Polymorphisms and Colorectal Tumor Risk

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Background & Aims: **Increasingly, studies of the relationship between common genetic variants and colorectal tumor risk are being proposed. To assess the evidence that any of these confers a risk, a systematic review and meta-analysis of published studies was undertaken.** ***Methods:*** **Fifty studies of the effect of common alleles of 13 genes on risk were identified. To clarify the impact of individual polymorphisms on risk, pooled analyses were performed.** ***Results:*** **Of the 50 studies identified, significant associations were seen in 16, but only 3 were reported in more than one study. Pooling studies, significant associations were only seen for 3 of the polymorphisms: adenomatosis polyposis coli (*APC*)-I1307K (odds ratio [OR] = 1.58, 95% confidence interval [CI]: 1.21–2.07); Harvey ras-1 variable number tandem repeat polymorphism (*HRAS1*-VNTR; OR = 2.50, 95% CI: 1.54–4.05); and methylenetetrahydrofolate reductase (*MTHFR*)$^{Val/Val}$ (OR = 0.76, 95% CI: 0.62–0.92). For tumor protein 53 (*TP53*), *N*-acetyl transferase 1 (*NAT1*), *NAT2*, glutathione-*S* transferase Mu (*GSTM1*), glutathione-*S* transferase Theta (*GSTT1*), and glutathione-*S* transferase Pi (*GSTP1*) polymorphisms, the best estimates are sufficient to exclude a 1.7-fold increase in risk of colorectal cancer.** ***Conclusions:*** ***APC*-I1307K, *HRAS1*-VNTR, and *MTHFR* variants represent the strongest candidates for low penetrance susceptibility alleles identified to date. Although their genotypic risks are modest, their high frequency in the population implies that they may well have considerable impact on colorectal cancer incidence. Determining precise risk estimates associated with other variants and gene-gene and gene-environment interactions will be contingent on further studies with sample sizes larger than typically used to date.**

After lung and breast cancer, colorectal cancer is the most common cause of death from malignant disease in Western countries. In England and Wales, there are approximately 30,000 new cases each year resulting in 17,000 deaths.[1,2] Worldwide, it has been estimated there are at least half a million new cases of colorectal cancer each year.[3] Although about 10% of colorectal cancer cases have some family history of the disease, only 2% to 6% can be ascribed to the rare, highly penetrant mutations in genes such as adenomatosis polyposis coli (*APC*), *MLH1*, and *MSH2*.[4–6] First-degree relatives of colorectal cancer cases have a twofold increase in risk compared with the general population,[7] most of which cannot be accounted for by *APC*, *MSH2*, or *MLH1*. Although part of the familial risk of colorectal cancer may be attributable to shared environment, there may be common, low penetrance genetic variants conferring susceptibility to colorectal cancer. Highly penetrant mutations in *APC* or the mismatch repair genes may cause a substantial proportion of colorectal cancers at young ages, but they are unlikely to be responsible for a high proportion of all cases. However, it is possible that low penetrance polymorphisms could do so.[7] It is difficult or impossible to identify such gene polymorphisms by linkage analysis, because the number of affected relative pairs required will be prohibitively large.[8] The commonest method for identifying common low penetrance alleles is through association studies. These are based on comparing the frequency of polymorphic genotypes in cases and controls. Alleles positively associated with the disease are analogous to risk factors in epidemiology and may be causally related to disease risk or in linkage disequilibrium with disease-causing variants (i.e., certain haplotype combinations of alleles at different loci occur more frequently than would be expected from random association).

There are a number of different methods of analyzing the risk associated with a specific variant.[9] For simple biallelic polymorphisms, the odds ratio (OR) of colorectal cancer can be derived by comparing allele frequencies in cases and controls. This approach is, however, less

Abbreviations used in this paper: ***APC***, adenomatosis polyposis coli; ***APO-E***, apolipoprotein-*E*; CI, confidence interval; ***CYP1A1***, cytochrome P450; FAP, familial adenomatous polyposis; ***GSTM1***, glutathione-*S* transferase Mu; ***GSTT1***, glutathione-*S* transferase Theta; ***HRAS1***-VNTR, Harvey ras-1 variable number tandem repeat polymorphism; ***mEPHX***, microsomal epoxide hydroxylase; methyl-THF, 5-methyltetrahydrofolate; ***MTHFR***, methylene tetrahydrofolate reductase; ***NAT***, *N*-acetyl transferase; OR, odds ratio; TNF, tumor necrosis factor; ***TP53***, tumor protein 53.

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powerful than a comparison of frequencies of the 3 genotypes among cases and controls using homozygosity of the "wild type allele" as the reference group. Where homozygotes are rare, it is common to combine the heterozygotes and homozygotes together, but this is only appropriate if a dominant model can be presumed. Similarly, combining heterozygotes with wild-type homozygotes is only appropriate if alleles act recessively. For multi-allelic loci, the frequency of certain genotypes can be low and the chi-squared statistics produced from analyses of such tables do not conform well to the asymptotic distribution. It is therefore common to group certain alleles together and analyze as a bi-allelic polymorphism. These groups are ideally defined on the basis of expressed phenotypes.

Most colorectal cancers develop from normal epithelium through sequentially worsening degrees of adenomatous dysplasia.[10] The molecular basis for this sequence is reasonably well established, requiring a series of at least 5 mutations.[10,11] Within this pathway, there are a number of different mechanisms by which low penetrance polymorphisms could either contribute directly to tumorigenesis or facilitate the acquisition of mutations, so affecting progression of normal colonic epithelium to carcinoma. The candidate genes that have been evaluated to date can be broadly delineated into those coding for: carcinogen metabolism enzymes, methylation enzymes, DNA repair proteins, microenvironmental modifiers, oncogenes and tumor suppressors, and others. Table 1 details the candidate gene polymorphisms that have been reviewed. For clarity, the position and standard nomenclature of each polymorphism is given.

Carcinogen Metabolism Genes

Colonic crypt cells express several of the important xenobiotic metabolizing enzymes. Hence, cancer susceptibility may result from differences in the expression of enzymes involved in the metabolism of aromatic and heterocyclic amines present in food.

The first and obligatory step in the activation of arylamines is *N*-hydroxylation by phase-I enzymes that include cytochrome P450A1 (*CYP1A1*). *CYP1A1* is induced by, and acts on, carcinogens found in tobacco smoke.[12,13]

The *N*-acetyl transferases, NAT1 and NAT2, are phase-II metabolizing enzymes detoxifying arylamines, some of which are derived from cooked meat. The action of NATs on these carcinogens can generate electrophilic ions capable of inducing DNA point mutations. The capacity to perform *N*-acetylation of arylamine chemicals is subject to extensive genetic variation. *NAT2* is responsible for inherited interindividual differences in the ability to acetylate certain drugs.[12,14] Slow acetylators are homozygous for low-activity alleles, and fast acetylators carry one or more high activity alleles. Variation in *NAT1* and *NAT2* may influence the local presentation of carcinogens such as arylamines found in cooked meat, to crypt cells. Studies in rats provide some support for this hypothesis, with carcinogen-induced aberrant intestinal crypt formation being highest in rapid acetylators.[15]

The glutathione-*S* transferases are a family of phase-II enzymes responsible for the detoxification of mutagenic electrophiles including polyaromatic hydrocarbons. Homozygotes for null alleles (deletion) of glutathione-*S* transferase Mu (*GSTM1*) and glutathione-*S* transferase Theta (*GSTT1*) have absent activity of the respective enzyme. DNA adduct formation and rates of somatic mutation have been reported to be increased in carriers of null alleles.[12,16]

Other metabolic genes that have been investigated as risk factors for colorectal cancer include microsomal epoxide hydroxylase (*mEPHX*),[17] involved in the detoxification of carcinogenic epoxides and steroidogenesis.[18] Two polymorphisms appear to affect enzyme activity.[18]

Methylation Genes

Global and gene-specific anomalies of DNA methylation contribute to loss of proto-oncogene and tumor suppressor expression. In colorectal cancer, this occurs during progression of adenomas to carcinomas.[19,20] There is some evidence that DNA methylation can be influenced by manipulating the availability of methyl group donors, such as folate.[21] Folate levels are influenced by 2 enzymes: 5,10-methylenetetrahydrofolate reductase (*MTHFR*) catalyzing the conversion of 5,10-methylene-tetrahydrofolate (required for purine and thymidine synthesis), to 5-methyltetrahydrofolate (methyl-THF), the primary circulatory form of folate necessary for methionine synthesis; and methionine synthetase (*MTR*) catalyzing transfer of a methyl group from methyl-THF to homocysteine. The Ala667Val polymorphism of *MTHFR* codes for an enzyme with reduced activity—homozygotes have about 30% of the normal enzyme activity and decreased levels of methyl-THF.[22] Although decreased levels of methyl-THF may adversely affect DNA methylation contributing to carcinogenesis, depletion of methylene-tetrahydrofolate interferes with thymidylate biosynthesis leading to deoxynucleotide pool imbalances. An accumulation of deoxyuridylate DNA and subsequent removal of this abnormal base may "liabilize" DNA to strand breaks.

Table 1. Polymorphisms Studied as Risk Factors for Colorectal Neoplasia

Class/gene	Nucleotide change	Amino acid change—nomenclature[a]	Effect on function	Method of detection
Carcinogen metabolism				
CYP1A1	A4889G (exon 7)	Ile462Val	?Increased activity	Creates *Nco*I site
	$T^{6235}C$ (3′ UTR)	None	?None	Creates *Msp*I site
NAT1	A1088T	None (*NAT1*10*)	?Increased activity	Allele-specific PCR
	C559T	Arg187Stop (*NAT1*14*)	Low activity allele	
	G560A	Arg187Gln (*NAT1*15*)	Low activity allele	
	C190T	Arg64Trp (*NAT1*17*)	Low activity allele	
	C97T	Arg33Stop (*NAT1*19*)	Low activity allele	
	A72T	Asp251Val (*NAT1*22*)	Low activity allele	
*NAT2**	C341T	Ile114Thr (*NAT2*5*)	Low activity allele	Abolishes *Kpn*I site
	G590A	Arg197Gln (*NAT2*6*)	Low activity allele	Abolishes *Taq*I site
	G857A	Gly286Glu (*NAT2*7*)	Low activity allele	Abolishes *Bam*HI site
	G191A	Arg64Gln (*NAT2*14*)	Low activity allele	Creates *Msp*I site (Metabolism of probe drug [Sulphamethiaszine, Dapsone Isoniazid])
GSTM1	Deletion	None (Null)	Absent activity	Allele-specific PCR GST activity GST immunoreactivity
GSTT1	Deletion	None (Null)	Absent activity	Allele-specific PCR
GSTP1	A1578G	Ile105Val (I_{105})	Reduced activity	Creates *Alw*261 site
	C2293T	Ala114Val (V_{114})		Creates *Aci*I site
mEPHX	Exon 3 T → C	Try113His	? Reduced activity	Abolishes *Eco*RV site
	Exon 4 A → G	His139Arg	? Increased activity	Creates *Rsa*I site
Methylation				
MTHFR	C677T	Ala667Val	Reduced activity	Creates *Hinf*I site
MTR	A2756G	Asp919Gly		Creates *Hae*III site
Microenvironmental modifiers				
PLA2G2A	Exon 1-5′ UTR C →G	None	Unlikely	SSCP
	Exon 3G → C	None	Unlikely	SSCP
APO-E		Cys112Arg (E4)	Altered lipoprotein metabolism	Allele-specific PCR, isoelectric focusing
Tumor suppressors/oncogenes				
APC	T3920A	Ileu1307Lys (I1307K) Gln1317Glu (E1317Q)	? Increased somatic Mutation	ARMS-PCR
	G3949C		Unknown	Creates *Pvu*II site
HRAS1	28-bp VNTR	None	? Altered transcription	Southern blot, PCR
TP53	Exon 3 G → C	Arg72Pro	Unknown	Allele-specific PCR
	Intron 3 16-bp dup		Unlikely	
	Intron 6 G → C		Unlikely	
MLH1	C2006T (Intron 12)	None	Unknown	ARMS-PCR
Other genes				
TNF-α	−380G → A	None	? Increased basal and	Creates *Nco*I site
TNF-β	−238G → A	None	Inducible TNF levels	Creates *Nco*I site

PCR, polymerase chain reaction; GST, glutathione-*S* transferase; SSCP, single-strand conformation polymorphism; ARMS, amplification refractory mutation system.

