Carcinogenesis vol.23 no.11 pp.1839–1849, 2002

A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer

Christoph Sachse[1]*, Gillian Smith[1]*, Murray J.V.Wilkie[1], Jennifer H.Barrett[2], Robin Waxman[3], Frank Sullivan[4], David Forman[3], D.Timothy Bishop[2], C.Roland Wolf[1,5] and the Colorectal Cancer Study Group**

[1]Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee DD1 9SY, [2]Cancer Research UK Genetic Epidemiology Laboratory, St James's University Hospital, Leeds LS9 7TF, [3]Centre for Cancer Research, Arthington House, Cookridge Hospital, Leeds LS16 6QB, [4]Tayside Centre for General Practice, University of Dundee, Dundee DD2 4AD, UK

[5]To whom correspondence should be addressed
Email: Margaret.Rooney@cancer.org.uk
*Contributed equally to this paper

****The Colorectal Cancer Study Group:** Jenny Barrett[2], D.Timothy Bishop[2], Alan R.Boobis[6], David Forman[3], R.Colin Garner[7], Nigel Gooderham[8], Tracy Lightfoot[7], Christoph Sachse[1], Gillian Smith[1], Robin Waxman[3] and C.Roland Wolf[1].

Addresses: [6]Department of Clinical Pharmacology, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, [7]Jack Birch Unit for Environmental Carcinogenesis, University of York, YO1 5DD [8]Department of Molecular Toxicology, Sir Alexander Fleming and Building, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK

Susceptibility to colorectal cancer, one of the most common forms of cancer in the Western world, has been associated with several environmental and dietary risk factors. Dietary exposure to food derived heterocyclic amine carcinogens and polycyclic aromatic hydrocarbons have been proposed as specific risk factors. Many polymorphic Phase I and Phase II drug metabolizing enzymes are responsible for the metabolism and disposition of these compounds and it is therefore possible that inheritance of specific allelic variants of these enzymes may influence colorectal cancer susceptibility. In a multicenter case–control study, 490 colorectal cancer patients and 593 controls (433 matched case–control pairs) were genotyped for common polymorphisms in the cytochrome P450 (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2C19 and CYP2D6), glutathione *S*-transferase (GSTM1, GSTP1 and GSTT1), sulfotransferase (SULT1A1 and SULT1A2), *N*-acetyl transferase 2 (NAT2), NAD(P)H:quinone oxidoreductase (NQO1), methylenetetrahydrofolate reductase (MTHFR), and microsomal epoxide hydrolase (EPHX1) genes. Matched case-control analysis identified alleles associated with higher colorectal cancer risk as carriage of *CYP1A1*2C* (OR = 2.15, 95% CI 1.36–3.39) and homozygosity for *GSTM1*2/*2* (OR = 1.53, 95% CI 1.16–2.02). In contrast, inheritance of the *CYP2A6*2* (OR = 0.51, 95% CI 0.28–1.06), *CYP2C19*2* (OR = 0.72, 95% CI 0.52–0.98) and the $EPHX1_{His113}$ alleles were associated with reduced cancer risk. We found no association with colorectal cancer risk with NAT2 genotype or any of the other polymorphic genes associated with the metabolism and disposition of heterocyclic amine carcinogens. This data suggests that heterocyclic amines do not play an important role in the aetiology of colorectal cancer but that exposure to other carcinogens such as polycyclic aromatic hydrocarbons may be important determinants of cancer risk.

Abbreviations: 95% CI, 95% confidence interval; AA, aromatic amine; CYP, cytochrome P450; EPHX1, microsomal epoxide hydrolase; GST, glutathione *S*-transferase; HA, heterocyclic amine; MTHFR, methylenetetrahydrofolate reductase; NAT2, *N*-acetyl transferase 2; NQO1, NAD(P)H:quinone oxidoreductase; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SULT, sulfotransferase.

Introduction

Colorectal cancer, second only to lung cancer, is a major cause of cancer death in the western world (1). Comparison of colorectal cancer incidence rates in different countries reveals marked regional differences in disease incidence, suggesting that environmental factors including local carcinogen exposure and diet may be important determinants of cancer risk. Migrants from countries with relatively low colorectal cancer incidence (e.g. Asian countries) to 'western' societies rapidly assume the incidence rate of their adopted country (2), supporting the hypothesis that regional dietary or carcinogen exposures are important risk factors.

Specific components of the western diet including meat consumption (particularly red and/or well-done meat) and dietary fat (particularly polyunsaturated fatty acids) have been proposed as risk factors which influence susceptibility to colorectal cancer (3–5). Data from both *in vitro* and *in vivo* studies suggest that exposure to heterocyclic amines (HAs) such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), aromatic amines (AAs) including 4-aminobiphenyl (4-ABP), and polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene significantly increase colorectal cancer risk (6–8). Dietary HAs are formed during frying and broiling of meat and fish by the pyrolysis of amino acids and proteins (9), whereas PAHs are derived from vegetable oils (10,11). Both AAs and PAHs are also present at significant levels in tobacco smoke (12). AA, HA and PAH compounds are known to be metabolized by a variety of Phase I and Phase II drug metabolizing enzymes, including cytochrome P450s, glutathione *S*-transferases and *N*-acetyl transferases, which catalyse the various activation and detoxification pathways illustrated in Figure 1.

Glutathione *S*-transferases GSTM1 and GSTT1 facilitate the inactivation of toxic compounds by forming hydrophilic glutathione conjugates. In contrast, a number of enzymes including CYP1A1, CYP1A2, NAT2, SULT1A1 and SULT1A2 activate HAs and PAHs to mutagenic products (13–16). Several additional enzymes including CYP1B1, CYP2C9, CYP2C19 and CYP2D6 (17–19) as well as microsomal epoxide hydrolase (EPHX1) and NADPH:quinone oxidoreductase (NQO1) have also been shown to metabolize these compounds using hepatic

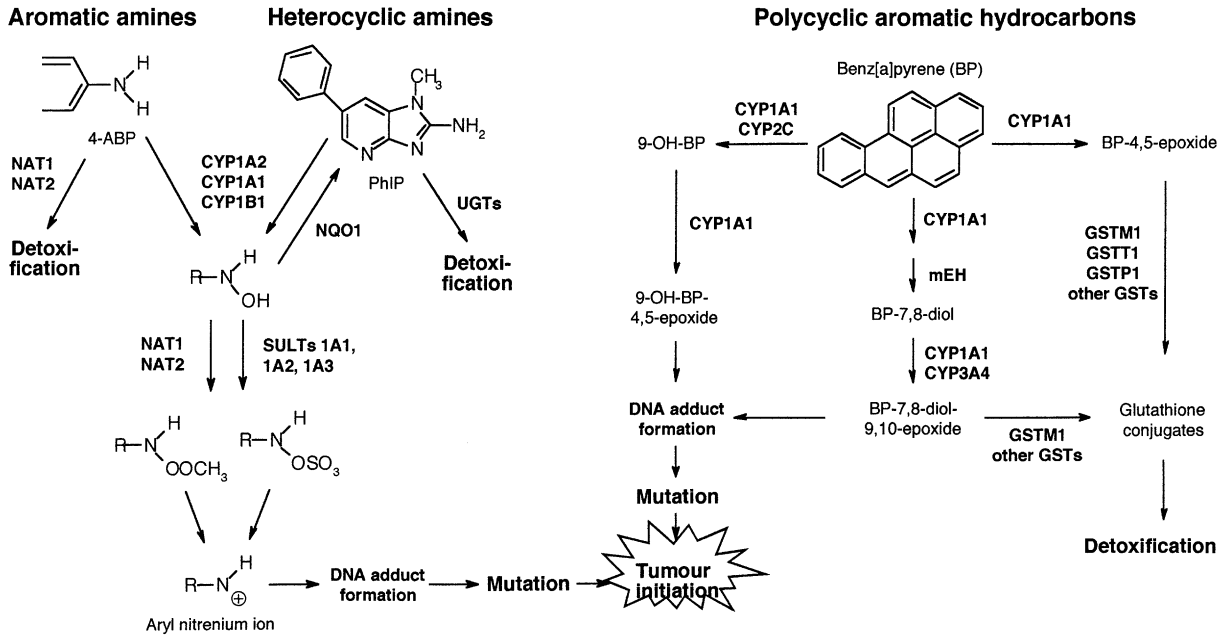

Fig. 1. Involvement of various polymorphic drug metabolizing enzymes in the metabolism and activation of AAs, HAs, and PAHs. For simplicity, not all pathways of metabolism are shown. UGTs, UDP glucuronosyltransferases.

microsomal preparations *in vitro* or using recombinant enzyme systems (20,21).

Dietary folate supplements have been shown to be protective against colorectal adenoma formation (22), while low folate diets in combination with high alcohol consumption have been associated with a significantly higher colorectal cancer risk (23). Methylenetetrahydrofolate reductase (MTHFR) catalyses the conversion of 5,10-methylenetetrahydrofolate, required for purine and thymidine synthesis, to 5-methylenetetrahydrofolate, required for the synthesis of methionine. A common polymorphism in MTHFR ($677_{C\rightarrow T}$) leading to reduced enzyme activity has previously been proposed to influence colorectal cancer susceptibility (24,25).

Extensive genetic polymorphism in these and other drug metabolizing enzymes results in a diverse population distribution of catalytic activities (26,27). For example, polymorphisms in GSTM1, GSTT1 and CYP2D6 result in 'null alleles' leading to a complete loss of enzyme function, while the other polymorphisms (e.g. CYP2C9, GSTP1) result in single amino acid changes with less predictable and often substrate-specific effects on catalytic activity. The inheritance of many DME alleles has now been associated with cancer susceptibility and is the subject of many recent review articles (28).

