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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEWS

Glutathione *S*-Transferase Polymorphisms and Colorectal Cancer: A HuGE Review

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The genes glutathione *S*-transferase *M1* (*GSTM1*) (chromosome 1p13.3) and glutathione *S*-transferase *T1* (*GSTT1*) (22q11.2) code for cytosolic enzymes glutathione *S*-transferase (GST)-μ and GST-θ, respectively, which are involved in phase 2 metabolism. Both genes may be deleted. There is geographic and ethnic variation in genotype frequencies for both genes. In developed countries, colorectal cancer is the second most common cancer. Colorectal cancer has been inconsistently associated with polycyclic aromatic hydrocarbons in diet and tobacco. Because GST enzymes are involved in polycyclic aromatic hydrocarbon metabolism, it has been postulated that genotype may modify colorectal cancer risk associated with polycyclic aromatic hydrocarbon exposure. No consistent associations between *GSTM1* or *GSTT1* genotype and colorectal cancer have been observed. However, most studies have methodological limitations. Few have investigated gene-environment interactions. No interactions between *GSTM1* or *GSTT1* genotype and smoking and colorectal cancer risk have been reported. One polyp study suggests an interaction between *GSTM1* genotype and smoking. Two studies suggest increased disease risk in subjects with high meat intake and GST nonnull genotype, contrary to the underlying hypothesis. One study suggests a strong inverse relation between colorectal adenomas and broccoli consumption, particularly in subjects who are *GSTM1* null. These finding require confirmation. Methods for determining *GSTM1* and *GSTT1* genotype are well established. Population testing is not currently justified. *Am J Epidemiol* 2000;151:7–32.

colorectal neoplasms; epidemiology; glutathione transferase; *GSTM1*; *GSTT1*

GENE

Four glutathione *S*-transferase (GST) isoenzyme classes have been identified—α, μ, π, and θ (1). Here we consider the two types most investigated in relation to colorectal cancer—GST-μ and GST-θ. These are summarized in table 1.

The glutathione *S*-transferase *M1* (*GSTM1*) and glutathione *S*-transferase *T1* (*GSTT1*) genes code for the cytosolic enzymes GST-μ and GST-θ respectively. These enzymes are involved in the conjugation reactions in phase 2 metabolism of xenobiotics (1), catalyzing reactions between glutathione and a variety of electrophilic compounds (2). It is thought that most GST substrates are xenobiotics or products of oxidative stress, including some environmental carcinogens (1). In particular, the enzymes detoxify the carcinogenic polycyclic aromatic hydrocarbons present in diet and tobacco smoke (3). They also conjugate isothiocyanates, which are potent inducers of enzymes that detoxify environmental mutagens (4), to glutathione, thereby diverting the isothiocyanates from the enzyme induction pathway to excretion (5). It has been postulated that the GST enzymes and the genes encoding these may be involved in susceptibility to cancer (6).

The genes coding for the enzymes GST-μ and GST-θ are polymorphic. There are three alleles at the *GSTM1* locus, located on chromosome 1p13.3: *GSTM1* null—a deletion, *GSTM1*a, and *GSTM1*b (6). *GSTM1*a and b differ by a substitution in one base pair. There is no evidence of functional differences between them (6). The *GSTT1* locus is located on chromosome 22q11.2 and is, in some instances, deleted (6). For both *GSTM1* and *GSTT1*, the hypothesized consequence of the null genotype is reduced conjugation activity or no conjugation activity. Evidence is lacking on whether heterozygosity in either *GSTM1* or *GSTT1* affects gene function.

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Abbreviations: GST, glutathione *S*-transferase enzyme; *GSTM1*, glutathione *S*-transferase *M1* gene; *GSTT1*, glutathione *S*-transferase *T1* gene; *NAT2*, *N*-acetyltransferase 2; RR, relative risk.

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TABLE 1. Glutathione *S*-transferase polymorphisms*

Gene	Chromosome location	Known alleles	Isoenzymes coded for	Function	Hypothesized consequence of null genotype
GSTM1	1p13.3	Null *GSTM1*a† *GSTM1*b†	GST-μ	Phase 2 metabolism of xenobiotics	Reduced or no conjugation activity
GSTT1	22q11.2	Null Present	GST-θ	Phase 2 metabolism of xenobiotics	Reduced or no conjugation activity

* The subfamilies *GSTA* and *GSTP* that code for the isoenzymes GST-α and GST-π also exist, but are not considered in this review.

† These two alleles differ by only one base pair. There is no evidence of functional differences between them.

GENE VARIANTS

We searched MEDLINE and EMBASE using the *Medical Subjects Headings* in *Index Medicus* heading "glutathione transferase" and the text words "GST" and "glutathione *S*-transferase" for papers published between 1993 and 1998. We also searched the Centers for Disease Control and Prevention Office of Genetics and Disease Prevention Medical Literature Search and reviewed reference lists in published articles. We identified relevant papers and critically appraised them. This section includes studies that reported genotype frequencies in a variety of groups of individuals who did not have cancer.