[a]For NAT2, only changes from the wild-type with functional significance are indicated. The substitutions at positions 191, 341, 590, and 857 are diagnostic for defective NAT2 function and hence, slow acetylator phenotype.

Microenvironmental Modifiers

Mutation of *PLA2G2A*, the secretory phospholipase A2 gene, dramatically increases the number of intestinal polyps that develop in the multiple intestinal neoplasia mouse, a murine model for familial adenomatous polyposis (FAP).[23] *PLA2G2A* probably exerts its action by altering the cellular microenvironment within the intestinal crypt,[24,25] possibly through prostaglandin synthesis.

It is well established that the colonic microenvironment is modified by bile acid excretion.[26] Apolipoprotein-*E* (*APO-E*) occupies a pivotal role in lipoprotein metabolism as one of the ligands involved in the receptor-mediated uptake of low-density lipoprotein and chy-

lomicron remnants.[27] Three common isoforms of *APO-E* exist—*E2*, *E3*, and *E4*.[26] Chylomicron and very low density lipoprotein clearance is faster and fecal bile acid output lower in individuals with the *APO-E4* allele.[27]

Oncogenes and Tumor Suppressors

The *HRAS* proto-oncogene VNTR minisatellite is located 1 kilobase downstream of *H-ras1* and is composed of 30 to 100 units of a 28–base pair consensus sequence. Over 30 alleles of the *H-ras*-VNTR have been described.[28] The 4 most common represent over 90% of alleles and serve as progenitors for rare alleles.[28] Rare alleles have been proposed as risk factors for a variety of cancers.[28] The basis of a relationship is, however, unclear. The Harvey ras-1 variable number tandem repeat polymorphism (*HRAS1*-VNTR) has been shown to modulate the expression of nearby genes by interacting with transcriptional regulatory elements, such as the rel/NF-κB family of regulatory factors. Alternatively, the association may result from linkage disequilibrium.[28]

Truncating *APC* mutations are responsible for FAP, and defective mismatch repair genes cause hereditary nonpolyposis colorectal cancer.[5,6] Polymorphisms in these genes have been identified, but because they are common or they are not predicted to alter protein structure, they have generally been considered to have little pathogenic potential. This may not be the case. Laken et al.[29] first drew attention to a possible relationship between the I1307K *APC* variant and colorectal cancer risk in the Ashkenazi population. *I1307K* was present in 6% of controls, compared with 10% in colorectal cancer patients. The mutation leads to creation of an A_8 tract instead of the normal A_3TA_4, possibly increasing the rate of somatic mutation,[29] so indirectly conferring susceptibility. Other *APC* variants that have been proposed as colorectal tumor risk factors include E1317Q.

Somatic mutations in the tumor suppressor gene *p53* are common as a mid-/late-stage event in colorectal cancer.[11] Overexpression of tumor protein 53 (TP53) protein induced by genotoxic damage results in G1 cell cycle arrest and induction of repair or apoptosis. Mutations in *TP53* that cause the Li-Fraumeni syndrome lead to decreased TP53 activity resulting in defective repair or apoptosis.[30] In addition to these types of mutations, a number of polymorphisms of *TP53* are recognized, although their functional significance is unknown.

Although polymorphism in any DNA repair gene is theoretically a potential risk factor for colorectal tumors, components of the mismatch repair machinery are the prime candidates. Many polymorphisms exist in *MSH2*, *MLH1*, and *MSH6* (http://www.nfdht.nl/database), although in most cases, functional effects are almost entirely unknown and/or alleles are rare.

Other Genes

The cytokine tumor necrosis factors beta (TNFβ) and alpha (TNFα) produced by activated macrophages have cytostatic and cytotoxic antitumor activity.[31,32] Polymorphic variation in TNF genes has been linked to level of TNFα expression and prognosis in gastric and lung cancer.[33]

In this systematic review, every effort has been made to ascertain all published studies that have examined the possible effects of polymorphic variation in the genes discussed on the risk of colorectal neoplasia in the context of an epidemiologic study. Although some genes have only been examined once, most have been evaluated as risk factors in several studies, often with discordant findings. Many of the studies are based on small sample sizes; therefore, to derive a more precise estimate of the risk associated with individual polymorphisms, results have been combined wherever possible in a meta-analysis. The underlying basis of meta-analysis is not directly to combine results from studies, but to obtain a relative measure of the observed effect supporting or rejecting a specific hypothesis. An advantage of this statistical procedure is the amalgamation of data collected and analyzed by different methods. The implications of the findings for future research on predisposition to colorectal neoplasia are discussed.

Methods

Literature Search

Published studies that had examined the relationship between polymorphisms and risk of colorectal neoplasia were identified using the electronic database MEDLINE (National Library of Medicine, Washington, DC) for the years 1983–1999 inclusive, using the search terms "colorectal_neoplasms" and "polymorphism(s)." A search was also made on individual candidate genes. Additional articles were ascertained through references cited in these publications. Care was taken to include only primary data or data that superseded earlier work. Characteristics of the studies were extracted from published articles and summarized in a consistent manner to aid comparison.

Statistical Analysis

ORs associated with individual polymorphisms were extracted from published reports or were computed from crude data. Pooled estimates of ORs were obtained using standard calculating the weighted-average of the logarithm of ORs.[34] Studies were analyzed jointly using both a fixed-effects and a random-effects model—if significant heterogeneity between

studies was present.[35] A random-effects model assumes that the studies in question are a random sample of a hypothetical population of studies taking into account within- and between-study variability. In cases in which a polymorphism is associated with distinctive phenotype, the principal analysis combined genotypes by phenotypic class; otherwise, ORs were computed for each genotype. Specific analyses considering confounding factors were not possible because the raw data were not available.

To ascertain the presence of publication bias, ORs were plotted in order according to the variance of the logOR estimate. Estimates from small studies that have less precision in estimating the underlying OR will scatter widely at the base of the graph, with a narrowing among larger studies. In the absence of publication bias, the plot resembles a symmetrical inverted funnel. Conversely, if there is bias, the funnel plot will be asymmetrical. To reduce the possibility of error in assessing publication by simple visual examination, funnel asymmetry was formally evaluated using the Egger et al.[36] regression asymmetry test. This test detects asymmetry by determining whether the intercept deviates significantly from zero in a regression of the standardized effect estimates against their precision. Failure of the standardized effect confidence interval (CI) to include zero indicates asymmetry and provides evidence of publication bias. Statistical manipulations were undertaken using the statistical program STATA version 6.0 (Stata Corporation, College Station TX) utilizing the META[37] and METABIAS[38] modules.

In any large, randomly mating population, in which there is no migration, or selection against a particular genotype and the mutation rate remains constant, the proportions of the various genotypes will remain unchanged from one generation to another. To test for population stratification, the distribution of genotypes in controls was tested for a departure from Hardy-Weinberg equilibrium by means of the chi-squared test.

The power of each study was computed as the probability of detecting an association between genotypes and colorectal cancer at the 0.05 level of significance, assuming a genotypic risk of 2 or 0.5. These estimates were performed on the basis of the method published by Fleiss et al.,[39] using the statistical program POWER (Epicenter Software, Pasadena, CA; http://icarus2.hsc.usc.edu/epicenter/).

Results

Individual Studies

Fifty studies were identified from the literature and are summarized in Table 2. All were essentially of similar design, although a variety of cases and controls have been analyzed. Fewer than 30% of the studies had 80% or greater power to demonstrate a twofold difference in allele frequency between cases and controls. The ORs of colorectal disease associated with the different polymorphisms in individual studies are given in Table 3 along with their corresponding 95% CIs. Where ORs were not reported, they have been derived from the raw data. Polymorphisms in 13 genes have been evaluated as potential colorectal cancer risk factors. Many of the results from different studies are discordant.

Carcinogen Metabolism Genes

Variation in *CYP1A1* defined by the *Ile*462*Val* and T6325C polymorphisms and colorectal cancer has only been evaluated in one small study of in situ cancer to date.[50] The risk of colorectal cancer was increased 7.9-fold in *Ile*462*Val* carriers, but confidence limits were wide (1.4–44.4), reflecting the size of the study. No association between colorectal cancer risk and the T6325C polymorphism was observed.

NAT2 has been as investigated as a colorectal tumor risk factor in 15 studies.[40,41,45,51,52,55,58,59,62,67,68,72,76,77,90] In 4 of the studies, NAT2 status was assigned on the basis of acetylator phenotype rather than genotype. The risk of colorectal cancer associated with rapid acetylator status was increased in 9 of the 15 studies, but was only statistically significant in the 3 small studies based on phenotyping.[40,41,62] The relationship between *NAT2* and colorectal adenoma risk has been examined in 2 studies,[58,62] but neither showed a significant association.

Six studies have examined the relationship between specific allelic variants of *NAT1* and risk of colorectal neoplasia.[55,59,70,72,81,91] Bell et al.[55] reported that possession of the *NAT1*10* allele was associated with a 1.92-fold increase in risk of colorectal cancer, but this was not confirmed in the studies reported by Chen et al.[72] and Yoshioka et al.[91] Probst-Hensch et al.[59] also found no relationship between the *NAT1*10* allele and risk of colorectal adenomas. Lin et al.[81] and Hubbart et al.[70] examined the relationship between other *NAT1* alleles and risk of colorectal cancer. Neither found support for an association between *NAT1* genotype defined by *NAT1*14/15* alleles and risk of colorectal cancer.

Twelve studies have examined the relationship between *GSTM1* status and colorectal cancer risk,[46,48,56,60,61,69,76,78,83–85,92] and one study examined colorectal adenoma risk.[57] Five studies showed that the *GSTM1*-deletion conferred an increase in colorectal cancer risk,[46,48,61,84,92] but this was only significant in 2 studies.[48,92] The study reported by Zhong et al.[48] found that the risk of colorectal cancer associated with *GSTM1*-deletion was primarily associated with proximal disease in a subgroup analysis, a finding not confirmed by other studies.[56,60,61,69,76] The relationship between *GSTM1* status and adenoma risk was examined by Lin et al.[57]; no relationship was seen.