There are now several studies investigating the influence of genetic polymorphism on colorectal cancer susceptibility. In most cases, these investigations have focussed on only a limited number of candidate genes such as *CYP1A1*, the *GSTs* and *NAT2* (Figure 1). Among previous studies, inheritance of the *CYP1A1*2C* allele has been associated with increased colon cancer risk [e.g. Kiss *et al.* (29): OR = 1.57, 95% CI 0.9–2.7], as has the *GSTM1* 'null' genotype [e.g. Zhong *et al.* (30): OR = 1.90, 95% CI 1.3–2.8], the *GSTT1* 'null' genotype [e.g. Deakin *et al.* (31): OR = 1.88, 95% CI 1.28–2.77], and the *NAT2* 'fast acetylator' genotype [e.g. Lang *et al.* (32): OR = 2.48, 95% CI 1.0–6.0, Roberts-Thomson *et al.* (33): OR = 1.9, 95% CI 1.0–3.3]. Many of these studies were relatively small, however, while a recent large study of 1542 colorectal cancer cases and 1860 controls (34) failed to show a significant effect of *GSTM1* deficiency or fast *NAT2* acetylation on colorectal cancer susceptibility. The ultimate determinant of 'genetic susceptibility' is likely to be complex, possibly arising from synergistic interactions between allelic variants of multiple genes including activating and detoxifying enzymes and the environment. This may explain why many previous studies have reported contradictory findings. These differences could arise from genetic differences in the populations studied, regional dietary differences and local carcinogen exposures. In addition, many previous studies have considered relatively small populations, leading to difficulties in assessing the true statistical significance of the data. There is therefore a need for larger, more comprehensive studies where multiple polymorphisms are studied simultaneously so that a true estimate of risk associated with these polymorphisms can be made within a defined population.

Here we describe a comprehensive case–control genotyping analysis, designed to investigate the influence of specific alleles of a wide range polymorphic carcinogen metabolizing enzymes on colorectal cancer susceptibility.

Materials and methods

Patient and control recruitment

A total of 490 patients with colorectal cancer were recruited in the period August 1997–February 2001 at either Ninewells Hospital, Dundee, Perth Royal Infirmary, Leeds General Infirmary, St James's Hospital, Leeds or York District Hospital. The study was approved by the Tayside Committee on Medical Research Ethics, the York Research Ethics Committee and the Leeds Health Authority/St James's and Seacroft University Hospitals Local Research Ethics Committee. Written informed consent was obtained from all study participants.

Patients (45–80 years) were invited to participate if they were Caucasian, had incident colorectal cancer (ICD-9 classification 153.0–153.9, 154.0–154.1), and had no history of familial adenomatous polyposis coli (FAP), inflammatory bowel disease, ulcerative colitis, diverticular disease or previous malignancy. Healthy population-based controls (GP controls), with no history of previous cancer, were recruited by age, sex and general practitioner matching of incident cases. Multiple GP controls were recruited for some cases, while no matching control was available for a minority of cases. In total, 490 cases and 593 controls were recruited, with 433 matched

Table I. Demographic and descriptive statistics of cases and controls

	Cases *n* (%)	Controls *n* (%)
Participants by centre:		
Dundee	145 (30)	249 (42)
Leeds	240 (49)	232 (39)
York	105 (21)	112 (19)
Total	490 (100)	593 (100)
Mean age in years (SD)	67.70 (8.50)	68.61 (8.89)
Sex:		
Male	297 (61)	322 (54)
Female	193 (39)	271 (46)
Duke's stage:		
A	57 (12)	–
B	162 (33)	–
C1	148 (30)	–
C2	25 (6)	–
Cancer site:		
Colon	277 (57)	–
Rectum	213 (43)	–

case–control pairs. The demographic and descriptive statistics of cases and controls are given in Table I.

All patients and controls were mentally competent to be interviewed by an experienced research nurse in order to provide dietary and lifestyle information which was recorded in a Food Frequency Questionnaire.

Molecular genetic analyses of polymorphisms

All patients and controls gave a 10 ml venous blood sample, which was stored in EDTA blood containers at –20°C. Genomic DNA was extracted from 200 ml of whole blood, using a QIAamp 96 spin blood kit (Qiagen), according to the Manufacturer's instructions and stored in 10 mM Tris–HCl, 1 mM EDTA at 4°C.

All study participants were genotyped for the most common and functionally relevant polymorphisms in drug metabolizing enzymes involved in HA and PAH carcinogen metabolism. A compilation of the alleles, primer sequences and reaction conditions for all genotyping methods are summarized in Table II and Table III.

PCR and PCR-RFLP assays (Table II) were adapted from literature methods (35–38) and used to identify the following alleles: *CYP1A2**1C ($-3858_{G\rightarrow A}$), *CYP1A2**1D (-2464_{delT}), *CYP1A2**1F ($-164_{C\rightarrow A}$), *CYP1B1*3 ($4326_{C\rightarrow G}$)*, *CYP2A6*2 ($479_{T\rightarrow A}$)*, *CYP2A6*3 (CYP2A6/CYP2A7 hybrid)*, *GSTT1*2*, *GSTM1*2*, *NQO1*2 ($Pro_{187}Ser$)*, *NQO1*3 ($Arg_{139}Trp$)*, *SULT1A1*2 ($Arg_{213}His$)*, *SULT1A2*2* (*$Asn_{235}Thr$*), *MTHFR*$_{Ala222Val}$, *EPHX1*$_{Tyr113His}$, *EPHX1*$_{His139Arg}$. All PCR-RFLP tests were modified for small scale reactions (10 µl), and were optimized for high-throughput analysis using the MADGE gel system (Madge Biosystems) allowing simultaneous analysis of 96 samples on one microtitre plate formatted polyacrylamide gel.

The presence of additional alleles was detected using novel high-throughput *TaqMan* allelic discrimination tests: *CYP1A1*2 (*$3801_{T\rightarrow C}$), *CYP1A1*2B* ($2455_{A\rightarrow G}$), *CYP2C9*2 ($430_{C\rightarrow T}$)*, *CYP2C9*3 ($1075_{A\rightarrow C}$)*, *CYP2C19*2 ($681_{G\rightarrow A}$)*, *CYP2D6*3 ($2549_{A\rightarrow del}$)*, *CYP2D6*4 ($1846_{G\rightarrow A}$)*, *GSTP1*$_{Ile105Val}$, *GSTP1*$_{Ala114Val}$, and the *NAT2* SNPs *NAT2*$_{481C\rightarrow T}$, *NAT2*$_{590G\rightarrow A}$, *NAT2*$_{803A\rightarrow G}$ and *NAT2*$_{857G\rightarrow A}$ (defining 'slow' *NAT2* alleles *NAT2*5A*, *NAT2*5B*, *NAT2*5C*, *NAT2*6A* and *NAT2*7B*, respectively). CYP2C9*2 and CYP2C19*2 genotyping analyses were performed using pre-developed assay reagents for allelic discrimination of cytochrome P450 (PE Biosystems), according to the manufacturer's instructions. Primer and probe sequences for all other Taqman genotyping assays are given in Table III. All TaqMan probes were synthesized by PE Applied Biosystems and oligonucleotide primers by MWG Biotech. Following PCR amplification, end-point fluorescence was read using an Applied Biosystems ABI PRISM 7700 Sequence Detector or 'Taqman'. Genotypes were assigned using Allelic Discrimination Software (Applied Biosystems SDS Software v1.7a). Appropriate controls representative of each genotype and multiple no template controls were included in each analysis.

Case–control analyses

Each polymorphism was initially tested for Hardy–Weinberg equilibrium in the control population. Genotype frequencies were then compared between all cases and controls using chi-squared tests using 3×2 contingency tables. For polymorphisms with a low variant allele frequency, homozygotes for the variant allele were combined with heterozygotes. For certain genes, genotypes were combined on the basis of a known phenotype–genotype relationship – for example, the combination of CYP2D6 alleles CYP2D6**3* and CYP2D6**4* were classified as 'poor metabolizers', all combinations with allele *CYP2D6*1* were termed 'extensive metabolizers'. Similarly *GSTT1*1/*1* + *GSTT*1/*2* were termed 'active metabolizers', while inheritance of the *GSTT1*2/*2* genotype identified an individual as 'deficient metabolizer'. Combinations of alleles NAT2**5A*, NAT2**5B*, NAT2**5C*, NAT2**6A* and NAT2**7B* were classified as 'slow acetylators', while all genotypes containing allele *NAT2*4* were classified as 'fast acetylators'. The *NQO1* genotype NQO1**1/*1* was classified as an 'active metabolizer', genotypes NQO1**1/*2* and NQO1**1/*3* were 'intermediate metabolizers' and genotypes NQO1**2/*2*, NQO1**3/*3*, and NQO1**2/*3* were 'slow metabolizers'. *EPHX1* 'fast' genotypes were $Y_{113}Y/H_{139}R$, $Y_{113}Y/R_{139}R$ and $Y_{113}H/R_{139}R$; 'intermediate' genotypes were $Y_{113}Y/H_{139}H$, $Y_{113}H/H_{139}R$ and $H_{113}H/R_{139}R$; and 'slow' genotypes were $Y_{113}H/H_{139}H$, $H_{113}H/H_{139}H$ and $H_{113}H/H_{139}R$.

For polymorphisms where the preliminary analysis demonstrated an association with colorectal cancer susceptibility (using a liberal cut-off of $P = 0.10$), a matched analysis was carried out on a subset of 433 matched case–control pairs using conditional logistic regression. Case–control pairs were matched for age, sex, study centre and general practitioner, factors not considered in the preliminary analysis.

Effect sizes are presented as odds ratios, with 95% confidence intervals, treating homozygosity for the most common allele as the baseline risk category.

Several possible synergistic genotype/genotype interactions are suggested by the various HA and PAH metabolic pathways (Figure 1) or have been proposed in previous studies. The following combinations of alleles were therefore analysed – *GSTM1* 'deficient' plus *NAT2* 'slow', *GSTM1* 'deficient' plus *GSTT1* 'deficient', *GSTM1* 'deficient' plus *CYP1A1 (CYP1A1*1/*2C + CYP1A1*2C/*2C)*, and *NAT2* 'slow' plus *CYP1A1 (CYP1A1*1/*2C + CYP1A1*2C/*2C)*. Based on the matched case–control analysis, a logistic model allowing for interaction was compared with a model assuming that each gene was an independent modifier of risk, using the likelihood ratio test.