The frequency of individuals who are homozygous for the *GSTM1* null genotype is summarized in table 2 (7–78), and those homozygous for the *GSTT1* null genotype are summarized in table 3 (8, 10, 11, 14, 29, 32, 35–37, 39, 47–49, 51–53, 55, 58, 59, 63, 65, 68, 73–75, 77, 78, 80–85). Many of the series were control groups in case-control studies of cancer. However, few could be described as truly population based; therefore, selection or participation biases may account for some of the variation between studies. Some of the studies have small numbers of participants. It is not always easy to establish ethnicity, nor is it necessarily sufficient to simply categorize individuals as belonging to one of the major ethnic groups (86); this limits the generalizability from, for example, one "white" population to another.

GSTM1

In African populations, the frequency of the *GSTM1* null genotype ranges from 23 to 48 percent; in Asian populations, from 33 to 63 percent; and in European populations, from 39 to 62 percent.

Published data from the Americas relate only to studies carried out in the United States; the range of reported frequencies is 23–62 percent. In African Americans and Blacks, the range is 23–41 percent, and in whites, it is 35–62 percent. In the two studies of subjects of Asian origin, the range was 32–53 percent, and in the three studies that included Hispanic/Mexican-American subjects, the range was 40–53 percent.

In two Australian series, the frequency is 51–54 percent. The highest frequencies have been reported in studies involving small numbers of subjects from parts of the South Pacific—64–100 percent (13). These studies differed from the others in that Southern blot analysis rather than polymerase chain reaction methodology was used.

GSTT1

The range of frequencies of the *GSTT1* null genotype is 16–64 percent in Asia, with frequencies of 44 percent or higher being reported in China, Japan, Korea, and the Singapore Chinese. Thus, in some Asian populations, it has been suggested that the frequency of *GSTT1* null deletions is similar to that of *GSTM1* null. However, in African, African-American, and white populations, the frequencies of *GSTT1* null are lower than those of *GSTM1* null. The range of frequencies in three African series is 15–26 percent, and in Europe, it is 10–21 percent. As was the case for *GSTM1*, data from the Americas relate only to the United States, where the range of frequencies is 10–36 percent. In whites, the range is 15–27 percent; in African Americans and Blacks, it is 22–29 percent; and in Mexican Americans, based on two studies, it is 10–12 percent. No data on Asian subjects in the United States are available. In three groups in Australia, the frequency of *GSTT1* null ranged from 9 to 19 percent.

Concordance between genotype and phenotype

Individuals lacking GST-μ or GST-θ activity can be identified by using phenotypic assays that classify individuals as active or inactive on the basis of a bimodal distribution. Use of polymerase chain reaction methodology indicates the presence or absence of the *GSTM1* or *GSTT1* alleles. Several studies have investigated concordance between genotype and phenotype; this can be a means of determining whether the appro-

priate section of DNA coding for the particular phenotype has been identified.

Four studies in Europe and one in the United States have demonstrated concordances between *GSTM1* genotype and GST-μ phenotype of 94 percent or greater (26, 33, 87–89). However, in one study in which genotype and phenotypic status were compared in 63 healthy Zimbabwean volunteers, concordance was lower, at 84 percent (90). This may have been due to the presence of 1) other mutations that affect protein expression, 2) compounds in the diet that may affect protein levels, or 3) mutations in the regions of the gene that bind to the primers during the polymerase chain reactions but that do not affect enzyme activity (90). Genotyping methods developed in populations of European origin may slightly underestimate the proportion of African populations with the *GSTM1* null genotype (90).

In two small studies (82, 91) and one larger one (83) in northern European populations, concordance between *GSTT1* genotype and conjugator status (phenotype) in excess of 95 percent was found.

DISEASE

Worldwide in 1996, there were an estimated 875,000 new cases of colorectal cancer (92). There is substantial geographic variation in incidence (figure 1) (93). Epidemiologic evidence suggests that much of the geographic variation reflects variations in environmental or lifestyle exposures, perhaps acting with variations in genetic factors. In developed countries, colorectal cancer is the second most common cancer, and in developing countries, it ranks sixth most common in men and fifth in women (94). In developed countries, the age-standardized rates (30–47 per 100,000 in men and 24–31 per 100,000 in women) are typically about four times higher than those in some developing countries (rates below 10 per 100,000 for both sexes) (93). The incidence of colorectal cancer is rising in most populations (95).

In most populations, cancer of the colon is more common than that of the rectum (93). The male:female ratio for colon cancer is approximately unity, and that for rectal cancer is 1.5 or greater (93). The incidence of colorectal cancer increases with age (93).

After exclusion of familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer, the risks of colorectal cancer to first-degree relatives of index patients with the disease is about twice that of the general population (96, 97). The genetic basis of this familial aggregation has not yet been characterized.