Table 2. Studies of Polymorphisms and Colorectal Tumor Risk

Reference	Place of study	Genes studied	Ethnicity	N	Cases[a]	N	Controls	Exposure assessment
Lang et al. 1986[40]	USA	*NAT2*	Mixed	43	Male prevalent CRC cases; mean age 41 (45–75); 65% white	41	Male hospital patients; mean age 41 (45–75); 78% white	
Ilett et al. 1987[41]	Australia	*NAT2* (SMZ)	Caucasian	49	Prevalent CRC cases	41	Hospital patients matched for age, gender, and smoking	Occupation (no exposure to known carcinogens)
Ceccherini-Neilli et al. 1988[42]	Italy	*HRAS1*-VNTR	Caucasian	62	CRC cases	108	Blood donors	
Wyllie et al. 1991[43]	UK	*HRAS1*-VNTR	Caucasian	46	Incident CRC cases	49	Healthy individuals; no family history of cancer	
Klingel et al. 1991[44]	Germany	*HRAS1*-VNTR	Caucasian	116	Unrelated CRC cases (5 with FAP); 50% male	122	Crohn's colitis patients; 38% male	
Ladero et al. 1991[45]	Spain	*NAT2*	Caucasian	109	Prevalent CRC cases (61 colon, 48 rectal); 48% male; mean age 66	96	Healthy individuals; mean age 43; 44% male	
Strange et al. 1993[46]	UK	*GSTM1*	Caucasian	26	CRC patients	49	Postmortem controls	
Rodriguez et al. 1993[47]	USA	*NAT2*	Mixed	44	CRC samples from 2 tissue repositories; mean age 63; 73% white, 27% black	28	Non-cancer colon samples from same source as cases; 21% male; 75% white, 23% black	
Krontiris et al. 1993[28]	USA	*HRAS1*-VNTR	Caucasian	13	CRC cases derived from a large cohort of different cancers (mean age 63.9)	652	Healthy individuals; mean age 50.5 (16.0)	
Zhong et al. 1993[48]	UK	*GSTM1*	Caucasian	196	CRC (prevalent) cases from one hospital	225	Subjects from a clinical chemistry department and volunteers	
Kawajiri et al. 1993[49]	Japan	*TP53*	Asian	84	CRC (prevalent) cases from one center	347	Unrelated healthy individuals; ~50% male	
Sivaraman et al. 1994[50]	USA	*CYP1A1*	Asian	23	In situ CRC cases	59	Population controls	
Shibuta et al. 1994[51]	Japan	*NAT2*	Asian	234	CRC cases; 53% male	329	Healthy volunteers	
Oda et al. 1994[52]	Japan	*NAT2*	Asian	36	Prevalent CRC cases; mean age 67.2 (38–81); 44% male	36	Autopsied controls matched for age	
Hall et al. 1994[53]	UK	*MLH1*	Caucasian	111	CRC cases (32 aged <45, 79 incident cases)	114	Spouse controls	
Sjalander et al. 1995[54]	Sweden	*TP53*	Caucasian	155	CRC cases from one hospital	206	Placental specimens	
Bell et al. 1995[55]	UK	*NAT1, NAT2*	Caucasian	202	CRC cases; mean age 63	112	Age- and sex-matched hospital patients	
Chevenix-Trench et al. 1995[56]	Australia	*GSTM1, GSTT1*	Caucasian	132	CRC cases	200	"unselected individuals" and "geriatric patients" without cancer or family history of cancer	
Lin et al. 1995[57]	USA	*GSTM1*	Mixed	447	Distal CRA cases ascertained through 2 Southern Californian medical centers; aged 50–74; no history of invasive cancer, previous bowel surgery, FAP, or severe gastrointestinal symptoms; 64% male; 55% white, 16% black, 17% Hispanic, 10% Asian, 2% other	488	Age- and sex-matched clinic patients with normal sigmoidoscopy; 675 male; 55% white, 16% black, 18% Hispanic, 11% Asian, 1% other	Diet, smoking
Probst-Hensch et al. 1995[58]		*NAT2*						
Probst-Hensch et al. 1996[59]		*NAT1*						
Deakin et al. 1996[60]	UK	*GSTM1, GSTT1*	Caucasian	252	Unrelated CRC cases from one hospital; mean age 66; 51% male	577	Hospital patients without malignancy or inflammatory disease from same hospital as cases; mean age 70 years; 48% male	
Katoh et al. 1996[61]	Japan	*GSTM1, GSTT1*	Asian	103	Consecutive CRC cases from 2 hospitals and 1 medical center; mean age 64.4; 65% male	126	Medical center screens; free of gastrointestinal symptoms and cancer; mean age 61.9; 57% male	

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Table 2 (continued). Studies of Polymorphisms and Colorectal Tumor Risk

Reference	Place of study	Genes studied	Ethnicity	N	Cases[a]	N	Controls	Exposure assessment
Roberts-Thomson et al. 1996[62]	Australia	*NAT2* (SMZ)	Caucasian	110	CRC cases	110	Patients with endoscopic/barium enema proven non-neoplastic gastrointestinal disease; FAP and IBD patients excluded; median age 69 (39–85)	Diet: meat intake
				89	CRA cases median age 69 (39–85)			
Tomlinson et al. 1996[63]	UK	*PLA2G2A*	NS	80	CRC cases	80	NS	
				30	CRA cases			
Chen et al. 1996[64]	USA	*MTHFR*	Caucasian (98%)	144	CRC cases; Nested case-control study using incident cases from the Health Professionals Follow-up Study; males; aged 40–75	627	Age-matched controls	Diet (stratified by folate, methionine intake, and alcohol consumption)
Kervinen et al. 1996[65]	Finland	*APO-E*	Caucasian	122	CRC (incident) cases from 1 hospital; mean age 68; 44% male	199	Population controls aged 58–61 years randomly selected from the same population as cases	
				135	CRA cases (incident); mean age ~62; 42% male			
Ma et al. 1997[66]	USA	*MTHFR*	Primarily Caucasian	202	CRC cases (79% colon); nested case-control study using incident cases from the Physicians' Health Study	326	Age-matched controls	Diet (stratified by alcohol and folate intake); smoking, multivitamin, and aspirin use
Hubbart et al. 1997[67]	UK	*NAT2*	Caucasian	275	CRC (consecutive operable) cases treated in 4 Scottish hospitals; aged 20–95	343	Healthy individuals aged 20–75 attending routine occupational screening clinics	
Welfare et al. 1997[68]	UK	*NAT2*	Caucasian (90%)	174	CRC patients (incident cases); aged 38–91	174	Age- and sex-matched population controls	Meat consumption, smoking
Laken et al. 1997[29]	USA	*APC*	Ashkenazim	211	CRC cases (172 cases from single center, mean age 68.1 and 39 others, mean age 59)	766	Individuals undergoing genetic testing for Tay-Sachs disease	
Gertig et al. 1998[69]	USA	*GSTM1, GSTT1*	Caucasian (98%)	212	CRC cases; nested case-control study using incident cases from the Physicians' Health Study; aged 40–84		Age-matched controls	
Hubbart et al. 1998[70]	UK	*NAT1*	Caucasian	260	CRC (consecutive operable) cases treated in 3 Scottish hospitals; aged 20–95	343	Healthy individuals attending routine occupational screening from same population	
Chen et al. 1998[71]	USA	*MTHFR, MTR*	Primarily Caucasian	257	CRA cases; Nested case-control study using incident cases from the Nurses' Health Study; females	713	Age-matched controls without CRAs	Diet (stratified by intake of folate, methionine, and alcohol intake); smoking
Chen et al. 1998[72]	USA	*NAT1, NAT2*	Caucasian (98%)	212	CRC cases; Nested case-control study using incident cases from the Physicians' Health Study		Age-matched controls	Meat intake
Harris et al. 1998[73]	Australia	*GSTP1*	Caucasian	131	CRC cases	199	"Unselected individuals" (n = 100; source: NS) and "geriatric patients" (n = 100; mean age 77 years) without cancer or a family history of cancer	
Frayling et al. 1998[74]	UK	*APC*	?Caucasian	164	CRC and CRA cases	NS	NS	
Grosse-Burn et al. 1998[75]	France	*HRAS1*-VNTR	Caucasian	142	CRC cases; median age 69 (24–90)	143	Individuals ascertained through genetic counseling clinics; median age 32 (5–66); 40% male; no family history of cancer in first-degree relative	

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Table 2 (continued). Studies of Polymorphisms and Colorectal Tumor Risk

Reference	Place of study	Genes studied	Ethnicity	N	Cases[a]	N	Controls	Exposure assessment
Slattery et al. 1998[76]	USA	*NAT2*	Mixed	1993	CRC cases ascertained within 131 days of diagnosis; FAP and IBD associated cancers excluded; aged 30–79	2410	Random population controls from same geography as cases	Diet and smoking (stratified by smoking and Western diet)
		GSTM1			91% white, 4% black, 4% Hispanic			
Lee et al. 1998[77,78]	Singapore	*GSTM1*	Asian	300	CRC cases (NAT2 analysis restricted to n = 216); mean age 47 (26–82); 59% male	187	Undergraduate students and blood donors (*NAT2* controls); mean age 27 (18–61); 73% male	
		NAT2				183	Hospital patients with no history of neoplasms ascertained through a clinical chemistry (*GSTM1* controls)	
Gil and Lehner 1998[79]	Portugal	*NAT2*	Caucasian	114	Colon cancer cases (unrelated) treated at 1 hospital; mean age 64.2 (11.0); 63% male	201	Individuals from same region as cases undergoing health checks; no history of cancer; mean age 46.4 (19.6)	
Park et al. 1998[80]	Korea	TNF-α TNF-β	Asian	136	CRC cases	325	Healthy unrelated individuals	
Lin et al. 1998[81]	USA	*NAT1* variants	Mixed	444	Distal CRA cases ascertained through 2 Southern Californian medical centers; aged 50–74; no history of invasive cancer, previous bowel surgery, FAP, or severe gastrointestinal symptoms; 64% male; 55% white, 16% black, 17% Hispanic, 10% Asian, 2% other	488	Age- and sex-matched clinic patients with normal sigmoidoscopy; 67% male; 55% white, 16% black, 18% Hispanic, 11% Asian, 1% other	
Woodage et al. 1998[82]	USA	*APC*	Ashkenazim	55	Community survey of 5081 volunteers. Cancer risk determined by comparing of cancer history in carriers and noncarriers			
Zhang et al. 1999[83]	Sweden	*GSTM1, GSTT1*	Caucasian	99	CRC cases; 58% male	109	Random individuals from same geographical region; free of gastrointestinal disease and history of tumors	
Welfare et al. 1999[84]	UK	*GSTM1, GSTT1, GSTP1*	Caucasian	178	CRC (incident) cases; median age 69 (38–91)	178	Age- and sex-matched individuals	
Abdel-Rahman et al. 1999[85]	Egypt	*GSTM1, GSTT1*	Arabic	66	CRC (incident cases) cases; mean age 42.8 (15.8); 62% male	55	Age-matched friends of other cancer cases; mean age 34.5 (9.0); 54% male	
Harrison et al. 1999[86]	UK	*mEPHX*	Caucasian	101	CRC (consecutive) cases	203	Blood donors from same Scottish region as cases; aged 18–65; 50% male	
Gryfe et al. 1999[87]	Canada	*APC*	Ashkenazim	404 72	CRC archival specimens CRA cases			
Slattery et al. 1999[88]	USA	*MTHFR*	Primarily Caucasian	1467	Colon cancer cases aged 30–79 ascertained within 131 days of diagnosis; FAP and IBD associated cancers excluded; 91% white, 4% black, 4% Hispanic	1821	Random age-matched population controls	Diet (stratified by folate, B_6, B_{12}, methionine, alcohol, and multivitamin intake); smoking
Ma et al. 1999[89]	USA	*MTR*	>95% Caucasian	356	CRC cases; nested case-control study using incident cases from the Physicians' Health Study (n = 212) and the Health Professionals Follow-up Study (n = 144)	346	Controls matched on age	Alcohol

(continued on following page)

Table 2 (continued). Studies of Polymorphisms and Colorectal Tumor Risk

Reference	Place of study	Genes studied	Ethnicity	N	Cases[a]	N	Controls	Exposure assessment
				130				PHS: levels of folate homocysteine, B12
Park et al. 1999[90]	Korea	*MTR*	Asian	136	CRC cases randomly selected postoperatively from 1 center; 51% male	325	Healthy unrelated individuals	
Yoshioka et al. 1999[91]	Japan	*NAT1, NAT2, GSTP1*[a]	Asian	106	CRC (consecutive) cases from 2 hospitals and 1 medical center; mean age 62.4 (12.0)	100	Local medical center screens; no gastrointestinal symptoms, current, or previous diagnosis of cancer; mean age 61.9; 57% male	
Gawronska-Szklarz et al. 1999[92]	Poland	*GSTM1*	Caucasian	28	CRC (without clinical features of hereditary tumors); 43% male; mean age 63.8 (9.4)	145	Healthy volunteers, 35% male, mean age 41.6 (15.4)	
				27	CRA cases, 48% male, mean age 60.0 (9.1)			

CRC, colorectal cancer; CRA, colorectal adenoma; IBD, inflammatory bowel disease; NS, not stated; ?, probable.
[a]*GSTM1* and *GSTT1* data detailed in Katoh et al.[61]

Conflicting findings have also been reported in studies of the relationship between *GSTT1* status and colorectal cancer risk. Two studies showed a significant association between deficiency and risk,[60,83] but either no relationship was seen or, in 5 other studies, the opposite was seen.[56,61,69,84,85] The study by Zhang et al.,[83] which showed the largest risk, may be methodologically flawed, as GSTT1 status was determined by analysis of colonic tissue—any contamination by tumor tissue showing allelic loss at *GSTT1* would invalidate the findings.