All analyses were carried out using the statistical analysis software Stata (Stata Statistical Software: Release 6.0. College Station, TX: Stata Corporation, 1999).

Results

Distribution of genotype frequencies in cases and controls

Results of the entire unmatched case–control analysis are presented in Table IV. All genotype distributions in the control group were in Hardy–Weinberg equilibrium with the exception of the *mEH*$_{Tyr113His}$ allele and *CYP1A1*2* (see Discussion).

In the unmatched case–control analysis, significant associations were found for carriage of the *CYP1A1*2C* allele (OR = 1.84, $P = 0.001$) and the *GSTM1*2/*2* 'null' genotype (OR = 1.33, $P = 0.021$). In contrast, variant genotypes of *CYP2A6* (OR = 0.57, $P = 0.070$) and *CYP2C19* (OR = 0.68, $P = 0.007$) were associated with lower colorectal cancer risk. Inheritance of the homozygous *mEH*$_{Tyr113Tyr}$ genotype was also found to be a significant protective factor (OR = 0.68, $P = 0.012$), although the $Tyr_{113}His$ allele distribution within our control population was not in Hardy–Weinberg equilibrium [observed (expected) frequencies were 270 (227) Tyr/Tyr, 193 (279) Tyr/His, 129 (86) His/His; $P < 0.0001$)]. mEH$_{Tyr113His}$ was therefore excluded from further matched case–control analysis.

There were no statistically significant differences in case and control allele frequencies for the other enzymes we investigated in this study (Table IV). In particular, we did not identify any statistically significant associations for any alleles of *CYP1A2* or *NAT2*, enzymes which are thought to be important determinants of the metabolic fate of heterocyclic amine carcinogens.

To confirm our initial observations, matched case–control analysis was performed for each of the genotypes which showed a significant effect in the unmatched analysis. As illustrated in Figure 2, inheritance of inactive or low activity alleles of *CYP2A6* (OR = 0.54, 95% CI 0.28–1.06) and

Table II. MADGE-gel based genotyping analysis

Gene Allele	Primer Name	Primer Sequence (5′→3′)	PCR Program
CYP1A2			
$C_{-164}A$	11F	TGAGGCTCCTTTCCAGCTCTCA	35× (30 s 94°C—
	4R	AGAAGCTCTGTGGCCGAGAAGG	10 s 58°C—1 min 72°C)
CYP1A2			
dT_{-4373}	dTF	TGAGCCATGATTGTGGCATA	35× (30 s 94°C—
	dTR	AGGAGTCTTTAATATGGACCCAG	10 s 49°C—1 min 72°C)
CYP1A2			
$G_{-3858}A$	R2	GCTACACATGATCGAGCTATAC	35× (30 s 94°C—
	R3	CAGGTCTCTTCACTGTAAAGTTA	10 s 52°C—1 min 72°C)
CYP1A2			
$T_{-740}G$	740F	CACTCACCTAGAGCCAGAAGCTC	35× (30 s 94°C—
	740R	AGAGCTGGGTAGCAAAGCCTGGA	10 s 49°C—1 min 72°C)
CYP1A2			
$C_{63}G$	chF	ATGAATGAATGAATGTCTC	38× (30 s 94°C—
	chR	CTCTGGTGGACTTTTCAG	10 s 49°C — 40 s 72°C)
CYP1A2			
$T_{1545}C$	F01	AGCCCTTGAGTGAGAAGATG	35× (30 s 94°C—
	R01	GGTCTTGCTCTGTCACTCA	10 s 58°C—1 min 72°C)
CYP2A6			
*2, *3	F03	CTGATCGACTAGGCGTGGTA	40× (30 s 94°C—
	R06	CGTCCTGGGTGTTTTCCTTC	10 s 51°C—1 min 72°C)
CYP1B1*2 $Leu_{432}Val$	323F	TAAGAATTTTGCTCACTTGC	33× (30 s 94°C—
	1B1R	GTTCTCCGGGTTAGGCCACTTAA	10 s 55°C—1 min 72°C)
mEH			
$Tyr_{113}His$	EH1	GACTTACACCAGAGGATCGATAAG	34× (30 s 94°C—
	EH2	GCCCTTCAATCTTAGTCTTGAAGTGACGGT	25 s 56°C — 40 s 72°C)
mEH			
$His_{139}Arg$	EH3	CCAGAGCCTGACCGTGCAGGG	35× (30 s 94°C—
	EH4	AACACCGGGCCCACCCTTGGC	10 s 58°C—1 min 72°C)
NQO1*2			
$Pro_{187}Ser$	55F	GAGACGCTAGCTCTGAACTGAT	35× (30 s 94°C—
	56R	ATTTGAATTCGGGCGTCTGCTG	10 s 58°C—1 min 72°C)
NQO1*3			
$Arg_{139}Trp$	464A	CTGGTCTTACCTCAATGATGTC	35× (30 s 94°C—
	464B	CCTGCATCAGTACAGACCACC	10 s 56°C—1 min 72°C)
SULT1A1*2 $Arg_{213}His$	7F	GTTGGCTCTGCAGGGTTTCTAGGA	35× (30 s 94°C—
	7R	CCCAAACCCCCTGCTGGCCAGCACCC	10 s 56°C—1 min 72°C)
SULT1A2*2 $Asn_{235}Thr$	S1A2F	GGTCGAGGAGCTGGCTCTAT	33× (30 s 94°C—
	S1A2R	CCTCATGAAGGGGGAGATGC	10 s 56°C—1 min 72°C)
GSTT1	T1P1F	TTCCTTACTGGTCCTCACATCTC	35× (1 min 93°C —
*2/*2	T1P2R	TCACCGGATCATGGCCAGCA	1 min 58°C—1 min 72°C)
	betaGF	CAACTTCATCCACGTTCACC	
	betaGR	GAAGAGCCAAGGACAGTTAC	
GSTT1	T1-0F	CAGTTGTGAGCCACCGTACCC	
*2	T1-0F	CAGTTGTGAGCCACCGTACCC	
	T1-0R	CGATAGTTGCTGGCCCCCTC	32× (30 s 94°C—
	T1-AF	CAGCACCCAGGGCATCAGCTTCTG	13s 69°C—1 min 72°C)
	T1-AR	GGTGAGCCAGTATCTCCCCAGACAC	
GSTM1	P1F	CGCCATCTTGTGCTACATTGCCCG	30× (1 min 95°C—
*2/*2	P2R	ATCTTCTCCTCTTCTGTCTC	1 min 52°C—1 min 72°C)
	P3R	TTCTGGATTGTAGCAGATCA	
GSTM1	tPA7	GGAAGTACAGCTCAGAGTTCTGCAGCACCCCTGC	35× (20 s 95°C—
*2	tPA13	TGTCTCCAGCACACAGCATGTTGTCGGTGAC	20 s 56°C—5 min 68°C)
	M2F10	AAGACAGAGGAAGGGTGCATTTGATA	
	M5R16	ACAGACATTCATTCCCAAAGCGACCA	

CYP2C19 (OR = 0.72, 95% CI 0.52–0.98) both had a protective effect while inheritance of the *GSTM1* 'null' genotype (OR = 1.53, 95% CI 1.16–2.02) and the *CYP1A1*2C* allele (OR = 2.15, 95% CI 1.36–3.39) identified individuals at increased risk of developing colorectal cancer.

Interactions between enzymes

Given that some of the genotypes tested were found to significantly influence colorectal cancer risk, we further investigated the joint effect of inheriting various genotype combinations including GSTM1 null/NAT2 slow, GSTM1 null/GSTT1 null and GSTM1 null/CYP1A1*2B (Table V). We found no evidence for significant interactions between any of the allele combinations tested.

Discussion

We have carried out a detailed and comprehensive study on the association of metabolic polymorphisms and colorectal cancer susceptibility. Epidemiological evidence suggests that specific environmental factors including dietary carcinogen exposure may be significant determinants of colorectal cancer

Table III. Taqman-based genotyping analysis

Gene Allele	Primer/probe Name	Primer and probe sequences Sequence (5′→3′)	Primer/probe concentration
CYP1A1			
E7	F	TGTTAAGTGAGAAGGTGATTATCTTTGG	300 nM
	R	GCAGGATAGCCAGGAAGAGAAAG	300 nM
	FAM	TATCGGTGAGACCATTGCCCGCT	100 nM
	VIC	TATCGGTGAGACCXTTGCCCGCT	100 nM
CYP1A1			
Msp 1	F	TGGTACCATTTTGTTTCACTGTAACC	300 nM
	R	GCGGCCCCAACTACTCAGA	900 nM
	FAM	CCACCTCCTGGGCTCACACGAT	100 nM
	VIC	CCACCTCCXGGGCTCACACGAT	100 nM
CYP2C9*3			
	F	GCCACATGCCCTACACAGATG	50 nM
	R	CATGGAGTTGCAGTGTAGGAGAAA	300 nM
	FAM	TCCAGAGATACCTTGACCTTCTCCCCA	100 nM
	VIC	TCCAGAGATACATTGACCTTCTCCCCA	100 nM
CYP2D6*3	F	AGGGCCGAGAGCATACTCG	300 nM
	R	GTCCCCGTCCTCCTGCAT	300 nM
	FAM	CAGGTCATCCTGTGCTCAGTTAGCAGC	100 nM
	VIC	CAGGTCATCCGTGCTCAGTTAGCAGC	100 nM
CYP2D6*4	F	ATGGGCAGAAGGGCACAA	300 nM
	R	AAGCCCGACTCCTCCTTCAG	900 nM
	FAM	CACCCCCAGGACGCCCCT	100 nM
	VIC	CACCCCCAAGACGCCCCT	100 nM
GSTP1			
a/b	F	CCTGGTGGACATGGTGAATG	50 nM
	R	CAACCCTGGTGCAGATGCT	300 nM
	FAM	CGCTGCAAATACATCTCCCTCATCTACA	100nM
	VIC	CGCTGCAAATACGTCTCCCTCATCTACA	100 nM
GSTP1			
a/c	F	GGGCAGTGCCTTCACATAGTC	300 nM
	R	GAGTAGGATGATACATGGTGGTGTCT	300 nM
	FAM	TCCTTGCCCGCCTCCTGC	100 nM
	VIC	TCCTTGCCCACCTCCTGC	100 nM
NAT2			
Kpn I	F	TGCATTTTCTGCTTGACAGAAGA	300 nM
	R	CTTTGGCAGGAGATGAGAATTAAGA	300 nM
	FAM	CTCTCCTGATTTGGTCCAGGTACCAGATT	100 nM
	VIC	CTCTCCTGATTTGGTCCAAGTACCAGATT	175 nM
NAT2			
Dde I	F	GGTGGGCTTCATCCTCACCTA	900 nM
	R	AGGTTTGGGCACGAGATTTCT	900 nM
	FAM	AAGTGCTGAAAAATATA	200 nM
	VIC	AAGTGCTGAGAAATATA	200 nM
NAT2			
Taq I	F	CATCTCCTGCCAAAGAAGAAACA	300 nM
	R	AAGGAACAAAATGATGTGGTTATAAATG	900 nM
	FAM	TTACGCTTGAACCTCGAACAATTGAAGATT	100 nM
	VIC	TTACGCTTGAACCTCAAACAATTGAAGATT	100 nM
NAT2			
Bam HI	F	GGAGAAATCTCGTGCCCAAA	900 nM
	R	TTAGTGAGTTGGGTGATACATACACAAG	900 nM
	FAM	AAGGGATTCATCACCAG	200 nM
	VIC	AGGGATCCATCACC	200 nM

