genetically determined predisposition to a broad but specific range of cancers. There was a prevailing concept at this time that inherited predisposition to cancer could occur only in a site-specific fashion. The acceptance of the notion that a single trait could result in predisposition to such a diverse spectrum of neoplasms represented a problem. Alternative explanations were that exposure to common environmental agents within families may have generated these clusters of cancers, or such clusters may simply represent rare chance aggregations in certain families. Strong support for the notion of inherited susceptibility to component cancers in families with LFS was subsequently provided by systematic studies of families and patient populations. A number of pieces of evidence indicating genetic predisposition emerged from these studies.

The first piece of evidence came from a follow-up study

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