Colorectal adenomatous polyps are thought to be precursors of colorectal cancer. While there is no direct evidence in support of the adenoma-carcinoma sequence, there is considerable indirect evidence from a range of epidemiologic, histopathologic, and molecular genetic studies (98).

Exposure of meats to pyrolysis temperatures produces heterocyclic amines and polycyclic aromatic hydrocarbons (99, 100). The World Cancer Research Fund/American Institute of Cancer Research panel recently concluded that consumption of red meat "probably" increases and intake of heavily cooked meats "possibly" increases the risk of colorectal cancer (101). In some studies, elevated risks of colorectal cancer have been associated with consumption of broiled or grilled meats and browning of the meat surface (102, 103). In a recent study, an increase in risk associated with higher levels of both a white meat and an overall meat mutagen index in men was found (104). However, in other studies, no association with consumption of broiled or grilled meats or browning of the meat surface was observed (105, 106).

High intake of alcohol may be associated with increased risk of colorectal lesions (98). With regard to dietary factors that may be protective, the World Cancer Research Fund/American Institute of Cancer Research panel concluded that there is "convincing" evidence that the consumption of vegetables decreases the risk of colon cancer and "possible" evidence that the consumption of nonstarch polysaccharides/fiber, starch, and carotenoids does so (101). In eight of 12 studies of colon cancer and all five studies of rectal cancer, high levels of consumption of cruciferous vegetables were associated with decreased risks (101). Cruciferous vegetables may have anticarcinogenic properties, since they contain isothiocyanates that induce enzymes that detoxify environmental mutagens (4, 5).

In addition to diet, the other major environmental source of exposure to polycyclic aromatic hydrocarbons is tobacco smoke. Most studies show a positive association between smoking and colorectal adenomas, but the association between smoking and colorectal cancer is less clear (107). However, in four recent, large cohort studies, smoking has been associated with colorectal cancer after a long latent period (108–111).

There is consistent evidence from observational studies that higher levels of physical activity are associated with a reduced risk of colon cancer (112).

While the evidence from observational studies suggests that regular use of aspirin or other nonsteroidal, anti-inflammatory drugs reduces the risk of colorectal cancer, no protective effect was found in the intervention trial in which US male physicians were given 325 mg aspirin on alternate days or a placebo for, on average, 5 years (113).

TABLE 2. Population frequency of *GSTM1 null genotype (studies published in 1993–1998)**

Study (reference no.) and year	Place of study	Type of subjects	No. of subjects	Frequency (%)	95% CI*
		Africa			
Anwar et al. (7), 1996	Egypt	Egyptian subjects with no history of schistosoma infection or malignancy (source of subjects not stated) matched to cases of bladder cancer on age and smoking history; 62% male	21	48	25.7, 70.2
Abdel-Rahman et al. (8), 1996	Egypt (Cairo)	Healthy individuals, serving as controls in a case-control study of bladder cancer (includes those subjects studied in Anwar et al., 1996 (7))	34	44	27.2, 62.1
McGlynn et al. (9), 1995	Ghana (Obuasi)	Healthy male gold miners	49	39	25.2, 53.8
Masimirembwa et al. (10), 1998	South Africa (Venda; Venda people)	Healthy subjects attending clinic for routine health care	96	23	15.0, 32.6
Masimirembwa et al. (10), 1998	Zimbabwe (Shona, Ndebele, and other minority African tribal groups)	Healthy students and staff of the University of Zimbabwe	148	24	17.7, 32.1
		Asia			
McGlynn et al. (9), 1995	China (Haimen City, Jiangsu Province)	Healthy, unrelated subjects aged 19–67 years participating in a cohort study of hepatocellular carcinoma; 70% male	116	41	31.5, 50.0
Hung et al. (11), 1997	China (Taipei City, and Taipei County)	Male controls frequency matched on age and ethnicity to cases of oral cancer, selected from household registration offices	123	58	48.5, 66.6
Rothman et al. (12), 1996	China	Men selected from those who participated in a benzidine-exposed cohort study, with negative urine cytology, matched to cases of bladder cancer on age and city; mean age, 63.2 years (±7)	43	60	44.4, 75.0
Lin et al. (13), 1994	Hong Kong	Volunteers aged 18–55 years of Chinese ethnic group without history of chronic disease or cancer, providing specimens to UCLA* Tissue Typing Laboratory for bone marrow donation	70	49	36.4, 60.8
Katoh et al. (14), 1996	Japan (Kitakyushu City)	Subjects who had visited local medical clinics for regular health checkups; no gastrointestinal symptoms and no current or previous diagnosis of cancer; mean age, 61.9 years (±16.8); 57% male	126	44	34.8, 52.8
Kihara et al. (15, 16), 1993, 1994	Japan (Kanagawa)	Healthy Japanese subjects attending for general health checkups, selected as controls in a case-control study of lung cancer, matched on age, sex, and smoking history to lung cancer patients; 76% male	201	45	38.3, 52.4
Morita et al. (17), 1997	Japan (Osaka)	Healthy subjects attending a periodical general health checkup and without history of malignancy	132	42	33.2, 50.6
Nakachi et al. (18), 1993	Japan (Saitama)	Subjects from the general population aged over 40 years included in a prospective cohort study in a Japanese town; mean age, 65.2 years (±8.3)	170	49	41.7, 57.2