risk. In particular, red meat consumption and dietary fat intake have been proposed to modify cancer risk. In confirmation of this hypothesis, in a recent multi-centre collaborative case–control study in three centres in the UK, we have demonstrated that red meat consumption and smoking are significantly associated with increased colorectal cancer risk (Barrett *et al.*, in press).

It is of particular interest that we did not find the major enzymes involved in the activation and disposition of HA carcinogens to be associated with altered colorectal cancer risk. In particular and in contrast to previous studies, we did not find an association with NAT2 genotype. The majority of previous studies reported an increased cancer risk for NAT2 'fast acetylators' [(e.g. Lang *et al.* (32): OR = 2.48, 95% CI 1.0–6.0; Roberts-Thomson *et al.* (33): OR = 1.9, 95% CI 1.0–3.3], although not all studies produced significant associations [e.g. Spurr *et al.* (55): OR = 1.22, 95% CI 0.7–2.3]. In addition, meta-analysis of six studies in Caucasian populations (56) gave an overall odds ratio of 1.31 (95% CI 1.08–1.59) suggesting that NAT2 genotype alone has only a small effect on colorectal cancer risk. In confirmation of this finding, a recent meta-analysis by Houlston and Tomlinson (57) found an odds ratio of 1.03 (95% CI 0.93–1.14) for the NAT2 fast acetylator genotype. It is important to note, however, that many meta-analyses have combined genotyping and phenotyping studies and have included data from different ethnic groups.

Several authors (33,58–60) have previously suggested that NAT2 fast acetylation is particularly associated with increased

Table IV. Unmatched case–control genotyping analysis (592 controls versus 490 cases)

Allele	–/– Genotype *n* (%) controls/cases	–/+ Genotype *n* (%) controls/cases	+/+ Genotype *n* (%) controls/cases	–/+ Genotype OR (95% CI)	+/+ Genotype OR (95% CI)	*P* Note
A. Cytochrome P450						
*CYP1A1*2* (m1)	**1/*1*	**1/*2*	**2/*2*			
	476 (80.4) / 389 (79.4)	111 (18.8)/90 (18.4)	5 (0.84)/11 (2.24)	0.99 (0.73–1.35)	2.69 (0.97–7.48)	0.164
	**1/*1*	**1/*2 + *2/*2*	–			
	as above	116 (19.6)/101 (20.6)	–	1.07 (0.79–1.44)	–	0.677
***CYP1A1*2B* (m2)**	****1/*1***	****1/*2B***	****2B/*2Bι***			
	539 (91.1)/415 (84.7)	**48 (8.11)/68 (13.9)**	**5 (0.84)/7 (1.43)**	**1.84 (1.25–2.72)**	**1.82 (0.61–5.46)**	**0.006[a]**
	****1/*1***	****1/*2B + *2B/*2B***	**–**			
	as above	**53 (8.95)/75 (15.3)**	**–**	**1.84 (1.27–2.67)**	**–**	**0.001**
*CYP1A2**1F	C/C	C/A	A/A			
	35 (5.90)/33 (6.73)	233 (39.3)/193 (39.4)	325 (54.8)/264 (53.9)	0.88 (0.53–1.46)	0.86 (0.52–1.42)	0.844[c]
*CYP1A2**1D	T/T	T/delT + delT/delT	–			
	510 (86.0)/430 (87.8)	83 (14.0)/60 (12.2)	–	0.86 (0.60–1.22)	–	0.397[c]
CYP1A2 *1C	G/G (only in –2464dT pos.)	G/A (only in –2464dT pos.)	A/A (only in –2464dT pos.)			
	72/51	1/9	0/0			
*CYP1B1*3*	**1/*1*	**1/*2*	**2/*2*			
	187 (31.5)/141 (28.8)	283 (47.7)/258 (52.7)	123 (20.7)/91 (18.6)	1.21 (0.92–1.59)	0.98 (0.69–1.39)	0.270[c]
*CYP2A6*2*	**1/*1*	**1/*2+*2/*2*	–			
	561 (94.6)/474 (96.7)	32 (5.40)/16 (3.27)	–	0.59 (0.32–1.08)	–	0.090[c]
CYP2A6	**1/*1*	**1/*2+*1/*3+*2/*2*	–			
	560 (94.4)/474 (3.27)	33 (5.56)/16 (3.27)	–	0.57 (0.31–1.05)	–	0.070[c]
*CYP2C9*2*	**1/*1*	**1/*2*	**2/*2*			
	442 (74.7)/376 (76.7)	138 (23.3)/102 (20.8)	12 (2.03)/12 (2.45)	0.87 (0.65–1.16)	1.18 (0.53–2.60)	0.571
*CYP2C9*3*	**1/*1*	**1/*3 + *3/*3*	–			
	517 (87.3)/422 (86.1)	75 (12.7)/68 (13.9)	–	1.11 (0.78–1.58)	–	0.559
CYP2C19*2	****1/*1***	****1/*2***	****2/*2ι***			
	423 (71.5)/385 (78.6)	**160 (27.0)/97 (19.8)**	**9 (1.52)/8 (1.63)**	**0.67 (0.50–0.89)**	**0.98 (0.39–2.48)**	**0.021**
	****1/*1***	****1/*2ι + *2/*2***	**–**			
	as above	**169 (28.6)/105 (21.4)**	**–**	**0.68 (0.52–0.90)**	**–**	**0.007**
*CYP2D6*3*	**1/*1*	**1/*3*	**3/*3*			
	566 (95.6) / 473 (96.7)	26 (4.39)/16 (3.27)	–	0.74 (0.39–1.38)	–	0.343
*CYP2D6*4*	**1/*1*	**1/*4*	**4/*4*			
	379 (64.0)/315 (64.4)	181 (30.6)/145 (29.7)	32 (5.41) /29 (5.93)	0.96 (0.74–1.26)	1.09 (0.65–1.83)	0.899
CYP2D6	Active	Deficient	–			
	554 (93.6)/456 (93.1)	38 (6.42)/34 (6.94)	–	1.09 (0.68–1.75)	–	0.733
B. Glutathione *S*-transferase						
GSTT1	**1/*1*	**1/*2*	**2/*2*			
	215 (36.3)/184 (37.6)	287 (48.4)/213 (43.5)	91 (15.4)/93 (19.0)	0.87 (0.67–1.13)	1.19 (0.84–1.69)	0.164[c]
	**1/*1 + *1/*2*	**2/*2*	–			
	502 (84.7)/397 (81.0)	91 (15.4)/93 (19.0)	–	1.29 (0.94–1.77)	–	0.113[c]
GSTM1	****1/*1ι + *1/*2***	****2/*2***	**–**			
	291 (49.1)/206 (42.0)	**302 (50.9)/284 (58.0)**	**–**	**1.33 (1.04–1.69)**	**–**	**0.021[c]**
GSTP1 $Ile_{105}Val$	Ile/Ile	Ile/Val	Val/Val			
	259 (43.8)/193 (39.4)	256 (43.2)/240 (49.0)	77 (13.0)/57 (11.6)	1.26 (0.97–1.63)	0.99 (0.67–1.47)	0.169
GSTP1 $Ala_{114}Val$	Ala/Ala	Ala/Val	Val/Val			
	479 (80.9)/401 (81.8)	102 (17.2)/84 (17.1)	11 (1.86)/5 (1.02)	0.98 (0.72–1.35)	0.54 (0.20–1.51)	0.522
C. Other genes						
NAT2	slow fast	–				
	348 (58.9)/304 (62.0)	243 (41.1)/186 (38.0)	–	0.88 (0.69–1.12)	–	0.291[d]
*NQO1*2*	Pro/Pro	Pro/Ser	Ser/Ser			
	398 (67.1)/316 (64.5)	173 (29.2)/157 (32.0)	22 (3.71)/17 (3.47)	1.14 (0.88–1.48)	0.97 (0.51–1.85)	0.592[c]
*NQO1*3*	Arg/Arg	Arg/Trp + Trp/Trp	–			
	535 (90.2)/452 (92.2)	58 (9.78)/38 (7.76)	–	0.78 (0.51–1.19)	–	0.243[c]
NQO1	fast	intermediate	slow			
	349 (58.9)/285 (58.2)	212 (35.8)/181 (36.9)	32 (5.40)/24 (4.90)	1.05 (0.81–1.35)	0.92 (0.53–1.59)	0.880[c]
*SULT1A1*2*	**1/*1*	**1/*2*	**2/*2*			
	275 (46.4)/217 (44.3)	255 (43.0)/209 (42.7)	63 (10.6)/64 (13.1)	1.04 (0.80–1.34)	1.29 (0.87–1.90)	0.444[c]
*SULT1A2*2*	**1/*1*	**1/*2*	**2/*2*			
	252 (42.5)/193 (39.4)	272(45.9)/231 (47.1)	69 (11.6)/66 (13.5)	1.11 (0.86–1.43)	1.25 (0.85–1.84)	0.485[c]
MTHFR $Ala_{222}Val$	Ala/Ala	Ala/Val	Val/Val			
	271 (45.8)/238 (48.6)	272 (46.0)/199 (40.6)	49 (8.28)/53 (10.8)	0.83 (0.65–1.07)	1.23 (0.81–1.88)	0.133
***EPHX1ι* $Tyr_{113}His$**	**Tyr/Tyr**	**Tyr/His**	**His/His**			
	270 (45.6) / 228 (46.6)	**193 (32.6)/187 (38.2)**	**129 (21.8)/74 (15.1)**	**1.15 (0.88–1.50)**	**0.68 (0.49–0.95)**	**0.012[b]**
EPHX1 $His_{139}Arg$	His/His	His/Arg	Arg/Arg			
	378 (63.7)/329 (67.1)	200 (33.7)/142 (29.0)	15 (2.53)/19 (3.88)	0.82 (0.63–1.06)	1.46 (0.74–2.88)	0.139[c]
EPHX1	Slow	Intermediate	Fast			
	246 (41.6)/197 (40.3)	240 (40.5)/208(42.5)	106 (17.9)/84 (17.2)	1.08 (0.83–1.41)	0.99 (0.70–1.39)	0.801