Three studies have investigated glutathione-*S* transferase Pi (*GSTP1*) status defined by the Ile105Val and Ala114Val polymorphisms as risk factors for colorectal cancer.[73,84,91] None has shown that either polymorphism confers an increased risk of colorectal cancer.

Two polymorphisms of *mEPHX* have been examined in one study.[18] $mEPHX^{Tyr113His}$ homozygosity was associated with a significant increase in risk of colorectal cancer. The $mEPHX^{His139Arg}$ polymorphism was not associated with risk of cancer.[18] There was, however, evidence of population stratification in the study as the distribution of Tyr113His genotypes shows a departure from Hardy-Weinberg equilibrium.

Methylation Genes

Four studies have examined the relationship between the C677T *MTHFR* polymorphism and colorectal cancer risk.[64,66,71,88,90] All showed an inverse relationship between $MTHFR^{Val/Val}$ genotype and colorectal cancer risk, although the relationship was only statistically significant in the study reported by Ma et al.[66] The study reported by Ma et al.[8] shows borderline departure from Hardy-Weinberg equilibrium, suggesting the possibility of population stratification. The polymorphism has also been evaluated as a risk factor for colorectal adenomas in one study.[71] No difference in the frequency of homozygosity was seen between 257 incident polyp cases and 713 controls in the study reported by Chen et al.[71] If an inverse relationship between $MTHFR^{Ala/Val}$ genotype and colorectal cancer risk exists, the fact that no relationship was seen with adenomas raises the possibility that its impact is only on late stage tumorigenesis. The 2 studies of the Asp/Gly polymorphism of methionine synthetase have shown no significant association with risk of colorectal cancers or adenomas.[71,89]

Oncogene and Tumor-Suppressor Genes

Five studies have examined the risk of colorectal cancer associated with rare alleles of the *HRAS1*-VNTR.[28,42–44,75] Four of the studies were based on Southern blot analysis,[28,42–44] whereas the most recently reported by Gosse-Brun et al.[75] was polymerase chain reaction–based. The OR of colorectal cancer was greater than unity in all of studies, but only statistically significant in 2.[44,75]

Since the original observation of a relationship between the *APC I1307K* and colorectal cancer risk made by Laken et al.,[29] 3 studies have reported a positive relationship between this variant and risk of colorectal neoplasia.[74,82,87] Frayling et al.[74] have also reported that the *E1317Q* mutation also confers an increased risk of colorectal tumor. *TP53* Arg72Pro, intron-6, and intron-3 polymorphisms have been evaluated in 2 studies—neither found any relationship.[49,54]

Table 3. Risks of Colorectal Tumor by Individual Polymorphism and Study

Gene and polymorphism	Type of colorectal neoplasm	Frequency of rare variant in controls	Risk group	OR	95% CI	Comment
Carcinogen metabolizing genes						
CYP1A1 $Ile^{462}Val$						
Sivaraman et al. 1994[50]	In situ Ca	0.03	Val-carrier	7.9	1.4–44.4	
CYP1A1 $T^{6235}C$						
Sivaraman et al.[50]	In situ Ca	0.02	C-carrier	5.7	0.5–66.3	
NAT2-rapid acetylator						
Lang et al.[40]	CRC	0.32	Phenotype	2.48	1.02–6.03	
Ilett et al.[41]	CRC	0.34	Phenotype	2.41	1.18–4.95	
Ladero et al.[45]	CRC	0.42	Phenotype	1.14	0.66–1.99	
Rodriguez et al.[47]	CRC	0.46	Genotype	0.96	0.37–2.49	
Shibuta et al.[51]	CRC	0.90	Genotype	0.83	0.48–1.41	
Oda et al.[52]	CRC	0.92	Genotype	1.00	0.19–5.32	
Bell et al.[55]	CRC	0.45	Genotype	1.12	0.71–1.79	
Probst-Hensch et al.[58,59]	Distal CRA	0.29	Genotype	1.10	0.84–1.44	Adjusted for age, gender, date of sigmoidoscopy, clinic attended, and ethnicity
Roberts-Thomson et al.[62]	CRC	NS	Phenotype	1.8	1.0–3.3	
	CRA			1.1	0.6–2.1	
Hubbard et al.[67]	CRC	0.41	Genotype	0.83	0.60–1.15	
Welfare et al.[68]	All CRC	0.43	Genotype	0.95	0.61–1.49	
	Proximal CC			0.66	0.29–1.47	
	Distal CC			0.85	0.36–2.0	
	Rectal Ca			1.42	0.58–3.5	
Chen et al.[72]	CRC	0.43	Genotype	0.81	0.53–1.25	Adjusted for age, smoking history, red meat intake, exercise, alcohol intake, and BMI
Slattery et al.[76]	CC in males	0.41	Genotype	1.0	0.8–1.2	Adjusted for age, energy intake, BMI, physical activity, dietary fiber, and smoking
	Proximal CC			1.1	0.8–1.3	
	Distal CC			0.9	0.7–1.2	
	CC in females			1.2	1.0–1.5	
	Proximal CC			1.0	1.0–1.6	
	Distal CC			1.1	0.9–1.5	
Lee et al.[77]	CRC	0.38	Genotype	1.08	0.70–1.67	
Gil & Lechner[79]	CRC	0.34	Genotype	1.23	0.78–1.98	
Yoshioka et al.[90]	CRC	0.93	Genotype	1.52	0.47–4.96	
*NAT1*10*						
Bell et al.[55]	CRC	0.29	T-carrier	1.92	1.18–3.15	
Probst-Hensch et al.[59]	Distal CRA	0.50	T-carrier	1.04	0.79–1.36	Adjusted for age, gender, date of sigmoidoscopy, clinic attended, and ethnicity
Chen et al.[72]	CRC	0.44	T-carrier	0.94	0.60–1.49	Adjusted for age, smoking history, red meat intake, exercise, alcohol intake, and BMI
Yoshioka et al.[91]	CRC	0.64	T-carrier	1.00	0.57–1.78	
*NAT1*14,15*						
Hubbard et al.[70]	CRC	0.04		1.35	0.60–3.00	
Lin et al.[81]	Distal CRA (Whites)	0.00	Carrier	—		
*NAT1*14, 15, 17, 19, 22*						
Lin et al.[81]	Distal CRA (Whites)	0.04	Carrier	0.93	0.48–1.80	
GSTM1 deletion						
Strange et al.[46]	Distal-CRC	0.41	Null phenotype	2.32	0.88–6.14	
Zhong et al.[48]	All CC	0.42	Null genotype	1.78	1.21–2.62	
	Proximal CC			3.38	1.91–6.00	
	Distal CC			1.66	1.03–2.66	
Chevenix-Trench et al.[56]	CRC	0.51	Null genotype	0.92	0.59–1.43	
	Proximal			0.87	0.42–1.80	
	Distal			0.94	0.58–1.52	
Lin et al.[57]	Distal CRC	NS	Null genotype	0.85	0.65–1.10	Adjusted OR
Deakin et al.[60]	CRC	0.55	Null genotype	0.95	0.71–1.28	
	Right-CC			0.79	0.50–1.25	
	Left-CC			1.06	0.63–1.79	
	Rectal cancer			1.16	0.75–1.81	
Katoh et al.[61]	All CRC	0.44	Null genotype	1.53	0.91–2.58	
	Proximal CC			1.19	0.61–2.31	

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Table 3 (continued). Risks of Colorectal Tumor by Individual Polymorphism and Study

Gene and polymorphism	Type of colorectal neoplasm	Frequency of rare variant in controls	Risk group	OR	95% CI	Comment
	Distal CC			2.03	0.93–2.66	
Gertig et al.[69]	CRC	0.53	Null genotype	1.0	0.7–1.5	Adjusted for age, smoking, BMI, physical activity, and alcohol
	Proximal			0.7	0.4–1.3	
	Distal			1.4	0.8–2.3	
Slattery et al.[76]	CC in males	0.55	Null genotype	1.0	0.8–1.2	Adjusted for age, energy intake, BMI, physical activity, dietary fiber, and smoking
	Proximal			1.1	0.9–1.4	
	Distal			1.0	0.7–1.2	
	CC in females			0.9	0.7–1.1	
	Proximal			1.0	0.6–1.1	
	Distal			1.0	0.7–1.2	
Lee et al.[78]	CRC	0.49	Null genotype	0.79	0.53–1.16	
Zhang et al.[83]	CRC	0.50	Null genotype	0.86	0.50–1.50	
Welfare et al.[84]	CRC	0.51	Null genotype	1.04	0.67–1.65	
Abdel-Rahman et al.[85]	CRC	0.67	Null genotype	0.56	0.23–1.3	
Gawronska-Szklarz et al.[92]	CRC	0.6	Null genotype	2.53	1.05–6.12	
	CRA		Null genotype	1.73	0.73–4.01	
GSTT1 deletion						
Chevenix-Trench et al.[56]	CRC	0.16	Null genotype	0.86	0.43–1.68	
	Proximal			0.37	0.08–1.68	
	Distal			1.03	0.51–2.10	
Deakin et al.[60]	All CRC	0.19	Null genotype	1.88	1.30–2.72	
	Right-sided CC			1.50	0.84–2.68	
	Left-sided CC			2.33	1.28–4.24	
	Rectal Ca			1.87	1.08–3.22	
Katoh et al.[61]	CRC	0.44	Null genotype	1.18	0.70–1.99	
Gertig et al.[69]	CRC	0.23	Null genotype	0.8	0.5–1.2	Adjusted for age, smoking, BMI, physical activity, and alcohol
	Proximal CRC			0.9	0.5–1.7	
	Distal CRC			0.6	0.3–1.2	
Zhang et al.[83]	CRC	0.20	Null genotype	4.49	2.42–8.34	
Welfare et al.[84]	CRC	0.17	Null genotype	1.21	0.63–2.0	
Abdel-Rahman et al.[85]	CRC	0.41	Null genotype	0.85	0.37–1.97	
GSTP1 Ile105Val						
Harris et al.[73]	CRC	0.34	Val-carrier	0.93	0.56–1.54	
Welfare et al.[84]	CRC	0.33	Val-carrier	1.01	0.65–1.60	
Yoshioka et al.[91]	CRC	0.27	Val-carrier	1.50	0.81–2.76	
GSTP1 Ala114Val						
Harris et al.[73]	CRC	0.07	Val-carrier	0.93	0.50–1.77	
Welfare et al.[84]	CRC	0.1	Val-carrier	1.00	0.50–1.90	
MEPHX Try113His						
Harrison et al.[18]	All CRC	0.31	Tyr/His	0.83	0.49–1.40	Departure from Hardy-Weinberg ($P = 0.04$)
			His/His	3.50	1.60–7.65	
			His-carrier	1.14	0.70–1.85	
	Proximal CRC		Tyr/His	1.34	0.60–2.98	
			His/His	3.82	1.21–12.08	
			His-carrier	1.62	0.75–3.53	
	Distal CRC		Tyr/His	0.65	0.35–1.20	
			His/His	3.39	1.47–7.81	
			His-carrier	0.97	0.56–1.68	
MEPHX His139Arg						
Harrison et al.[18]	All CRC	0.15	His/Arg	0.77	0.43–1.36	
			Arg/Arg	2.58	0.63–10.56	
			Arg-carrier	0.86	0.50–1.49	
	Right-sided CRC		His/Arg	1.19	0.52–2.72	
			Arg/Arg	7.00	1.51–32.56	
			Arg-carrier	1.50	0.70–3.21	
	Left-sided CRC		His/Arg	0.61	0.30–1.21	
			Arg/Arg	0.89	0–6.40	
			Arg-carrier	0.62	0.32–1.21	
Methylation genes						
MTHFR Ala677Val						