Kato et al. (19), 1996	Japan (Tokyo)	Cancer-free patients examined by gastric endoscopy and diagnosed with benign gastric diseases, age and sex matched with gastric cancer cases; 55% male	120	51	41.6, 60.1
Hori et al. (20), 1997	Japan (Tokyo)	Healthy Japanese controls participating in a case-control study of esophageal carcinoma; 61% male	70	41	29.8, 53.8
Zhao et al. (21), 1995	Malaysia (Kuala Lumpur)	Healthy students and employees of the university (Indian—age 19–57 years, 62% male; Malay—age 18–55 years, 52% male)	Indians Malays	139 146	33 62	25.4, 41.6 53.2, 69.6
Lee et al. (22), 1994	Singapore	Chinese undergraduates and blood donors	187	63	55.8, 70.0
Lee et al. (23), 1998	Singapore	Patients with no history of neoplasms	183	49	41.1, 56.1
Lin et al. (13), 1994	Taiwan	Volunteers aged 18–55 years, of Chinese ethnic group, without history of chronic disease or cancer, providing specimens to UCLA Tissue Typing Laboratory for bone marrow donation	100	45	35.0, 55.3
Yu MW et al. (24), 1995	Taiwan	Men who were selected as controls in a nested case-control study of hepatocellular carcinoma; mean age, 51.6 years	150	63	55.1, 71.0
		Europe			
Okkels et al. (25), 1996	Denmark (Aarhus)	Hospitalized patients of Danish ethnic background with noncancerous diseases of the urinary tract, acting as controls in a case-control study of bladder cancer; mean age, 68 years (±11); 58% male	202	50	42.4, 56.6
Vistisen et al. (26), 1997	Denmark (Greater Copenhagen)	Male controls from a case-control study of testicular cancer frequency matched to cases on year of birth, selected from the Danish National Population Registry and born in 1943–1973	148	49	41.0, 57.7
Mikelsaar et al. (27), 1994	Estonia	Healthy, unrelated volunteers, aged 18–56 years	151	50	42.1, 58.6
Hirvonen et al. (28), 1993	Finland	Blood donors (n = 115) and other volunteers (n = 27)	142	44	35.4, 52.2
Jourenkova et al. (29), 1997	France	"Caucasian" hospitalized patients without previous or current malignant disease, frequency matched on age, sex, and hospital to cases with lung carcinoma; 95% male	172	52	44.6, 60.0
Coutelle et al. (30), 1997	France (Bordeaux)	French "Caucasian" male alcoholics recruited from an alcoholism clinic, without clinically diagnosed cancer or alcohol-related medical complication; mean age, 42 years	37	49	31.9, 65.6
Maugard et al. (31), 1998	France (Nantes)	"Caucasian" female blood donors, and "Caucasian" females recruited through a hospital department; mean age, 47 years (±13)	437	51	46.5, 56.0
Lin et al. (13), 1994	Germany	White volunteers, aged 18–55 years, without history of chronic disease or cancer, providing specimens to UCLA Tissue Typing Laboratory for bone marrow donation	101	39	29.1, 48.8
Jahnke et al. (32), 1996	Germany	Not specified	216	52	45.0, 58.7