[a]Not in Hardy–Weinberg equilibrium ($P = 0.01$); [b]not in Hardy–Weinberg equilibrium ($P < 0.0001$), [c]$n = 593$ controls, [d]$n = 591$ controls.

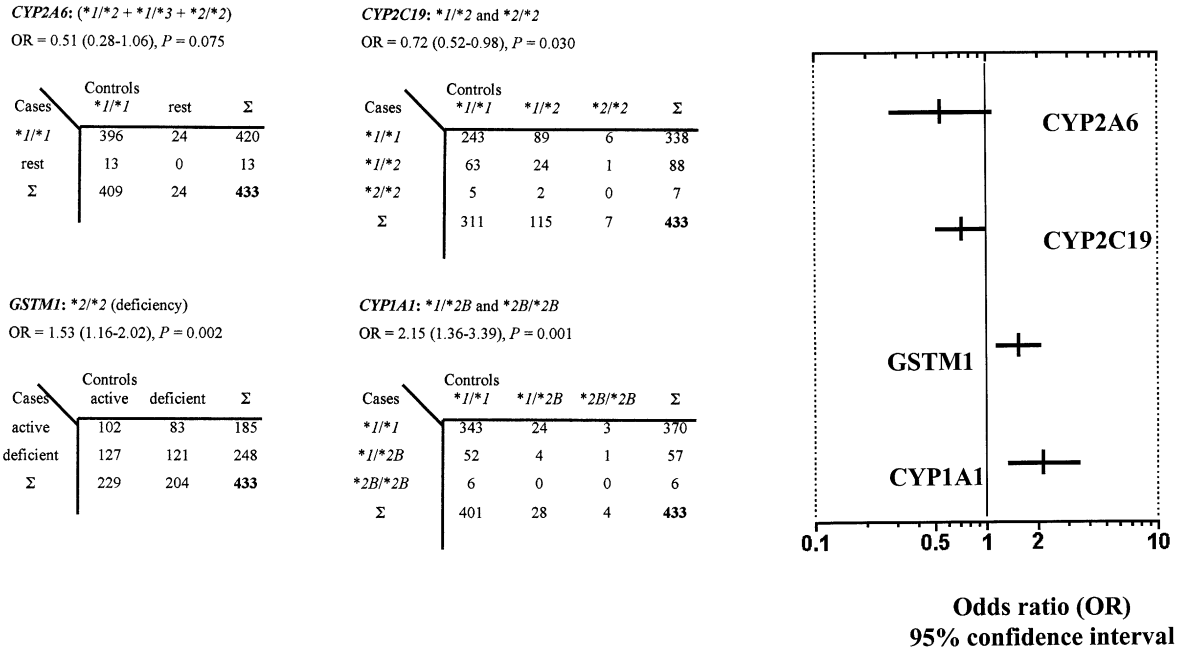

Fig. 2. Matched case-control genotyping analysis (433 case–control pairs). Only genotypes which showed an association in the unmatched analysis (i.e. with $P = 0.1$ in Table IV) were considered.

Table V. Analysis of potential interaction between susceptibility genotypes

Genotype	OR	(95% CI)	*P* for interaction
GSTM1 'deficient'	1.52	(1.15–2.01)	0.91
NAT2 'slow'	1.11	(0.84–1.46)	
GSTM1 'deficient'	1.54	(1.17–2.03)	0.94
GSTT1 'deficient'	1.27	(0.88–1.84)	
GSTM1 'deficient'	1.53	(1.16–2.03)	0.44
*CYP1A1 (*1/*2B + *2B/*2B)*	2.15	(1.36–3.41)	
NAT2 'slow'	1.10	(0.83–1.46)	0.92
*CYP1A1 (*1/*2B + *2B/*2B)*	2.13	(1.35–3.37)	

colorectal cancer risk in individuals who consume well-done meat, as a direct consequence of increased exposure to heterocyclic amine carcinogens. We have confirmed the association between red meat consumption and colorectal cancer risk in our UK population, although our data does not identify NAT2 genotype as a significant modifier of increased cancer risk, suggesting that heterocyclic amine carcinogens are not the primary causative agents (Barrett *et al.*, in press). In addition, we did not find any association between 'doneness' of meat, taken as a crude estimate of heterocyclic amine exposure and cancer risk.

CYP1A2 is another candidate gene of particular interest because of its role in the activation of heterocyclic amines. One common *CYP1A2* polymorphism (CYP1A2*F) has previously been associated with higher enzyme activity in smokers (37) suggesting that genetically determined variation in CYP1A2 expression may influence susceptibility to smoking related cancers. Recently, we reported that multiple *CYP1A2* SNPs are in linkage disequilibrium (61), although the functional effect of each of the *CYP1A2* alleles on CYP1A2 phenotype is still not clear. In this study, we screened for the most common CYP1A2 alleles, CYP1A2*1F and CYP1A2*1D, (and additionally for CYP1A2*1C in samples with CYP1A2*1D since these sites are in linkage disequilibrium), but failed to detect any influence of CYP1A2 genotype on colorectal cancer risk.

Our analysis did however identify specific alleles of *CYP1A1 (CYP1A1*2C)*, *CYP2A6 (CYP2A6*2)*, *CYP2C19* (CYP2C19*2) and *GSTM1* (GSTM1*2) as statistically significant determinants of colorectal cancer risk, confirming a role for environmental mutagens in disease etiology (Figure 2, Table IV). Interestingly, some of these alleles have been implicated in the metabolism of PAH carcinogens (Figure 1). For example, CYP1A1 activates PAH substrates to reactive epoxide intermediates, which are then detoxified by GSTM1. The GSTM1*2 allele is a gene deletion resulting in loss of enzyme function in GSTM1*2/*2 homozygotes (39). Our finding that inheritance of the GSTM1*2 allele is significantly associated with increased colorectal cancer risk [OR 1.53 (95% CI 1.16–2.02)] is consistent with our earlier study (30) which reported an odds ratio of 1.90 (95% CI 1.3–2.8).

In contrast to GSTM1, the functional consequences of the CYP1A1*2C allele, arising from an isoleucine to valine substitution in Exon 7 of CYP1A1 are less well defined. Schwarz *et al.* (40) reported that the protein encoded by the CYP1A1*2C allele had a reduced ability to form phenols and quinones as well as the carcinogenic epoxide metabolites from benzo[*a*]pyrene. In contrast, Crofts *et al.* (41) proposed an increased inducibility phenotype for *CYP1A1*2C* (but not for *CYP1A1*2A*), which may rationalize our observed increase in cancer risk, particularly in smokers. CYP1A1 has previously been considered as a susceptibility factor for several cancers (28). Although most previous studies focused on lung cancer, the CYP1A1*2C genotype was identified as a significant risk factor for colorectal cancer by Kiss *et al.* (29) (OR = 1.57, 95% CI 0.9–2.7) but not by Ishibe *et al.* (42) (OR = 1.08, 95% CI 0.65–1.8). Consistent with our data, Sivaraman *et al.* (43) reported a higher colorectal cancer risk associated with inheritance of the *CYP1A1*2* allele in a Japanese population. There was some evidence of deviation from Hardy–Weinberg equilibrium for the *CYP1A1*2C* allele in our control population

($P = 0.01$), with fewer heterozygotes observed than expected (Table IV). The reasons for this are not clear, but may be attributable to the close proximity of the nucleotide changes defining the CYP1A1*2C and CYP1A1*4 alleles which may influence PCR efficiency in the minority of individuals inheriting both alleles.

In contrast to CYP1A1, the microsomal epoxide hydrolase (mEH) $His_{113}His$ genotype was associated with a decreased colorectal cancer risk in our unmatched case–control analysis (OR = 0.68, 95% CI 0.49–0.95, $P = 0.012$), consistent with data from a recent study of 464 colorectal adenomas (44), but in contrast to the results of Harrison *et al.* (45) who described a higher colorectal cancer risk for the mEH_{His113} allele. In our population, the $mEH_{Tyr113His}$ control genotype distribution was not in Hardy–Weinberg equilibrium ($n = 592$; 45.6% Tyr/Tyr, 32.6% Tyr/His, 21.8% His/His; $P < 0.0001$). This disequilibrium of the $mEH_{Tyr113His}$ genotype has also been reported by Cortessis *et al.* (44) ($n = 277$; 50.2% Tyr/Tyr, 33.0% Tyr/His, 16.8% His/His; $P = 0.0001$), but not by Harrison *et al.* (45) ($n = 203$; 44.8% Tyr/Tyr, 48.8% Tyr/His, 6.40% His/His). Keicho *et al.* (46) suggested that this may be due to a silent polymorphism found in Japanese subjects in close proximity to $mEH_{Tyr113His}$ ($AAG_{119}AAA$) in the primer binding region of commonly used genotyping assays, which could result in anomalous genotyping results. Although plausible, this mechanism would not explain the apparent discrepancies between the studies of Harrison *et al.* (45) and Cortessis *et al.* (44), who used the same genotyping assay (47) and it remains to be determined whether the $AAG_{119}AAA$ allele exists in Caucasian populations. We did, however, observe a statistically significant difference in *mEH* allele frequencies between colorectal cancer patients and controls, indicating that inheritance of the $mEH_{Tyr113His}$ allele represents a susceptibility factor for colorectal cancer.