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Table 3 (continued). Risks of Colorectal Tumor by Individual Polymorphism and Study

Gene and polymorphism	Type of colorectal neoplasm	Frequency of rare variant in controls	Risk group	OR	95% CI	Comment
Chen et al.[64]	CRC	0.34	Val/Val	0.57	0.30–1.06	Adjusted for age, family history, and intakes of folate, methionine, and alcohol
Ma et al.[66]	CRC	0.35	Val/Val	0.45	0.24–0.86	Ala/Ala as reference group
				0.46	0.25–0.84	Val/Ala and Ala/Ala as reference group Adjusted ORs Departure from Hardy-Weinberg ($P = 0.04$)
Chen et al.[71]	CRA	0.32	Val/Val	1.35	0.84–2.17	Adjusted for age, family history, smoking status, BMI, and intakes of folate, methionine, alcohol, fiber, and saturated fat. Val/Ala and Ala/Ala as reference group
Slattery et al.[88]	Colon cancer	0.33	Val/Val	0.9	0.7–1.1	Ala/Ala as reference group
	Distal cancer		Val/Val	1.0	0.7–1.3	Adjusted for age, BMI, long-term vigorous activity, energy intake, dietary fiber, and current smoking
	Proximal cancer		Val/Val	0.8	0.6–1.1	
Park et al.[90]	CRC	0.43	Val/Val	0.87	0.53–1.39	Departure from Hardy-Weinberg ($P = 0.048$)
MTR Asp919Gly						
Chen et al.[71]	CRA	0.19	Gly/Gly	0.66	0.26–1.70	Adjusted for age, family history, smoking status, BMI, and intakes of folate, methionine, alcohol, fiber, and saturated fat. Gly/Asp and Asp/Asp as reference group
Ma et al.[89]	CRC (PHS)	0.18	Asp/Gly	1.04	0.71–1.53	
			Gly/Gly	0.63	0.24–1.64	
	CRC (HPFS)	0.21	Asp/Gly	0.71	0.42–1.20	
			Gly/Gly	0.53	0.14–1.93	
Microenvironmental modifiers						
APOE						
Kervinen et al.[65]	All CRC	0.18	*APOE4*-carrier	0.69	0.42–1.13	
	All CRA			0.58	0.35–0.95	
	Proximal CRC			0.35	0.14–0.86	
	Proximal CRA			0.36	0.14–0.89	
	Distal CRC			0.90	0.52–1.57	
	Distal CRA			0.68	0.39–1.18	
PLA2G2A-Exon 1						
Tomlinson et al.[63]	CRC	0.23	1/2	1.02	0.46–2.30	
			2/2	0.97	0.18–5.16	
			2-carrier	1.02	0.47–2.20	
	CRA		1/2	1.78	0.57–5.54	
			2/2	2.67	0.38–18.73	
			2-carrier	1.90	0.64–5.61	
PLA2G2A-Exon 3						
Tomlinson et al.[63]	CRC	0.08	1/2	1.61	0.59–4.35	
			2/2	0.93	0.13–6.87	
			2-carrier	1.19	0.56–2.83	
	CRA		1/2	0.69	0.13–3.53	
			2/2	2.40	0.32–18.00	
			2-carrier	1.06	0.30–3.79	
Tumor suppressors/oncogenes						
APC-I1307K						
Laken et al.[29]	All CRC	0.06	Carrier	1.78	1.05–3.03	
	CRC aged <66 yrs			4.76	2.44–9.29	
	CRC aged >66 yrs			1.08	0.50–2.35	
	CRC with FH			5.95	2.37–14.95	
Frayling et al.[74]	Colorectal neoplasm	0.08	Carrier	6.60	1.33–32.82	
Woodage et al.[82]	CRC		Carrier	1.9	0.84–4.2	
Gryfe et al.[87]	Colorectal neoplasm	ND	Carrier	1.48	1.08–2.04	Comparison made using published control data[82]
	CRC					

(continued on following page)

Table 3 (continued). Risks of Colorectal Tumor by Individual Polymorphism and Study

Gene and polymorphism	Type of colorectal neoplasm	Frequency of rare variant in controls	Risk group	OR	95% CI	Comment
APC-E1307Q						
Frayling et al.[74]	CRA	Carrier	0.003	9.51	1.90–47.60	
MSH2^{2006}C → T variant						
Hall et al.[53]	CRC	0.12	Het	1.10	0.60–2.02	
			Hom	—	—	
			Carrier	1.13	0.62–2.08	
TP53 Arg72Pro						
Kawajiri et al.[49]	CRC	0.35	Arg/Pro	0.78	0.46–1.30	
			Pro/Pro	1.68	0.85–3.33	
			Pro-carrier	0.95	0.59–1.53	
Sjalander et al.[54]	CRC	0.29	Arg/Pro	1.30	0.83–2.03	Genotype distribution of controls—departure from Hardy-Weinberg ($P = 0.05$)
			Pro/Pro	1.25	0.64–2.43	
			Pro-carrier	1.29	0.85–1.95	
TP53 intron 6 *Msp*I						
Sjalander et al.[54]	CRC	0.15	G/A het	1.09	0.67–1.79	
			A/A hom	0.50	0.14–1.78	
			A-carrier	1.00	0.62–1.61	
TP53 16-bp dupl						
Sjalander et al.[54]	CRC	0.15	A1/A2	0.50	0.29–0.88	Presence of 16-bp duplication designated by A2 allele
			A2/A2	0.49	0.14–1.79	
			A2-carrier	0.50	0.30–0.85	
HRAS1-VNTR						
Ceccherini-Nelli et al.[42]	CRC	0.02	Carrier	1.31	0.29–5.97	
Wyllie et al.[43]	CRC	0.02	Carrier	3.95	0.8–19.55	
Klingel et al.[44]	CRC	0.06	Carrier	1.98	1.00–3.92	
Krontiris et al.[28]	CRC	0.06	Carrier	2.84	0.95–8.55	
Gosse-Brun et al.[75]	CRC	0.01	Carrier	7.36	1.66–32.70	
Other genes						
TNF-α–*308T A*						
Park et al.[80]	CRC	0.12	TA-het	0.73	0.44–1.21	
			AA-hom	0.55	0.06–4.96	
			A-carrier	0.72	0.44–1.19	
TNF-β						
Park et al.[80]	CRC	0.32	Het	1.74	1.11–2.73	
			Hom	2.89	1.54–5.42	
			Carrier	1.94	1.26–2.98	

BMI, body mass index; CC, colon cancer; CRA, colorectal adenoma; CRC, colorectal cancer; ND, not determined; bp, base pair(s).

Polymorphic variation in only one mismatch repair gene, *MLH1*, has been examined to date.[53] No association was seen.

Other Genes

The −238G →A TNF-α polymorphism has been shown in one study to be associated with an increased risk of colorectal cancer.[80] There was no association with the −308G →A TNF-β polymorphism.

Meta-analysis

For the meta-analysis, data from studies of 11 of the genes were suitable for pooling. The study by Zhang et al.[83] was omitted from the meta-analysis of *GSTM1* and *GSTT1*, as in this study colonic tissue was assayed. Table 4 details the studies that were combined for each of the polymorphic variants and the results of each analysis in relation to risk of colorectal cancer. In the absence of heterogeneity between studies, pooled estimates of risk were based on the fixed-effects model. There were statistically significant associations between colorectal cancer risk and 3 of the polymorphic variants: *APC*-I1307K (OR = 1.58, 95% CI: 1.21–2.07); *HRAS1*-VNTR rare alleles, and *MTHFR*$^{Val/Val}$ (OR = 0.76, 95% CI: 0.62–0.92). There was no obvious evidence from visual diagnostic appraisal or formal analysis of findings for publication bias in these studies. The risk of colorectal cancer associated with rare alleles of the *HRAS1*-VNTR remained increased if the analysis was restricted to the 4 studies based on Southern analysis.[28,42–44]

In the pooled analysis, NAT2 rapid acetylator status determined by phenotyping was associated with an increased risk; however, no relationship was seen in the studies based on genotyping. Hence in the combined

Table 4. Results of Meta-analyses of Polymorphisms and Risk of Colorectal Cancer

Gene/polymorphism	Pooled studies (reference no.)	Risk group	OR	95% CI	Significance	Remarks
NAT2	40,41,45,61	Rapid acetylator-phenotype	1.70	1.23–2.37	0.001	Test of heterogeneity $\chi^2 = 3.60$; 3 df; $P = 0.31$
	47,51,52,55,67,68,72,76,77,79,90	Rapid acetylator-genotype	1.03	0.93–1.14	0.77	
	40,41,45,47,51,52,55,61,67,68,72,76,77,79,90	Rapid acetylator-genotype/phenotype	1.08	0.97–1.19	0.14	
*NAT1*10*	55,72,91	T-carrier	1.22	0.92–1.63	0.17	
GSTM1 deletion	48,56,60,61,69,76,78,84,85,92	Null genotype	1.10	0.99–1.21	0.07	
	46,48,56,60,61,69,76,78,84,85,92	Null genotype/phenotype	1.09	0.98–1.20	0.11	
GSTT1 deletion	56,60,61,69,83–85,92	Null	1.16	0.86–1.52	0.29	
GSTP1 Ile105Val	73,84,91	Ile/Val	1.14	0.76–1.72	0.52	
		Val-carrier	0.98	0.69–1.40	0.92	
GSTP1 Ala114Val		Val-carrier	0.98	0.68–1.42	0.93	
MTHFR Val677Val	64,66,88,90	Val/Val	0.77	0.64–0.93	0.006	Ala/Ala and Ala/Val genotypes as reference Test of heterogeneity $\chi^2 = 2.05$; 3 df; $P = 0.56$
MTR	89	Asp/Gly	0.92	0.67–1.25	0.55	
		Gly/Gly	0.59	0.27–1.27	0.19	
APC-I1307K	29,82,87	Carrier	1.61	1.22–2.11	0.001	Test of heterogeneity $\chi^2 = 0.48$; 2 df; $P = 0.78$
HRAS1-VNTR	28,42–44,75	Carrier (all studies)	2.50	1.54–4.05	0.0002	Test of heterogeneity $\chi^2 = 3.52$; 4 df; $P = 0.48$
	28,42–44	Carrier (alleles defined by Southern blotting)	2.20	1.32–3.67	0.001	Test of heterogeneity $\chi^2 = 1.26$; 3 df; $P = 0.74$
TP53 Arg72Pro	49,54	Arg/Pro	1.05	0.74–1.47	0.26	
		Pro/Pro	1.44	0.89–2.34	0.14	
		Pro-carrier	1.13	0.82–1.55	0.46	

NOTE. Estimates obtained from fixed-effects model.
df, degrees of freedom.

analysis, there was no significant evidence for an association. Although there was no statistical evidence for publication bias, this could not be excluded. Combining the 2 studies that examined NAT2 status and risk of colorectal adenomas,[58,62] there was no evidence for a relationship (OR = 1.08; 95% CI: 0.85–1.39). A minority of studies estimated the risk of colorectal cancer associated with NAT2-rapid acetylator status by site, distinguishing between colon and rectal cancers.[68,76,78] Using these studies, the risks of colon and rectal cancers were 1.09 (0.95–1.25; $P = 0.24$) and 1.05 (0.70–1.58; $P = 0.82$), respectively.