Table continues

TABLE 2. Continued

Study (reference no.) and year	Place of study	Type of subjects	No. of subjects	Frequency (%)	95% CI
Brockmöller et al. (33), 1993	Germany (Berlin)	1) "Caucasian" hospitalized controls with bronchopneumonia, asthma, bacterial or viral pneumonia, or other noncancer diagnoses; age 32–84 years; 54% male	155	53	44.7, 61.0
		2) "Caucasian" hospitalized controls without clinical evidence of pulmonary disease; mostly from ICU* with cardiovascular disease; 65% male	200	50	42.4, 56.6
Brockmöller et al. (34), 1994	Germany (Berlin)	Subjects hospitalized for reasons other than cancer	400	51	45.7, 55.8
Kempkes et al. (35), 1996	German (Ruhr area)	Newborn infants	170	54	46.3, 61.8
Brockmöller et al. (36), 1996	Germany (former West Berlin)	Hospitalized subjects without malignant disease, known or considered not to be of African, Asian, or Mediterranean origin	373	51	46.3, 56.7
Oude Ophuis et al. (37), 1998	Netherlands (Nijmegen)	Healthy blood donors; mean age, 34.3 years (±11.5); 37% male	207	52	44.7, 58.7
Moreira et al. (38), 1996	Portugal (Lisbon)	Healthy caucasoid blood donors	84	52	41.2, 63.4
Esteller et al. (39), 1997	Spain (Barcelona)	Women free from clinical or histologic malignancy, selected randomly from those attending an annual gynecologic cancer screening program in a Barcelona hospital; aged 44–76 years	60	47	33.7, 60.0
Gonzalez et al. (40), 1998	Spain (Asturias)	Healthy "Caucasian" blood donors participating in a case-control study of head and neck cancer; 75% male	200	52	44.3, 58.6
Alexandrie et al. (41), 1994	Sweden	Convenience sample comprising laboratory staff, welders, and chimney sweeps; aged less than 65 years; 91% male	329	53	47.3, 58.4
Ichiba et al. (42), 1994	Sweden	Male city council employees; median age, 42 years (range, 19–62)	34	53	35.1, 70.2
Warholm et al. (43), 1994	Sweden	Welders (n = 129) and laboratory staff (n = 79); age range, 22–79 years (median age, 47); 85% male	208	52	44.9, 58.9
Fryer et al. (44), 1993	UK*	Subjects without evidence of malignancy (no further details)	89	44	33.3, 54.7
Chern et al. (45), 1994	UK (Bristol)	Controls matched to cases of bladder cancer	74	53	40.7, 64.4
Daly et al. (46), 1993	UK (Newcastle)	1) Controls from the urology department participating in a case-control study of bladder cancer, who had cytoscopy to exclude bladder tumor; 87% male	52	60	45.1, 73.0
		2) Healthy volunteers from staff and students of Newcastle University; 41% male	58	53	39.9, 66.7
Duncan et al. (47), 1995	UK (North Staffordshire)	Hospitalized subjects without clinical or histologic evidence of malignant or inflammatory disease; mean age, 59.1 years (±18.6)	373	54	49.2, 59.6

Elexpuru-Camiruaga et al. (48), 1995; Deakin et al. (49), 1996	UK (North Staffordshire)	Hospitalized subjects without malignancy or inflammatory pathologies		577	55	50.6, 58.9
Inskip et al. (50), 1995	UK (North Staffordshire)	Hospitalized subjects with various pathologies		244	59	53.0, 65.6
Hand et al. (51), 1996; Yengi et al. (52), 1996	UK (North Staffordshire)	Subjects with tension headaches (25%), inguinal hernia (about 25%), varicose veins (about 25%), benign breast lumps (about 15%), or hiatus hernia (about 10%)		211	57	50.4, 64.1
Sarhanis et al. (53), 1996	UK (North Staffordshire)	Unrelated white women undergoing hysterectomy and bilateral salpingoophorectomy for benign disease or with benign breast lumps or mild iron deficiency		312	62	55.9, 67.0
Zhong et al. (54), 1993	UK (Sheffield, Edinburgh, and Potters Bar)	Hospitalized subjects and volunteers		225	42	35.3, 48.5
Heagerty et al. (55), 1996	UK (Staffordshire, Cornwall, and Hampshire)	White hospital in- and outpatients with a variety of nonmalignant and noninflammatory diseases; mean age, 70 years; 47% male		561	55	50.3, 58.7
Heagerty et al. (56), 1994	UK (West Midlands)	Unrelated white adults participating in a case-control study of cutaneous tumors, without clinical or histologic evidence of cancer or inflammatory pathology; mean age, 67 years; 55% male		153	52	43.4, 59.8
		North America				
Kelsey et al. (57), 1997	US	Female control subjects free of cancer selected from the Nurses' Health Study cohort, >95% "Caucasian," matched to incident and prevalent cases of breast cancer on at least year of birth		484	50	45.2, 54.3
Gertig et al. (58), 1998	US	Sample of subjects not diagnosed with colorectal cancer in Physicians' Health Study, matched on year of birth and smoking history with cases of colorectal cancer		221	53	46.1, 59.7
Chen CL et al. (59), 1996	US	Healthy volunteers	American Blacks	203	28	21.6, 34.3
			American Whites	213	54	46.6, 60.4
Lin et al. (13), 1994	US (multicenter)	Black volunteers aged 18–55 years without history of chronic disease or cancer, providing specimens to UCLA Tissue Typing Laboratory for bone marrow donation		87	31	21.5, 41.9
García-Closas et al. (60), 1997	US (Boston, MA)	Friends or spouses of patients with lung cancer ($n = 167$), friends or spouses of patients undergoing cardiac surgery ($n = 83$), or patients undergoing other thoracic surgery ($n = 208$)		446	52	47.3, 56.7
Lin et al. (13), 1994	US (California)	Newborn offspring of Hispanic mothers (cord blood)		108	53	42.9, 62.5
Abdel-Rahman et al. (8), 1996	US (Galveston-Houston area, TX)	Healthy volunteers participating in a case-control study of lung cancer		80	51	39.8, 62.6
Lin et al. (13), 1994	US (Los Angeles, CA)	Blood donors (predominantly white)		98	49	38.7, 59.3