The CYP2A6 polymorphism is of particular relevance to cancer epidemiology as the enzyme is known to metabolize a range of carcinogens including nitrosamines and Aflatoxin B1 (48,49). Inheritance of the CYP2A6*3 allele has been proposed to influence desire to smoke (50), and may therefore be a significant determinant of cancer susceptibility in smokers. Both the population frequency and the optimal assay conditions required to uniquely identify the *CYP2A6*3* allele have been substantially revised in recent years, following the realization that published genotyping methods significantly over-estimated CYP2A6 allele frequencies. Using the revised genotyping assay of Chen *et al.* (51), *CYP2A6*3* was found in only one of 1083 subjects (allele frequency 0.046% in pooled cases and controls) in the current study. This is consistent with the data of Oscarson *et al.* (52) where *CYP2A6*3* was not found in 100 Spanish Caucasians, and suggests that routine screening for the presence of *CYP2A6*3* allele is not justified in Caucasian populations. Consistent with our own findings, *CYP2A6*2* was the only variant *CYP2A6* allele which was found in the majority of previously published studies. A very recent report, however, suggests that the most frequent variant CYP2A6 allele in Caucasian populations which may be the most significant determinant of interindividual differences in CYP2A6 activity is *CYP2A6*9*, a SNP in the TATA box motif of the *CYP2A6* promoter which has been associated with reduced CYP2A6 activity (53). Studies on the influence of this allele on colorectal cancer susceptibility are in progress.

CYP2C19 has not previously been extensively studied as a determinant of cancer susceptibility and no carcinogenic CYP2C19 substrates have been investigated or identified. There are no studies to date on the influence of *CYP2C19* genotype on colorectal cancer susceptibility; although recent data suggests an under-representation of the *CYP2C19*2* allele in bladder cancer patients (54). While the bladder cancer data was not statistically significant, it is consistent with our observation that the *CYP2C19*2* allele associated with reduced CYP2C19 activity was under-represented in colorectal cancer.

CYP2C9 genotype was not found to be a determinant of colorectal cancer susceptibility in our population. We assayed for the presence of two allelic variants of CYP2C9 – CYP2C9*2 which contains a single arginine to cysteine substitution ($Arg_{144}Cys$) and CYP2C9*3 in which an isoleucine residue is replaced by a leucine residue ($Ile_{359}Leu$) (65). Both these alleles have been associated with decreased CYP2C9 activity towards a variety of substrates including warfarin (66). Like CYP2C19, CYP2C9 is not known to metabolize carcinogens or mutagens although murine Cyp2c enzymes have been implicated in PAH metabolism (67). A recent report suggests that there may be an interaction between the *CYP2C9* and *UGT1A6* polymorphisms both of which metabolize non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and which are known to be protective against colon cancer (68).

The MTHFR $Ala_{222}Val$ polymorphism has been proposed to alter folate metabolism and, as a consequence, influence DNA synthesis and repair (24). Consistent with previous reports, we did not find an association between *MTHFR* genotype and colorectal cancer susceptibility although, interestingly, alcohol consumption has been proposed to have a modifying effect on MTHFR genotype. We are currently correlating MTHFR genotype with alcohol and folate consumption in our study population (Forman *et al.*, manuscript in preparation).

In addition, our data did not indicate that inheritance of allelic variants of *GSTT1*, *CYP1B1*, *CYP2D6*, *NQO1* and *GSTP1* influenced colorectal cancer susceptibility (Table IV). We failed to find any significant association for the *GSTT1* 'null' genotype (OR = 1.19, 95% CI 0.84–1.69 for GSTT1*2/*2 vs. GSTT1*1/*1 and OR = 1.29, 95% CI 0.94–1.77 for the 'null' vs. 'active' genotypes). This is comparable to previous reports of GSTT1 allele frequencies in colon cancer (63) (OR = 0.86, 95% CI 0.4–1.8) and colorectal cancer (64) (OR = 1.21, 95% CI 0.63–2.00), although one previous colorectal cancer study described a significantly increased cancer risk associated with the GSTT1 null genotype (31) (OR = 1.88, 95% CI 1.28–2.77). In contrast to our data, the $CYP1B1_{Leu432Val}$ polymorphism was previously associated with an increased risk of colorectal cancer (OR = 1.93, 95% CI 1.15–3.24) in a German population (69). CYP1B1 is known to be expressed in the GI tract and over-expressed in colorectal tumours (70) where one can speculate that it may contribute to local carcinogen activation. While GSTP1 and CYP2D6 genotypes have not been consistently associated with altered colorectal cancer susceptibility, the $NQO1_{C609T}$ allele has been associated with increased colorectal cancer risk (OR = 2.9, 95% CI 1.19–6.97), an association which was more pronounced in individuals with tumours containing K-ras mutations (71) (OR = 6.5, 95% CI 1.39–34.9). We have performed a detailed characterization of mutations in APC, K-ras and p53 in tumours from our cancer patients and are currently correlating genotype with tumour mutation spectra.

There are several additional drug metabolizing enzymes which have been shown to be involved in carcinogen activation

in vitro, e.g. CYP2B6, CYP3A4 and CYP3A5 (72,73), for which allelic variants have only recently been reported and detailed information on allele frequencies is not yet available. The *CYP3A4*1B* allele (an A to G substitution in the CYP3A4 gene promoter) is very rare in Caucasians; in this study, we did not find any *CYP3A4*1B* alleles in 207 samples randomly chosen from cases and controls (data not shown). Inheritance of the CYP3A4*2 allele has previously been associated with susceptibility to prostate cancer (74) and treatment-associated leukaemia (75), although the sequence change associated with the CYP3A4*2 allele was not associated with altered enzyme activity in *in vitro* experiments (76). The functional relevance of additional *CYP3A4* alleles is not yet clear. Recently, a number of CYP2B6 alleles have been described (77) but which have not yet been studied as modifiers of cancer risk.

To date, more than 25 alleles of NAT1 have been reported (78). Due to limited information about the population frequencies and functional consequences of the various NAT1 alleles, we did not include NAT1 genotyping analysis in the current study.

In addition to the sulfotransferases SULT1A1 and SULT1A2, which showed no significant association with colorectal cancer risk in our population, SULT1A3 has also been implicated in the metabolism of HA carcinogens (16). The extent to which genetic polymorphisms influence SULT1A3 activity is not yet fully understood. Similarly, Malfatti and Felton (79) have suggested a role for the UDP-glucuronosyltransferases UGT1A1, UGT1A4 and UGT1A9 in PhIP glucuronidation. The role of UDP-GT polymorphisms on cancer susceptibility will be an interesting area for further study.

Combined effects and interactions

From the pathways of HA and PAH carcinogen metabolism illustrated in Figure 1, one might predict that inheritance of various allele combinations would lead to an increased cancer risk. From our data, assuming no interaction between genotypes, the theoretical maximum combined odds ratio for the *GSTM1* 'deficient' plus (*CYP1A1*1/*2B* or **2B/*2B*) genotype compared with the baseline *GSTM1* 'active' plus *CYP1A1*1/*1* would be 3.30 [1.53 (CYP1A1) × 2.15 (GSTM1)]. To investigate whether such combined effects occurred, we tested several interactions between the different susceptibility genotypes (Table V). Interactions were chosen based on the metabolic pathways of HA and PAH carcinogens and were genotype combinations which had previously been investigated in other epidemiological studies or were suggested by *in vitro* studies. For example, Vaury *et al.* (80) described increased CYP1A1 inducibility in *GSTM1* deficient compared with *GSTM1* active cell lines. In contrast, however, we found no evidence for significant interactions between the various susceptibility genotypes tested in our study population. Recently, a further synergistic interaction of the GSTM1 null allele *GSTM1*2*/*2 with the GSTM3**A/*B* allele (a 3 bp deletion in intron 6) has been proposed (81).

In conclusion, therefore, we have performed a comprehensive study of the influence of inheriting various alleles of HA and PAH carcinogen metabolizing enzymes on susceptibility to colorectal cancer and have shown that inheritance of the cytochrome P450 *CYP1A1*2B* allele or the glutathione *S*-transferase *GSTM1*2/*2* 'null' allele confers an increased risk of disease, while inheritance of the *CYP2C19*2*, *CYP2A6*2* or *EPHX1* $His_{113}His$ alleles appears to be protective.

Certain of these data are novel and others are consistent with previous reports. Where our results contradict previous findings, this could indicate differences in environmental or dietary factors between study groups.

The influence of specific dietary and lifestyle factors (e.g. smoking and red meat consumption) on the development of colorectal cancer and the interaction of these factors with susceptibility genotypes has been proposed to be a critical determinant of colorectal cancer susceptibility (33). In order to address these issues, we are currently correlating the genotyping data presented here with individual dietary and environmental carcinogen exposures.

Acknowledgements

All authors wish to acknowledge the magnificent efforts of our team of research nurses in Dundee, Leeds and York who were responsible for all patient and control recruitment. We are indebted to all general practitioners who allowed us to approach their patients in order to recruit our control populations. We acknowledge financial support from the Food Standards Agency (Contracts T01003, TO1004 and T01005).