There was no evidence from the meta-analysis to indicate that either GSTM1 or GSTT1 deficiency is significantly associated with an increased risk of colorectal cancer. It has, however, been proposed that any association between GSTM1 deficiency and colorectal cancer might be confined to proximal disease.[48] There was little evidence from a pooled analysis to support this notion. The cancer risk associated with proximal disease was 1.14 (95% CI: 0.85–1.57, $P = 0.38$), and that with distal disease was 1.14 (95% CI: 0.96–1.29, $P = 0.14$). Similarly, there was no evidence to support the postulate that GSTT1 deficiency might confer significant susceptibility to proximal disease, the combined OR being 1.10 (95% CI: 0.72–1.67, $P = 0.44$).

Interaction Between Polymorphisms

The possibilities of gene-gene interactions impacting on colorectal cancer risk have been explored in some of the studies of enzymes involved in carcinogen metabolism. A possible gene-gene interaction between *NAT1*-rapid (defined by the *NAT1*10* allele) and *NAT2*-rapid acetylator status was reported by Bell et al.[55] The interaction was associated with a 2.2-fold elevated risk of colorectal cancer, but this was nonsignificant (95% CI: 0.8–6.1). No support for this notion was seen in the study reported by Chen et al.[72] (OR = 0.78, CI: 0.48–1.27), or in the study of colorectal adenoma risk reported by Probst-Hensch et al.[59] (OR = 1.11,

95% CI: 0.79–1.56). Similarly, no interaction between *NAT1* status, defined by *NAT1*14* and *NAT1*15* alleles, and *NAT2*-rapid acetylator status was found by Hubbard et al.[70] A possible interaction between *GSTM1* and *GSTT1* was evaluated in 3 studies reported by Gertig et al.,[69] Abdel-Rahman et al.,[85] and Deakin et al.[60] No interaction between the 2 loci was seen in these studies. Paradoxically, an interaction between *NAT2*-slow acetylator and *GSTT1*-null status was reported by Welfare et al.[84] (OR = 2.33, 95% CI: 1.1–5.0).

Polymorphism-Environment Interaction

Dietary folate, methionine, and alcohol intake have been associated in some, but not all, epidemiologic studies with risk of colorectal neoplasia.[93,94] How these dietary factors might impact on colorectal cancer risk is not well established, but one unifying mechanism is that they act in concert through DNA methylation. Although modest depletion of dietary folate may not be sufficient to cause hypomethylation in vivo, high levels of alcohol might reduce levels of *S*-adenosyl methionine sufficient to impact on patterns of DNA methylation. There is some support for this from the studies of $MTHFR^{Asp/Val}$, in which the inverse relationship between homozygosity and cancer risk was not seen in individuals with diets deficient in methionine or rich in alcohol.[64,66,88] The adverse effect of alcohol on risk was greater in homozygotes and the protective effect of genotype diminished by folate deficiency. No significant interaction between genotype and folate, methionine, or alcohol intake on polyp formation was, however, seen,[71] suggesting any effect on cancer risk is post-adenoma.

Diets high in animal fat and red meat have been proposed to increase the risk of colorectal neoplasia.[95,96] *NAT* is of particular interest in respect to metabolism of polycyclic amines derived from cooked meats. Positive associations between red meat intake, *NAT2* acetylator status, and colorectal neoplasia have been reported in the studies by Lang et al.,[40] Chen et al.,[72] Probst-Hensch et al.,[58] Roberts-Thomson et al.,[62] and Welfare et al.[68] Other lifestyle factors suggested as risk factors for colorectal neoplasia include cigarette smoking, although the evidence is equivocal.[97–99] Higher levels of DNA damage have been observed in individuals with *GSTM1* and *GSTT1* null genotypes in heavy smokers compared with nonsmokers. There is, however, no evidence to date from the studies of Katoh et al.,[61] Gertig et al.,[69] or Slattery et al.[76] to support an interaction between *GSTM1* and *GSTT1* and smoking on colorectal cancer risk. Welfare et al.[68] purported that there is an interaction between NAT2-status and smoking, but this was not supported by Slattery et al.[76]

Discussion

Research into genetic susceptibility to colorectal cancer has, until recently, largely focused on the identification of genes causing dominantly inherited diseases such as FAP, hereditary nonpolyposis colorectal cancer, and, to a lesser extent, Peutz-Jeghers syndrome and juvenile polyposis. Mutations in the genes in their classical guise (resulting in a truncated protein) are associated with a significant risk of colorectal cancer, but carriers in the general population are rare; hence, the overall impact of such mutations on cancer incidence will be small. As opportunities to identify further high penetrance genes diminish, increasing attention is being paid to the possibility that common low penetrance genes might in part determine susceptibility to colorectal cancer.

Substantial research has been performed evaluating the polymorphisms in a number of genes as low penetrance colorectal tumor susceptibility alleles. Has this all been worthwhile? There is no clear evidence that any is strongly associated with risk of colorectal cancer or adenomas. Of the 24 variants examined to date, few have been reported as statistically significant in more than one study—defined by a P value of 0.05. Given the issue of multiple testing in many reports, it is clearly advantageous when interpreting any purported association to stipulate low P values, or to replicate findings in independent samples, or ideally both. The issue of if it is appropriate to correct for multiple testing and on what basis this should be undertaken is a thorny issue arousing considerable debate. Boehringer et al.[100] have argued that a correction should be based on the number of polymorphisms analyzed in any one gene rather than on the total number of polymorphisms analyzed if more than one gene is evaluated.

In some studies, the failure to demonstrate a relationship may in part be a consequence of lack of power. It is unlikely that any common predisposition allele will confer much more than a twofold difference in the risk of colorectal tumors. Fewer than 40% of studies had 80% power to show a twofold difference in risk at the 0.05 significance level. To overcome this lack of power, a meta-analysis was undertaken by pooling the data from published studies. There are, however, caveats to this statistical procedure. In any systematic review, publication bias is clearly of great concern. The most common scenario is that negative findings may go unreported. We cannot exclude this possibility for the 3 polymorphisms that seem to confer a risk of colorectal cancer (*HRAS1*-VNTR, $MTHFR^{Ala/Val}$, and *APCI1307K*). Furthermore, many studies do not describe the ethnicity of cases or

controls, and it is assumed that each polymorphism is functional with respect to risk in each study population. This is likely to be the case with *MTHFR* and presumably also, at least in part, with *APCI1307K*. If, however, the polymorphism is a neutral marker for another variant, the assumption may well not apply, because linkage disequilibrium can be population-dependent. The studies of the risks associated with *APCI1307K* are unique in this respect, having been performed exclusively in the Ashkenazi population. Another potential issue in combining data relates to designation of individual polymorphisms. In some genes, multiple polymorphisms can be detected by the same assay. This is clearly a problem with microsatellite or minisatellite polymorphisms, such as the *HRAS1*-VNTR, in which assigning alleles can be problematic. The choice of the cutoff between rare and common alleles is crucial, and allele definition may be different between studies. The low resolution of the Southern blot method used in early studies of the *HRAS1*-VNTR may have failed to distinguish some rare alleles.[101] This, coupled with post hoc analysis of data in studies, suggests that the relationship between rare alleles and risk of colorectal cancer should be revisited.

Ideally, quality scoring of studies should be used to determine which studies are included in any meta-analysis.[102] This was not undertaken because existing scales have not been validated and it is also unclear how they could readily be applied to the published studies of polymorphisms and colorectal tumor risk.[103]

If genetic susceptibility to colorectal cancer is mediated through polymorphic variation, it is probable that combinations of genotypes should be more discriminating as risk factors than a single locus genotype, especially if a multiplicative model of interaction operates. Some investigators have performed subgroup analyses; however, in many cases the data were "mined" to detect a significant finding.

An important lesson from current studies is that greater attention should be paid to design in future studies. It has been argued that in association studies based on genotyping, it is unnecessary to take into account factors such as age or concomitant disease, because they do not influence genotype.[104] The age-structure of cases and controls is, however, a source of potential bias, albeit a minor one, because genotypes may display age-dependency. For example, the population frequencies of *APO-E* genotypes show age-dependency.[105] Furthermore, age is relevant to determining the probable exposure to carcinogens. A difference in the ages between cases and controls is therefore a potential source of bias. Considering the issues of concomitant disease, a number of the studies were based on a comparison of cases and noncolon cancer disease controls. The use of healthy population controls is preferable, because it is conceivable that the polymorphism might confer susceptibility to noncancer diseases.

The issue of population stratification in case-control studies and resulting false positive results is also of great concern. If population subdivisions exist, it will be possible to find associations between disease and arbitrary markers that are unlinked to causative loci. These associations occur because of population subdivision and nonrandom mating, leading to variation in the marker frequency within the population as a result of founder effects and/or genetic drift. The severity of spurious association becomes an increasing problem with increasing study size. To avoid the problem of such spurious associations, it is essential that any potential confounding effect of population stratification be allowed for in the design and analysis of a study. This requires the identification of subpopulations in terms of factors that can influence both disease and marker allele frequencies. Provided cases and controls are well-matched, differences in the frequency of genotypes will only be seen at predisposition loci. Hence, stratification can be detected by typing a series of unlinked markers chosen from a panel known to exhibit differences in allele frequency between populations.[106] The appropriate Bonferroni correction would be required to assess the statistical significance of any putative association. One method of circumventing the problem of occult population stratification is to use family-based controls. The most common approach is the transmission disequilibrium test, which assesses the evidence for preferential transmission of one allele over the other from heterozygous parents.[107] To obviate the requirement for parental genotypes, allied statistics based on the use of sibling genotypes have been devised.[108,109]

Despite these concerns, it is possible to draw a number of conclusions from the evidence. The *APC*-I1307K, *HRAS1*-VNTR rare alleles, and *MTHFR*$^{Ala/Val}$ variants seem to represent the strongest candidates for low penetrance susceptibility alleles of the polymorphisms evaluated to date. Although the genotypic risks associated with these variants are modest, because they are not uncommon, they will have considerable impact on the overall burden of colorectal cancer. For example, *APC-I1307K* may account for up to 6% of all Ashkenazi Jewish colorectal cancer. For polymorphisms of *NAT1*, *NAT2*, *GSTM1*, and *GSTT1*, the best estimate of risk obtained from the meta-analysis is sufficient to exclude a colorectal cancer risk greater than 1.7-fold. For the remainder—*GSTP1*, *CYP1A1*, *mEPHX*, *MTR*, and secre-

tory phospholipase A2 (*PLAG2A*)—the estimates of risk are too imprecise and, although there is no good evidence to implicate them to date, moderate increases in risk of colorectal cancer cannot be excluded. To derive precise estimates of the risks associated with variants of these genes and to investigate gene-gene and gene-environment interactions will require much larger sample sizes than have been used in published studies.