Table continues

TABLE 2. Continued

Study (reference no.) and year	Place of study	Type of subjects		No. of subjects	Frequency (%)	95% CI
Lin et al. (13), 1994	US (Los Angeles, CA)	Volunteers aged 18–55 years from various ethnic groups, without history of chronic disease or cancer, providing specimens to UCLA Tissue Typing Laboratory for bone marrow donation	White (Jewish)	227	47	40.5, 53.9
			White (Non-Jewish)	71	52	39.9, 64.1
			White ("Mixed")	83	54	42.9, 65.2
			Indian	61	36	24.2, 49.4
			Japanese	78	51	39.7, 62.8
			Korean	183	53	45.5, 60.4
			Philippino	101	59	49.2, 69.1
			Hispanic	81	49	38.1, 60.7
London et al. (61), 1995	US (Los Angeles County, CA)	Population-based controls aged 40–84 years selected from driver's license records or Medicare records, frequency matched with lung cancer cases on age, ethnicity, and sex	Total	716	44	39.9, 47.3
			African Americans	251	27	21.7, 33.0
			"Caucasians"	465	52	47.4, 56.7
Yu MC et al. (62), 1995	US (Los Angeles County, CA)	Male participants aged over 35 years in a multiethnic survey ($n = 108$) and male and female controls, aged 25–64 years, from a study of bladder cancer ($n = 43$); overall, 93% male	Whites	74	51	39.4, 63.1
			Blacks	40	23	10.8, 38.5
			Asians	37	32	18.0, 49.8
Helzlsouer et al. (63), 1998	US (Maryland)	White women selected from a research specimen blood bank; matched to breast cancer cases on age, menopausal status, time from last menstrual period, and date of blood donation		112	46	37.0, 56.1
Cheng et al. (64), 1995	US (Massachusetts)	Friends and spouses of lung cancer cases and cardiovascular patients acting as controls in case-control study of lung cancer; mean age, 58 years (±12); 40% male; 95% "Caucasian"		78	55	43.4, 66.4
Bailey et al. (65), 1998	US (Nashville, TN)	"Caucasian" and African-American women hospitalized for reasons other than cancer, matched by age and race to breast cancer cases	"Caucasians"	162	62	53.8, 69.2
			African Americans	59	41	28.1, 54.3
Ambrosone et al. (66), 1995	US (New York State, NY)	Population-based female controls randomly selected from motor vehicle lists or health care finance administration lists and matched to postmenopausal breast cancer cases on age and county of residence		233	50	43.6, 56.8

Bell et al. (67), 1993	US (North Carolina)	1) Controls from urology clinics with no history of cancer, frequency matched to bladder cancer cases on race, age, and sex	American Blacks	12	33	9.9, 65.1
			American Whites	199	48	41.1, 55.4
		2) Healthy, unrelated, paid volunteers	American Blacks	168	35	27.9, 42.8
			American Whites	298	50	44.2, 55.8
Chen H et al. (68), 1996	US (North Carolina)	White patients without history of cancer recruited in urology clinics with similar age and sex distributions to bladder cancer cases		201	48	40.7, 54.9
Slattery et al. (69), 1998	US (North Carolina, Utah, and Minnesota)	Controls randomly selected to meet age and sex distribution of cases of colon cancer, from medical care program lists, Social Security lists, and driver's license lists and by random digit dialing; 55% male		1,949	55	52.4, 56.8
Park et al. (70), 1997	US (Philadelphia, PA, and New York, NY)	Healthy controls—friends, spouses, and spousal family members of cancer patients and subjects receiving non-disease-related dental treatment and outpatients receiving treatment for non-cancer-related hearing or vision problems or nonrespiratory allergic reactions at the Ear, Nose, and Throat Clinic, and hospital inpatients treated for trauma-related injuries; mean age, 60.9 years; 65% male		133	51	42.3, 59.9
Wiencke et al. (71), 1997	US (San Francisco Bay area, CA)	White subjects selected by random digit dialing and frequency matched with adult glioma cases on age and gender; mean age, 53.2 years (±1.2); 52% male		157	50	41.6, 57.8
Nazar Stewart et al. (72), 1993	US (Seattle, WA)	Subjects aged 30–80 years undergoing major lung surgery or deceased individuals being autopsied, with cause for surgery or cause of death unrelated to tobacco smoking; mean age, 63 years (±9); 74% male; all smokers		29	48	29.4, 67.5
Trizna et al. (73), 1995	US (Texas)	Blood donors and relatives of head and neck cancer patients, matched with cases of head and neck cancer on age, sex, and ethnicity		42	48	32.0, 63.6
Kelsey et al. (74), 1997	US (Texas)	Convenience sample without history of cancer, recruited from community centers, cancer screening programs, churches, and employee groups, frequency matched with lung cancer cases on gender, ethnicity, and age	Total	278	32	26.6, 37.8
			Mexican Americans	146	40	32.4, 48.8
			African Americans	132	23	15.9, 30.8
Trizna et al. (75), 1998	US (Texas)	Blood donors matched on age (±5 years), sex, and race to cases of glioma; average age of cases, 43.2 years; about 87% non-Hispanic white, about 13% African American, about 4% Hispanic		90	43	32.9, 54.2