References

1. Parkin,D.M., Whelan,S.L., Ferlay,J., Raymond,L. and Young,J. (eds). (1997) *Cancer Incidence in Five Continents*. IARC Scientific Publications vol VII, 143 pp. IARC, Lyon.
2. Le Marchand,L. (1999) Combined influence of genetic and dietary factors on colorectal cancer incidence in Japanese Americans. *J. Natl Cancer Inst. Monogr.*, **26**, 101–105.
3. Bartsch,H., Nair,J. and Owen,R.W. (1999) Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. *Carcinogenesis*, **20**, 2209–2218.
4. Giovannucci,E., Rimm,E.B., Stampfer,M.J., Colditz,G.A., Ascherio,A. and Willett,W.C. (1994) Intake of fat, meat and fibre in relation to risk of colon cancer in men. *Cancer Res.*, **54**, 2390–2397.
5. de Verdier,M., Hagman,U., Peters,R.K., Steineck,G. and Overvik,E. (1991) Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm. *Int. J. Cancer*, **49**, 520–525.
6. Nagao,M. and Sugimura,T. (1993) Carcinogenic factors in food with relevance to colon cancer development. *Mutat. Res.*, **90**, 43–51.
7. Kadlubar,F., Kaderlik,R.K., Mulder,G.J., *et al.* (1995) Metabolic activation and DNA adduct detection of PhIP in dogs, rats and humans in relation to urinary bladder and colon carcinogenesis. *Princess Takamatsu Symp.*, **23**, 207–213.
8. Sugimura,T. (2000) Nutrition and dietary carcinogens. *Carcinogenesis*, **21**, 387–395.
9. Kadlubar,F.F., Butler,M.A., Kaderlik,K.R., Chou,H.C. and Lang,N.P. (1992) Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. *Environ. Health Perspect.*, **98**, 69–74.
10. Dennis,M.J., Massey,R.C., Cripps,G., Venn,I., Howarth,N. and Lee,G. (1991) Factors affecting the polycyclic aromatic hydrocarbon content of cereals, fats and other food products. *Food Addit. Contam.*, **8**, 517–530.
11. Sugimura,T. and Sato,S. (1983) Mutagens–carcinogens in foods. *Cancer Res.*, **43**, 2415–2421.
12. DeMarini,D.M., Shelton,M.L. and Levine,J.G. (1995) Mutation spectrum of cigarette smoke condensate in Salmonella: comparison to mutations in smoking-associated tumors. *Carcinogenesis*, **16**, 2535–2542.
13. Boobis,A.R., Gooderham,N.J., Edwards,R.J., Murray,S., Lynch,A.M., Yadollahi-Farsani,M. and Davies,D.S. (1996) Enzymic and interindividual differences in the human metabolism of heterocyclic amines. *Arch. Toxicol.*, **18**, 286–302.
14. Gooderham,N.J., Murray,S., Lynch,A.M., *et al.* (1996) Heterocyclic amines: evaluation of their role in diet associated human cancer. *Br. J. Clin. Pharmacol.*, **42**, 91–98.
15. Crofts,F.G., Sutter,T.R. and Strickland,P.T. (1998) Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by human cytochrome P4501A1, P4501A2 and P4501B1. *Carcinogenesis*, **19**, 1969–1973.
16. Yamazoe,Y., Nagata,K., Yoshinari,K., Fujita,K., Shiraga,T. and Iwasaki,K. (1999) Sulfotransferase catalyzing sulfation of heterocyclic amines. *Cancer Lett.*, **143**, 103–107.
17. Gooderham,N.J., Murray,S., Lynch,A.M., Yadollahi-Farsani,M., Zhao,K., Boobis,A.R. and Davies,D.S. (2001) Food-derived heterocyclic amine mutagens: variable metabolism and significance to humans. *Drug Metab. Dispos.*, **29**, 529–534.

18. Shimada,T., Hayes,C.L., Yamazaki,H., Amin,S., Hecht,S.S., Guengerich,F.P. and Sutter,T.R. (1996) Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res*., **56**, 2979–2984.
19. Oda,Y., Aryal,P., Terashita,T., Gillam,E.M., Guengerich,F.P. and Shimada,T. (2001) Metabolic activation of heterocyclic amines and other procarcinogens in Salmonella typhimurium umu tester strains expressing human cytochrome P4501A1, 1A2, 1B1, 2C9, 2D6, 2E1 and 3A4 and human NADPH-P450 reductase and bacterial O-acetyltransferase. *Mutat. Res*., **492,** 81–90.
20. Yang,S.K. (1988) Stereoselectivity of cytochrome P-450 isoenzymes and epoxide hydrolase in the metabolism of polycyclic aromatic hydrocarbons. *Biochem. Pharmacol*., **37**, 61–70.
21. Joseph,P. and Jaiswal,A.K. (1994) NAD (P)H:quinone oxidoreductase1 (DT diaphorase) specifically prevents the formation of benzo[*a*]pyrene quinone-DNA adducts generated by cytochrome P4501A1 and P450 reductase. *Proc. Natl Acad. Sci. USA*, **91**, 8413–8417.
22. Giovannucci,E., Stampfer,M.J., Colditz,G.A., Rimm,E.B., Trichopoulos,D., Rosner,B.A., Speizer,F.E. and Willett,W.C. (1993) Folate, methionine and alcohol intake and risk of colorectal adenoma. *J. Natl Cancer Inst*., **85**, 875–884.
23. Giovannucci,E., Rimm,E.B., Ascherio,A., Stampfer,M.J., Colditz,G.A. and Willett,W.C. (1995) Alcohol, low-methionine–low-folate diets and risk of colon cancer in men. *J. Natl Cancer Inst.*, **87**, 265–273.
24. Chen,J., Giovannucci,E., Kelsey,K., Rimm,E.B., Stampfer,M.J., Colditz,G.A., Spiegelman,D., Willett,W.C. and Hunter,D.J. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res*., **56**, 4862–4864.
25. Ulrich,C.M., Kampman,E., Bigler,J., *et al.* (2000) Lack of association between the C677T MTHFR polymorphism and colorectal hyperplastic polyps. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 427–433.
26. Idle,J.R., Armstrong,M., Boddy,A.V., *et al.* (1992) The pharmacogenetics of chemical carcinogenesis. *Pharmacogenetics*, **2**, 246–258.
27. Wolf,C.R., Smith,C.A. and Forman,D. (1994) Metabolic polymorphisms in carcinogen metabolising enzymes and cancer susceptibility. *Br. Med. Bull.,* **50**, 718–731.
28. Vineis,P., Malats,N., Lang,M., d'Errico,A., Caporaso,N., Cuzick,J. and Bofetta,P. (eds). (1999) *Metabolic Polymorphisms and Susceptibility to Cancer.* IARC Scientific Publications, vol 148. IARC, Lyon.
29. Kiss,I., Sandor,J., Pajkos,G., Bogner,B., Hegedus,G. and Ember,I. (2000) Colorectal cancer risk in relation to genetic polymorphism of cytochrome P450 1A1, 2E1 and glutathione-*S*-transferase M1 enzymes. *Anticancer Res*., **20**, 519–522.
30. Zhong,S., Wyllie,A.H., Barnes,D., Wolf,C.R. and Spurr,N.K. (1993) Relationship between the GSTM1 polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis*, **14**, 1821–1824.
31. Deakin,M., Elder,J., Hendrickse,C., *et al.* (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.
32. Lang,N.P., Chu,D.Z., Hunter,C.F., Kendall,D.C., Flammang,T.J. and Kadlubar,F.F. (1986) Role of aromatic amine acetyltransferase in human colorectal cancer. *Arch. Surg*., **121**, 1259–1261.
33. Roberts-Thomson,I.C., Ryan,P., Khoo,K.K., Hart,W.J., McMichael,A.J. and Butler,R.N. (1996) Diet, acetylator phenotype and risk of colorectal neoplasia. *Lancet*, **347**, 1372–1374.
34. Kampman,E., Slattery,M.L., Bigler,J., Leppert,M., Samowitz,W., Caan,B.J. and Potter,J.D. (1999) Meat consumption, genetic susceptibility and colon cancer risk: a United States multicenter case–control study. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 15–24.
35. Nakajima,M., Yokoi,T., Mizutani,M., Shin,S., Kadlubar,F.F. and Kamataki,T. (1994) Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 413–421.
36. Nakajima,M., Yokoi,T., Mizutani,M., Kinoshita,M., Funayama,M. and Kamataki,T. (1999) Genetic polymorphism in the 5′-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. *J. Biochem.,* (Tokyo) **125**, 803–808.
37. Sachse,C., Brockmöller,J., Bauer,S. and Roots,I. (1999) Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 1A2 (CYP1A2) gene tested with caffeine. *Br. J. Clin. Pharmacol.,* **47**, 445–449.
38. Huang,J.D., Guo,W.C., Lai,M.D., Guo,Y.L. and Lambert,G.H. (1999) Detection of a novel cytochrome P-450 1A2 polymorphism (F21L) in *Chinese. Drug Metab. Dispos*., **27**, 98–101.
39. Ketterer,B., Harris,J.M., Talaska,G., Meyer,D.J., Pemble,S.E., Taylor,J.B., Lang,N.P. and Kadlubar,F.F. (1992) The human glutathione *S*-transferase supergene family, its polymorphism and its effects on susceptibility to lung cancer. *Environ. Health Perspect*., **98**, 87–94.
40. Schwarz,D., Kisselev,P., Cascorbi,I., Schunck,W.H. and Roots,I. (2001) Differential metabolism of benzo[*a*]pyrene and benzo[*a*]pyrene-7,8-dihydrodiol by human CYP1A1 variants. *Carcinogenesis*, **22**, 453–459.
41. Crofts,F., Taioli,E., Trachman,J., Cosma,G.N., Currie,D., Toniolo,P. and Garte,S.J. (1994) Functional significance of different human CYP1A1 genotypes. *Carcinogenesis*, **15**, 2961–2963.
42. Ishibe,N., Stampfer,M., Hunter,D.J., Hennekens,C. and Kelsey,K.T. (2000) A prospective study of cytochrome P450 1A1 polymorphisms and colorectal cancer risk in men. *Cancer*, *Epidemiol. Biomarkers Prev*., **9**, 855–856.
43. Sivaraman,L., Leatham,M.P., Yee,J., Wilkens,L.R., Lau,A.F. and Le Marchand,L. (1994) CYP1A1 genetic polymorphisms and in situ colorectal cancer. *Cancer Res*., **54**, 3692–3695.
44. Cortessis,V., Siegmund,K., Chen,Q., *et al.* (2001) A case–control study of microsomal epoxide hydrolase, smoking, meat consumption, glutathione *S*-transferase M3 and risk of colorectal adenomas. *Cancer Res*., **61**, 2381–2385.
45. Harrison,D.J., Hubbard,A.L., MacMillan,J., Wyllie,A.H. and Smith,C.A.D. (1999) Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. *Br. J. Cancer*, **79**, 168–171.
46. Keicho,N., Emi,M., Kajita,M., Matsushita,I., Nakata,K., Azuma,A., Ohishi,N. and Kudoh,S. (2001) Overestimated frequency of a possible emphysema-susceptibility allele when microsomal epoxide hydrolase is genotyped by the conventional polymerase chain reaction-based method. *J. Hum. Genet*., **46**, 96–98.
47. Hassett,C., Aicher,L., Sidhu,J.S. and Omiecinski,C.J. (1994) Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum. Mol. Genet.,* **3**, 421–428.
48. Forrester,L.M., Neal,G.E., Judah,D.J., Glancey,M.J. and Wolf,C.R. (1990) Evidence for involvement of multiple forms of cytochrome P-450 in aflatoxin B1 metabolism in human liver. *Proc. Natl Acad. Sci. USA*, **87**, 8306–8310.
49. Yamazaki,H., Inui,Y., Yun,C.H., Guengerich,F.P. and Shimada,T. (1992) Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of *N*-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. *Carcinogenesis*, **13**, 1789–1794.
50. Tyndale,R.F. and Sellers,E.M. (2001) Variable CYP2A6-mediated nicotine metabolism alters smoking behavior and risk. *Drug Metab. Dispos*., **29**, 548–552.
51. Chen,G.F., Tang,Y.M., Green,B., Lin,D.X., Guengerich,F.P., Daly,A.K., Caporaso,N.E. and Kadlubar,F.F. (1999) Low frequency of CYP2A6 gene polymorphism as revealed by a one-step polymerase chain reaction. *Pharmacogenetics*, **9**, 327–332.
52. Oscarson,M., McLellan,R.A., Gullsten,H., Agundez,J.A., Benitez,J., Rautio,A., Raunio,H., Pelkonen,O. and Ingelman-Sundberg,M. (1999) Identification and characterisation of novel polymorphisms in the CYP2A locus: implications for nicotine metabolism. *FEBS Lett*., **460**, 321–327.
53. Pitarque,M., von Richter,O., Oke,B., Berkkan,H., Oscarson,M. and Ingelman-Sundberg,M. (2001) Identification of a single nucleotide polymorphism in the tata box of the CYP2A6 gene: impairment of its promoter activity. *Biochem. Biophys. Res. Commun*., **284**, 455–460.
54. Brockmöller,J., Cascorbi,I., Kerb,R. and Roots,I. (1996) Combined analysis of inherited polymorphisms 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 Res*., **56**, 3915–3925.
55. Spurr,N.K., Gough,A.C., Chinegwundoh,F.I. and Smith,C.A. (1995) Polymorphisms in drug-metabolizing enzymes as modifiers of cancer risk. *Clin. Chem*., **41**, 1864–1869.
56. D'Errico,A., Malats,N., Vineis,P. and Bofetta,P. (1999) Review of studies of selected metabolic polymorphisms and cancer. In: Vineis,P. *et al.* (eds). *Metabolic Polymorphisms and Susceptibility to Cancer.* IARC Scientific Publications, vol. 148, Chapter 23, pp. 323–393. IARC, Lyon.
57. Houlston,R.S. and Tomlinson,I.P.M. (2001) Polymorphisms and colorectal tumor risk. *Gastroenterology*, **121**, 282–301.
58. Lang,N.P., Butler,M.A., Massengill,J., Lawson,M., Stotts,R.C., Hauer-Jensen,M. and Kadlubar,F.F. (1994) Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiol. Biomarkers Prev*., **3**, 675–682.
59. Welfare,M.R., Cooper,J., Bassendine,M.F. and Daly,A.K. (1997) Relationship between acetylator status, smoking and diet and colorectal cancer risk in the north-east of England. *Carcinogenesis*, **18**, 1351–1354.