The notion that inter-individual differences in colorectal cancer risk may in part be attributable to polymorphic variation is an attractive hypothesis. Alleles of other genes such as the DNA repair genes or other metabolic enzymes represent candidates for future studies. At present, association studies in colorectal cancer are restricted to evaluating polymorphisms in a small number of candidate genes. In the medium term, polymorphisms for all genes will be identified. This, and the introduction of reliable, high-density oligonucleotide arrays (or alternative methods) will allow allelic association studies to be conducted on a genome-wide basis. The detection of low penetrance colorectal cancer susceptibility genes will then only be restricted by the availability of large cohorts of well-characterized cases and controls. The polymorphism studies that have been conducted to date have important lessons for the design and execution of future studies. It is clear that in addition to conducting studies using sample sizes commensurate with the detection of low penetrance polymorphisms, attention should be paid to adequate matching of cases and controls to avoid problems of population stratification and other sources of potential bias.

References

1. HMSO. Cancer Statistics. Registrations. England and Wales. London, UK: HMSO, 1989.
2. HMSO. Mortality Statistics. Cause. England and Wales. London, UK: HMSO, 1992.
3. Finlay IG, Pickford IR. Colorectal carcinoma. In: Lawson DH, ed. Current medicine. 4th ed. Edinburgh, UK: Churchill Livingstone, 1994:51–64.
4. Burt RW, Bishop DT, Lynch HT, Rozen P, Winawer SJ. Risk and surveillance of individuals with heritable factors for colorectal cancer. WHO Collaborating Centre for the Prevention of Colorectal Cancer. Bull World Health Organ 1990;68:655–665.
5. Jarvinen HJ. Epidemiology of familial adenomatous polyposis in Finland: impact of family screening on the colorectal cancer rate and survival. Gut 1992;33:357–360.
6. Lynch HT, Smyrk T, Lynch JF. Overview of natural history, pathology, molecular genetics and management of HNPCC (Lynch Syndrome). Int J Cancer 1996;69:38–43.
7. Houlston RS, Peto J. Genetics of common cancers. In: Eeles RA, Ponder B, Easton DE, Horwich A, eds. Inherited predisposition to cancer. 1st ed. London, UK: Chapman Hall Medical, 1996:208–226.
8. Risch N, Merikangas K. The future of genetic studies of complex human disease. Science 1996;273:1516–1517.
9. Sham P. Statistics in human genetics. 1st ed. London, UK: Arnold Publications, 1998.
10. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–767.
11. Ilyas M, Tomlinson IPM. Genetic pathways in colorectal cancer. Histopathology 1996;28:389–399.
12. Smith G, Stanley LA, Sim E, Strange RC, Wolf CR. Metabolic polymorphisms and cancer susceptibility. Cancer Surv 1995; 25:27–65.
13. Kawajiri K. CYP1A1. In: Vineis P, Malatas N, Lang M, d'Errico A, Caporosa N, Cuzick J, Bofetta P, eds. Metabolic polymorphisms and susceptibility to cancer. Lyon, France: IARC Sci Publ, 1999: 159–172.
14. Hirvonen A. Polymorphic NATs and cancer predisposition. In: Vineis P, Malatas N, Lang M, d'Errico A, Caporosa N, Cuzick J, Bofetta P, eds. Metabolic polymorphisms and susceptibility to cancer. Lyon, France: IARC Sci Publ, 1999:251–270.
15. Feng Y, Fretland AJ, Rustan TD, Jiang W, Becker WK, Hein DW. Higher frequency of aberrant crypt foci in rapid than slow acetylator inbred rats administered the colon carcinogen 3,2′-dimethyl-4-aminobiphenyl. Toxicol Appl Pharmacol 1997;147: 56–62.
16. Strange RC, Fryer AA. The glutathione S-transferases: influence of polymorphism on cancer susceptibility. In: Vineis P, Malatas N, Lang M, d'Errico A, Caporosa N, Cuzick J, Bofetta P, eds. Metabolic polymorphisms and susceptibility to cancer. Lyon, France: IARC Sci Publ, 1999:231–249.
17. Wormhoudt LW, Commandeur JN, Vermeulen NP. Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit Rev Toxicol 1999;29:59–124.
18. Harrison DJ, Hubbard AL, MacMillan J, Wyllie AH, Smith CA. Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. Br J Cancer 1999;79:168–171.
19. Breivik J, Gaudernack G. Genomic instability, DNA methylation, and natural selection in colorectal carcinogenesis. Semin Cancer Biol 1999;9:245–254.
20. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A 1999;96:8681–8686.
21. Kim YI, Pogribny IP, Salomon RN, Choi SW, Smith DE, James SJ, Mason JB. Exon-specific DNA hypomethylation of the p53 gene of rat colon induced by dimethylhydrazine. Modulation by dietary folate. Am J Pathol 1996;149:1129–1137.
22. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.
23. Cormier RT, Hong KH, Halberg RB, Hawkins TL, Richardson P, Mulherkar R, Dove WF, Lander ES. Secretory phospholipase Pla2g2a confers resistance to intestinal tumorigenesis. Nat Genet 1997;17:88–91.
24. Dragani TA, Manenti G. Mom1 leads the pack. Nat Genet 1997; 17:7–8.
25. Riggins GJ, Markowitz S, Wilson JK, Vogelstein B, Kinzler KW. Absence of secretory phospholipase A2 gene alterations in human colorectal cancer. Cancer Res 1995;55:5184–5186.
26. Reddy BS, Wynder EL. Metabolic epidemiology of colon cancer. Faecal bile acids and neutral sterols in colon cancer patients and patients with adenomaous polyps. Cancer 1977;39:2533–2539.
27. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1–21.
28. Krontiris TG, Devlin B, Karp DD, Robert NJ, Risch N. An associ-

ation between the risk of cancer and mutations in the HRAS1 minisatellite locus. N Engl J Med 1993;329:517–523.
29. Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostrer H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D, Jhanwar S, Winawer S, Offit K, Luce MC, Kinzler KW, Vogelstein B. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. Nat Genet 1997;17:79–83.
30. Evans SC, Lozano G. The Li-Fraumeni syndrome: an inherited susceptibility to cancer. Mol Med Today 1997;3:390–395.
31. Sung SSJ, Bjorndahl JM, Wang CY, Kao HT, Fu SM. Production of tumour necrosis factor/cachectin by human T cell lines and peripheral blood lymphocytes stimulated by phorbol myristrate acetate and anti-CD3 antibody. J Exp Med 1988;167:937–953.
32. Shimura T, Hagihura M, Takebe K, Munkhbat B, Odaka T, Kata H, Nagamachi Y, Tsuji K. The study of tumour necrosis factor beta polymorphism in lung cancer patients. Cancer 1994;73: 1184–1188.
33. Shimura T, Hagihura M, Takebe K, Munkhbat B, Odaka T, Kata H, Nagamachi Y, Tsuji K. 10.5kb homozygote of tumour necrosis factor-beta gene is associated with a better prognosis in gastric cancer patients. Cancer 1995;75:1450–1453.
34. Breslow NE, Day NE. Statistical methods in cancer research., Vol. 1 & 2. Lyon, France: IARC Sci Publ., 1987.
35. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials1986;7:177–188.
36. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple graphical test. BMJ 1997;315: 629–634.
37. Sharpe S, Sterne S. META. Stata Technical Bulletin 38 and 42. Available at: http://www.stata.com. 1998. Accessed May 2000.
38. Steichen T. METABIAS. Stata Technical Bulletin 41. Available at: http://www.stata.com. 1998. Accessed May 2000.
39. Fleiss JL, Tytun A, Uray HK. A simple approximation for calculating sample sizes for comparing independent proportions. Biometrics 1980;36:343–346.
40. Lang NP, Chu DZ, Hunter CF, Kendall DC, Flammang TJ, Kadlubar FF. Role of aromatic amine acetyltransferase in human colorectal cancer. Arch Surg 1986;121:1259–1261.
41. Ilett KF, David BM, Detchon P, Castleden WM, Kwa R. Acetylation phenotype in colorectal carcinoma. Cancer Res 1987;47: 1466–1469.
42. Ceccherini-Nelli L, De Re V, Viel A, Molaro G, Zilli L, Clemente C, Boiocchi M. Ha-ras-1 restriction fragment length polymorphism and susceptibility to colon adenocarcinoma. Br J Cancer 1987; 56:1–5.
43. Wyllie FS, Wynford-Thomas V, Lemoine NR, Williams GT, Williams ED, Wynford-Thomas D. Ha-ras restriction fragment length polymorphisms in colorectal cancer. Br J Cancer 1988;57:135–138.
44. Klingel R, Mittelstaedt P, Dippold WG, Meyer zum Buschenfelde KH. Distribution of Ha-ras alleles in patients with colorectal cancer and Crohn's disease. Gut 1991;32:1508–1513.
45. Ladero JM, Gonzalez JF, Benitez J, Vargas E, Fernandez MJ, Baki W, Diaz-Rubio M. Acetylator polymorphism in human colorectal carcinoma. Cancer Res 1991;51:2098–2100.
46. Strange RC, Matharoo B, Faulder GC, Jones P, Cotton W, Elder JB, Deakin M. The human glutathione S-transferases: a case-control study of the incidence of the GST1 0 phenotype in patients with adenocarcinoma. Carcinogenesis 1991;12:25–28.
47. Rodriguez JW, Kirlin WG, Ferguson RJ, Doll MA, Gray K, Rustan TD, Lee ME, Kemp K, Urso P, Hein DW. Human acetylator genotype: relationship to colorectal cancer incidence and arylamine N-acetyltransferase expression in colon cytosol. Arch Toxicol 1993;67:445–452.
48. Zhong S, Wyllie AH, Barnes D, Wolf CR, Spurr NK. Relationship between the GSTM1 genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis 1993;14: 1821–1824.
49. Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi S. Germ line polymorphisms of p53 and CYP1A1 genes involved in human lung cancer. Carcinogenesis 1993;14:1085–1089.
50. Sivaraman L, Leatham MP, Yee J, Wilkens LR, Lau AF, Le Marchand L. CYP1A1 genetic polymorphisms and in situ colorectal cancer. Cancer Res 1994;54:3692–3695.
51. Shibuta K, Nakashima T, Abe M, Mashimo M, Mori M, Ueo H, Akiyoshi T, Sugimachi K, Suzuki T. Molecular genotyping for N-acetylation polymorphism in Japanese patients with colorectal cancer. Cancer 1994;74:3108–3112.
52. Oda Y, Tanaka M, Nakanishi I. Relation between the occurrence of K-ras gene point mutations and genotypes of polymorphic N-acetyltransferase in human colorectal carcinomas. Carcinogenesis 1994;15:1365–1369.
53. Hall NR, Taylor GR, Finan PJ, Kolodner RD, Bodmer WF, Cottrell SE, Frayling I, Bishop DT. Intron splice acceptor site sequence variation in the hereditary non-polyposis colorectal cancer gene hMSH2. Eur J Cancer 1994;30A:1550–1552.
54. Sjalander A, Birgander R, Athlin L, Stenling R, Rutegard J, Beckman L, Beckman G. P53 germ line haplotypes associated with increased risk for colorectal cancer. Carcinogenesis 1995;16: 1461–1464.
55. Bell DA, Stephens EA, Castranio T, Umbach DM, Watson M, Deakin M, Elder J, Hendrickse C, Duncan H, Strange RC. Polyadenylation polymorphism in the acetyltransferase 1 gene (NAT1) increases risk of colorectal cancer. Cancer Res 1995; 55:3537–3542.
56. Chenevix-Trench G, Young J, Coggan M, Board P. Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. Carcinogenesis 1995;16:1655–1657.
57. Lin HJ, Probst-Hensch NM, Ingles SA, Han CY, Lin BK, Lee DB, Frankl HD, Lee ER, Longnecker MP, Haile RW. Glutathione transferase (GSTM1) null genotype, smoking, and prevalence of colorectal adenomas. Cancer Res 1995;55:1224–1226.
58. Probst-Hensch NM, Haile RW, Ingles SA, Longnecker MP, Han CY, Lin BK, Lee DB, Sakamoto GT, Frankl HD, Lee ER, Lin HJ. Acetylation polymorphism and prevalence of colorectal adenomas. Cancer Res 1995;55:2017–2020.
59. Probst-Hensch NM, Haile RW, Li DS, Sakamoto GT, Louie AD, Lin BK, Frankl HD, Lee ER, Lin HJ. Lack of association between the polyadenylation polymorphism in the NAT1 (acetyltransferase 1) gene and colorectal adenomas. Carcinogenesis 1996; 17:2125–2129.
60. Deakin M, Elder J, Hendrickse C, Peckham D, Baldwin D, Pantin C, Wild N, Leopard P, Bell DA, Jones P, Duncan H, Brannigan K, Alldersea J, Fryer AA, Strange RC. Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. Carcinogenesis 1996;17:881–884.
61. Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, Ookuma R, Bell DA. Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. Carcinogenesis 1996;17: 1855–1859.
62. Roberts-Thomson IC, Ryan P, Khoo KK, Hart WJ, McMichael AJ, Butler RN. Diet, acetylator phenotype, and risk of colorectal neoplasia. Lancet 1996;347:1372–1374.
63. Tomlinson IP, Beck NE, Neale K, Bodmer WF. Variants at the secretory phospholipase A2 (PLA2G2A) locus: analysis of associations with familial adenomatous polyposis and sporadic colorectal tumours. Ann Hum Genet 1996;60:369–376.
64. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. A methylenetetrahy-

drofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4864.
65. Kervinen K, Sodervik H, Makela J, Lehtola J, Niemi M, Kairaluoma MI, Kesaniemi YA. Is the development of adenoma and carcinoma in proximal colon related to apolipoprotein E phenotype? Gastroenterology 1996;110:1785–1790.
66. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–1102.
67. Hubbard AL, Harrison DJ, Moyes C, Wyllie AH, Cunningham C, Mannion E, Smith CA. N-acetyltransferase 2 genotype in colorectal cancer and selective gene retention in cancers with chromosome 8p deletions. Gut 1997;41:229–234.
68. Welfare M, Cooper J, Bassendine MF, Daly AK. Relationship between acetylator status, smoking, diet and colorectal cancer risk in the north-east of England. Carcinogenesis 1997;18: 1351–1354.
69. Gertig DM, Stampfer M, Haiman C, Hennekens CH, Kelsey K, Hunter DJ. Glutathione S-transferase GSTM1 and GSTT1 polymorphisms and colorectal cancer risk: a prospective study. Cancer Epidemiol Biomarkers Prev 1998;7:1001–1005.
70. Hubbard AL, Moyes C, Wyllie AH, Smith CA, Harrison DJ. N-acetyl transferase 1: two polymorphisms in coding sequence identified in colorectal cancer patients. Br J Cancer 1998;77:913–916.
71. Chen J, Giovannucci E, Hankinson SE, Ma J, Willett WC, Spiegelman D, Kelsey KT, Hunter DJ. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis 1998;19:2129–2132.
72. Chen J, Stampfer MJ, Hough HL, Garcia-Closas M, Willett WC, Hennekens CH, Kelsey KT, Hunter DJ. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res 1998;58:3307–3311.
73. Harris MJ, Coggan M, Langton L, Wilson SR, Board PG. Polymorphism of the Pi class glutathione S-transferase in normal populations and cancer patients. Pharmacogenetics 1998;8:27–31.
74. Frayling IM, Beck NE, Ilyas M, Dove-Edwin I, Goodman P, Pack K, Bell JA, Williams CB, Hodgson SV, Thomas HJ, Talbot IC, Bodmer WF, Tomlinson IP. The APC variants I1307K and E1317Q are associated with colorectal tumors, but not always with a family history. Proc Natl Acad Sci U S A 1998;95:10722–10727.
75. Gosse-Brun S, Sauvaigo S, Daver A, Larra F, Kwiatkowski F, Bignon YJ, Bernard-Gallon D. Association between H-ras minisatellite and colorectal cancer risk. Anticancer Res 1998;18: 2611–2616.
76. Slattery ML, Potter JD, Samowitz W, Bigler J, Caan B, Leppert M. NAT2, GSTM1, cigarette smoking and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1998;7:1079–1084.
77. Lee EJD, Zhao B, Seow-Choen F. Relationship between polymorphism of n-acetyltransferase gene and susceptibility to colorectal carcinoma in a chinese population. Pharmacogenetics 1998; 8:513–517.
78. Lee EJD, Zhao B, Seow-Choen F, Balakrishman A, Chan SH. Genetic polymorphism of conjugating enzymes and cancer risk: GSTM1, GSTT1, NAT1 and NAT2. J Toxicol Sci 1998;23(suppl II):140–142.
79. Gil JP, Lechner MC. Increased frequency of wild-type arylamine-N-acetyltransferase allele NAT2*4 homozygotes in Portugese patients with colorectal cancer. Carcinogenesis 1998;19: 37–41.
80. Park KS, Mok JW, Rho SA, Kim JC. Analysis of TNFB and TNFA NcoI RFLP in colorectal cancer. Mol Cells 1998;8:246–249.
81. Lin HJ, Probst-Hensch NM, Hughes NC, Sakamoto GT, Louie AD, Kau IH, Lin BK, Lee DB, Lin J, Frankl HD, Lee ER, Hardy S, Grant DM, Haile RW. Variants of N-acetyltransferase NAT1 and a case-control study of colorectal adenomas. Pharmacogenetics 1998;8:269–281.
82. Woodage T, King SM, Wacholder S, Hartge P, Struewing JP, McAdams M, Laken SJ, Tucker MA, Brody LC. The APCI1307K allele and cancer risk in a community-based study of Ashkenazi Jews. Nat Genet 1998;20:62–65.
83. Zhang H, Ahmadi A, Arbman G, Zdolsek J, Carstensen J, Nordenskjold B, Soderkvist P, Sun XF. Glutathione S-transferase T1 and M1 genotypes in normal mucosa, transitional mucosa and colorectal adenocarcinoma. Int J Cancer 1999;84:135–138.
84. Welfare M, Monesola Adeokun A, Bassendine MF, Daly AK. Polymorphisms in GSTP1, GSTM1, and GSTT1 and susceptibility to colorectal cancer. Cancer Epidemiol Biomarkers Prev 1999; 8:289–292.
85. Abdel-Rahman SZ, Soliman AS, Bondy ML, Wu X, El-Badawy SA, Mahgoub KG, Ismail S, Seifeldin IA, Levin B. Polymorphism of glutathione S-transferase loci GSTM1 and GSTT1 and susceptibility to colorectal cancer in Egypt. Cancer Lett 1999;142:97–104.
86. Harrison DJ, Hubbard AL, MacMillan J, Wyllie AH, Smith CA. Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. Br J Cancer 1999;79:168–171.
87. Gryfe R, Di Nicola N, Lal G, Gallinger S, Redston M. Inherited colorectal polyposis and cancer risk of the APC I1307K polymorphism. Am J Hum Genet 1999;64:378–384.
88. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999;8:513–518.
89. Ma J, Stampfer MJ, Christensen B, Giovannucci E, Hunter DJ, Chen J, Willett WC, Selhub J, Hennekens CH, Gravel R, Rozen R. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:825–829.
90. Park KS, Mok JW, Kim JC. The 677C > T mutation in 5,10-methylenetetrahydrofolate reductase and colorectal cancer risk. Genet Test 1999;3:233–236.
91. Yoshioka M, Katoh T, Nakano M, Takasawa S, Nagata N, Itoh H. Glutathione S-transferase (GST) M1, T1, P1, N-acetyltransferase (NAT) 1 and 2 genetic polymorphisms and susceptibility to colorectal cancer. Sangyo Ika Daigaku Zasshi 1999;21:133–147.
92. Gawronska-Szklarz B, Lubinski J, Klandy J, Kurzawski G, Bieliki D, Wojcicki M, Sych Z, Musial HD. Polymorphism of GSTM1 gene in patients with colorectal cancer and colonic polyps. Exp Toxicol Pathol 1999;51:321–325.
93. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate intake and carcinogenesis of the colon and rectum. Int J Epidemiol 1991;20:368–374.
94. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–273.
95. Bingham SA. High-meat diets and cancer risk. Proc Nutr Soc 1999;58:243–248.
96. Giovannucci E, Goldin B. The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. Am J Clin Nutr 1997;66:1564S–1571S.
97. Newcomb PA, Storer BE, Marcus PM. Cigarette smoking in relation to risk of large bowel cancer in women. Cancer Res 1995;55:4906–4909.
98. Sturmer T, Glynn RJ, Lee IM, Christen WG, Hennekens CH. Lifetime cigarette smoking and colorectal cancer incidence in the Physicians' Health Study I. J Natl Cancer Inst 2000;92: 1178–1181.
99. Tavani A, Gallus S, Negri E, Franceschi S, Talamini R, La Vecchia C. Cigarette smoking and risk of cancers of the colon and

rectum: a case-control study from Italy. Eur J Epidemiol 1998; 14:675–681.
100. Boehringer S, Epplen JT, Krawczak M. Genetic association studies of bronchial asthma—a need for Bonferroni correction? Hum Genet 2000;107:197.
101. Firgaira FA, Seshadri R, McEvoy CR, Dite GS, Giles GG, McCredie MR, Sothery MC, Venter DJ, Hopper JL. HRAS1 rare minisatellite alleles and breast cancer in Australian women under age forty years. J Natl Cancer Inst 1999;91:2107–2111.
102. Sacks HS, Berrier J, Reitman D, Ancona-Beck VA, Chalmers TC. Meta-analyses of randomized controlled trials. N Engl J Med 1987;316:450–455.
103. Dickersin K, Berlin JA. Meta-analysis: state-of-science. Epidemiol Rev 1992;4:154–176.
104. Wolf CR, Smith CA, Bishop T, Forman D, Gough AC, Spurr NK. CYP2D6 genotyping and the association with lung cancer susceptibility. Pharmacogenetics 1994;4:104–106.
105. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1–21.
106. Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. Am J Hum Genet 1999;65:220–228.
107. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 1993;52: 506–516.
108. Horvath S, Laird NM. A discordant-sibship test for disequilibrium and linkage: no need for parental data. Am J Hum Genet 1998;63:1886–1897.
109. Knapp M. The transmission/disequilibrium test and parental-genotype reconstruction: the reconstruction-combined transmission/disequilibrium test. Am J Hum Genet 1999;64:861–870.

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