Table continues

TABLE 2. Continued

Study (reference no.) and year	Place of study	Type of subjects	No. of subjects	Frequency (%)	95% CI
Chen C et al. (76), 1996	US (western Washington State)	Population-based controls selected by random digit dialing, frequency matched with cases of anal cancer on age and gender; 24% male	360	57	51.4, 61.9
		Oceania			
Butler et al. (77), 1997	Australia (Adelaide)	White blood donors	200	54	46.8, 61.1
Chenevix-Trench et al. (78), 1995	Australia (Queensland)	Unselected controls and geriatric patients (without cancer or family history of cancer) participating in a case-control study of colorectal cancer	200	51	43.4, 57.6
Lin et al. (13), 1994	Cook Islands	No further details†	49	81	
Lin et al. (13), 1994	Kiribati	No further details†	37	100	
Lin et al. (13), 1994	Samoan ethnic group	Volunteers aged 18–55 years of Samoan ethnic group, from the South Pacific and Los Angeles, CA, without history of chronic disease or cancer, providing specimens to UCLA Tissue Typing Laboratory for bone marrow donation	24	88	67.6, 97.3
Lin et al. (13), 1994	Tolai, Papua New Guinea	No further details†	49	64	

* *GSTM1*, glutathione *S*-transferase *M1* gene; CI, confidence interval; UCLA, University of California, Los Angeles; ICU, intensive care unit; UK, United Kingdom.
† The frequencies in these populations are quoted by Lin et al. (13). The original study is thought to be by Board et al. (79), and the analysis was by Southern Blot assay.

ASSOCIATIONS

The studies appraised in this section were identified by using the search strategy described earlier, with the addition of *Medical Subject Headings* in *Index Medicus* headings and text words relevant to colorectal cancer or polyps.

GSTM1 and colorectal cancer

The eight available case-control studies of *GSTM1* and colorectal cancer (14, 23, 49, 54, 58, 69, 77, 78) and one of colorectal adenomas (114) are summarized in table 4 and discussed below in order of publication. In four of the studies (14, 58, 69, 114), exposure to environmental and lifestyle factors was assessed; this is discussed in the Interactions section of this paper.

The results of the colorectal cancer studies are inconsistent: Three suggested no association (49, 58, 77), three suggested a slightly lower risk in those with the *GSTM1* null genotype (23, 69, 78), and two, an increased risk associated with this genotype (14, 54). The study of colorectal adenomas suggests a slightly lower risk in those with *GSTM1* null genotype (114).

In the first reported study of colorectal cancer and *GSTM1*, Zhong et al. (54) found a significantly raised relative risk associated with the *GSTM1* null genotype among 196 cases from an Edinburgh hospital and 225 controls from Sheffield, Edinburgh, and Potters Bar (relative risk (RR) = 1.8, 95 percent confidence interval (CI): 1.2, 2.6). This is the only study in which a statistically significant association was observed. The risk was especially elevated for those with a proximal tumor (RR = 3.4, 95 percent CI: 1.9, 6.0).

Chenevix-Trench et al. (78) investigated 132 patients with colorectal adenocarcinoma and 200 controls in Australia. Of the controls, 100 were "unselected," and no further information on them was presented; the remainder were geriatric patients without cancer or a family history of cancer. The relative risk of colorectal cancer associated with the *GSTM1* null genotype was 0.9 (95 percent CI: 0.6, 1.4). When the analysis was restricted to cases with a proximal tumor, the RR was also 0.9 (95 percent CI: 0.4, 1.8). The proportions of cases aged less than 70 and over 70 years who were *GSTM1* null were not significantly different. The authors acknowledge that their study had fewer cases, a smaller proportion of cases with proximal tumors, and a higher proportion of controls who carried the null genotype than did the study by Zhong et al. (54), and, hence, there may have been inadequate statistical power to detect a relation of the type observed in the earlier study.

In a Japanese study of 103 consecutive colorectal adenocarcinoma patients and 126 subjects with no gastrointestinal symptoms or current or previous diagnosis of cancer who visited local medical clinics for regular medical checkups, an RR of 1.5 (95 percent CI: 0.9, 2.6) associated with the *GSTM1* null genotype was observed (14). For proximal cases, the RR was 1.2 (95 percent CI: 0.6, 2.3), and for distal cases, it was 2.0 (95 percent CI: 1.0, 3.9).

In another study in the United Kingdom of 252 colorectal cancer patients and 577 patients without malignancy or inflammatory pathologies recruited through the same hospital, Deakin et al. (49) found an RR of 1.0 (95 percent CI: 0.7, 1.3) associated with the *GSTM1* null genotype. For tumors of the right colon, the RR was 0.8 (95 percent CI: 0.5, 1.2); for those of the left colon, it was 1.1 (95 percent CI: 0.6, 1.8); and for those of the rectum, it was 1.2 (95 percent CI: 0.8, 1.8).