60. Chen,J., Stampfer,M.J., Hough,H.L., Garcia-Closas,M., Willett,W.C., Hennekens,C.H., Kelsey,K.T. and Hunter,D.J. (1998) A prospective study of *N*-acetyltransferase genotype, red meat intake and risk of colorectal cancer. *Cancer Res.*, **58**, 3307–3311.
61. Sachse,C., Chana,U., Smith,G., Lightfoot,T.J., Barrett,J., Scollay,J., Boobis,A.R., Wolf,C.R. and Gooderham,N.J. (2002) Polymorphisms of the cytochrome P450 1A2 gene (CYP1A2): frequency in colorectal cancer cases and controls, linkage disequilibrium and functional impact on caffeine metabolism. Submitted to the *British Journal of Clinical Pharmacology.*
62. Sprenger,R., Schlagenhaufer,R., Kerb,R., Bruhn,C., Brockmöller,J., Roots,I. and Brinkmann,U. (2000) Characterization of the glutathione *S*-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype–phenotype correlation. *Pharmacogenetics*, **10**, 557–565.
63. Chenevix-Trench,G., Young,J., Coggan,M. and Board,P. (1995) Glutathione *S*-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. *Carcinogenesis*, **16**, 1655–1657.
64. Welfare,M., Adeokun,A.M., Bassendine,M.F. and Daly,A.K. (1999) Polymorphisms in GSTP1, GSTM1 and GSTT1 and susceptibility to colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 289–292.
65. Stubbins,M.J., Harries,L.W., Smith,G., Tarbit,M.H. and Wolf,C.R. (1996) Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics,* **6**, 429–439.
66. Aithal,G.P., Day,C.P., Kesteven,P.J. and Daly,A.K. (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet*, **353**, 717–719.
67. Meehan,R.R., Speed,R.M., Gosden,J.R., *et al.* (1988) Chromosomal organization of the cytochrome P450-2C gene family in the mouse: a locus associated with constitutive aryl hydrocarbon hydroxylase. *Proc. Natl Acad. Sci. USA*, **85**, 2662–2666.
68. Bigler,J., Whitton,J., Lampe,J.W., Fosdick,L., Bostick,R.M. and Potter,J.D. (2001) CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. *Cancer Res.*, **61**, 3566–3569.
69. Fritsche,E., Bruning,T., Jonkmanns,C., Ko,Y., Bolt,H.M. and Abel,J. (1999) Detection of cytochrome P450 1B1 Bfr I polymorphism: genotype distribution in healthy German individuals and in patients with colorectal carcinoma. *Pharmacogenetics*, **9**, 405–408.
70. Murray,G.I., Taylor,M.C., McFadyen,M.C., McKay,J.A., Greenlee,W.F., Burke,M.D. and Melvin,W.T. (1997) Tumor-specific expression of cytochrome P450 CYP1B1 *Cancer Res.*, **57**, 3026–3031.
71. Lafuente,M.J., Casterad,X., Trias,M., Ascaso,C., Molina,R., Ballesta,A., Zheng,S., Wiencke,J.K. and Lafuente,A. (2000) NAD (P)H:quinone oxidoreductase-dependent risk for colorectal cancer and its association with the presence of K-ras mutations in tumors. *Carcinogenesis*, **21**, 1813–1819.
72. Code,E.L., Crespi,C.L., Penman,B.W., Gonzalez,F.J., Chang,T.K. and Waxman,D.J. (1997) Human cytochrome P4502B6: interindividual hepatic expression, substrate specificity and role in procarcinogen activation. *Drug Metab. Dispos.*, **25**, 985–993.
73. Kuehl,P., Zhang,J., Lin,Y., *et al.* (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat. Genet*, **27**, 383–391.
74. Rebbeck,T.R., Jaffe,J.M., Walker,A.H., Wein,A.J. and Malkowicz,S.B. (1998) Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. *J. Natl Cancer Inst.*, **90**, 1225–1229.
75. Felix,C.A., Walker,A.H., Lange,B.J., *et al.* (1998) Association of CYP3A4 genotype with treatment-related leukaemia *Proc. Natl Acad. Sci. USA*, **95,** 13176–13181.
76. Westlind,A., Lofberg,L., Tindberg,N. Andersson,T.B. and Ingelman-Sundberg,M. (1999) Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5′-upstream regulatory region. *Biochem. Biophys. Res. Commun.*, **259**, 201–205.
77. Lang,T., Klein,K., Fischer,J., Nussler,A.K., Neuhaus,P., Hofmann,U., Eichelbaum,M., Schwab,M. and Zanger,U.M. (2001) Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics*, **11**, 399–415.
78. Hein,D.W., Grant,D.M. and Sim,E. (2000) Update on consensus arylamine *N*-acetyltransferase gene nomenclature. *Pharmacogenetics*, **10**, 291–292.
79. Malfatti,M.A. and Felton,J.S. (2001) *N*-glucuronidation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and *N*-hydroxy-PhIP by specific human UDP-glucuronosyltransferases. *Carcinogenesis*, **22**, 1087–1093.
80. Vaury,C., Laine,R., Noguiez,P., de Coppet,P., Jaulin,C., Praz,F., Pompon,D. and Gueret,M. (1995) Human glutathione *S*-transferase M1 null genotype is associated with a high inducibility of cytochrome P450 1A1 gene transcription. *Cancer Res.*, **55**, 5520–5523.
81. Loktionov,A., Watson,M.A., Gunter,M., Stebbings,W.S., Speakman,C.T. and Bingham,S.A. (2001) Glutathione-S-transferase gene polymorphisms in colorectal cancer patients: interaction between GSTM1 and GSTM3 allele variants as a risk-modulating factor. *Carcinogenesis*, **22**, 1053–1060.

Received May 23, 2002; revised July 11, 2002; accepted July 12, 2002