In a study reported only in abstract form, Butler et al. (77) compared the frequency of *GSTM1* genotypes between 219 white adults with sporadic colorectal cancer and 200 white blood donors in Australia. The relative risk of colorectal cancer associated with the *GSTM1* null genotype was 1.0 (95 percent CI: 0.7, 1.4).

Gertig et al. (58) conducted a case-control study nested within the Physicians' Health Study in the United States. A total of 212 men with colorectal cancer were matched on year of birth and smoking history to men without colorectal cancer. An RR of 1.0 (95 percent CI: 0.7, 1.5) was associated with the *GSTM1* null genotype (adjusted for body mass index, physical activity, and alcohol use). The RRs were not substantially different when the analysis was stratified by age (≤60 years, >60 years). For proximal cancer, the adjusted RR was 0.7 (95 percent CI: 0.4, 1.3), and for distal cancer, it was 1.4 (95 percent CI: 0.8, 2.3).

Lee et al. (23) investigated the frequency of *GSTM1* polymorphisms among Chinese subjects resident in Singapore. A total of 300 cases of colorectal carcinoma were compared with 183 patients without history of neoplasms recruited from a Clinical Chemistry Department. The RR associated with the *GSTM1* null genotype was 0.8 (95 percent CI: 0.5, 1.1). For tumors of the right side, the RR was 1.2 (95 percent CI: 0.6, 2.5); for those of the left side, it was 1.0 (95 percent CI: 0.5, 2.1), and for rectosigmoid tumors, it was 0.7 (95 percent CI: 0.5, 1.0). In individuals with poorly differentiated tumors, the frequency of the null genotype was 67 percent; in moderately differentiated tumors, it was 41 percent; and in well-differentiated tumors, it was 43 percent.

In a large, multicenter case-control study in the United States, Slattery et al. (69) compared 1,567 cases and 1,889 controls randomly selected from medical care program lists, driver's license lists, and Social Security lists and by random digit dialing. The crude

TABLE 3. Population frequency of *GSTT1** null genotype (studies published in 1993–1998)

Study (reference no.) and year	Place of study	Type of subjects	No. of subjects	Frequency (%)	95% CI*
		Africa			
Abdel-Rahman et al. (8), 1996	Egypt (Cairo)	Healthy individuals serving as controls in a case-control study of bladder cancer	34	15	5.0, 31.1
Masimirembwa et al. (10), 1998	South Africa (Venda; Venda people)	Healthy subjects attending clinic for routine health care	71	20	11.2, 30.9
Masimirembwa et al. (10), 1998	Zimbabwe (Shona, Ndebele, and other minority African tribal groups)	Healthy staff and students of the University of Zimbabwe	123	26	18.5, 34.7
		Asia			
Nelson et al. (80), 1995	China (Anhui Province)	Employees of a plant, including spray painters and administrative staff	45	64	48.8, 78.1
Hung et al. (11), 1997	China (Taipei City and Taipei County)	Male controls, frequency matched on age and ethnicity to cases of oral cancer, selected from household registration offices	123	53	43.6, 61.9
Katoh et al. (14), 1996	Japan (Kitakyushu City)	Subjects who had visited local medical clinics for regular health checkups; no gastrointestinal symptoms and no current or previous diagnosis of cancer; mean age, 61.9 years (±16.8); 57% male	126	44	35.6, 53.6
Nelson et al. (80), 1995	Korea	Lead-acid battery workers from three different factories	103	60	50.1, 69.7
Lee et al. (81), 1995	Singapore and Malaysia	Chinese subjects recruited from undergraduates of National University of Singapore and blood donors (age range, 18–53 years; 73% male); Malays (age range, 18–55 years; 56% male) and Indians (age range, 18–57 years; 64% male) recruited from the staff and students of the University of Malaya	Chinese Malays Indians	187 167 152	58 38 16	50.9, 64.4 30.9, 46.2 10.9, 23.3
		Europe			
Jourenkova et al. (29), 1997	France	Hospitalized "Caucasian" patients without previous or current malignant disease, frequency matched on age, sex, and hospital to cases with lung carcinoma; 95% male	172	16	11.1, 22.7
Jahnke et al. (32), 1996	Germany	Not specified	216	13	8.8, 18.2
Kempkes et al. (82), 1996	Germany (Dortmund)	Healthy adults	40	15	5.7, 29.8
Kempkes et al. (35), 1996	Germany (Ruhr area)	Newborn infants	170	18	12.7, 24.9
Brockmöller et al. (36), 1996	Germay (former West Berlin)	Hospitalized subjects without malignant disease, known or considered not to be of African, Asian, or Mediterranean origin	360	21	16.8, 25.4

Oude Ophuis et al. (37), 1998	Netherlands (Nijmegen)	Healthy blood donors, mean age, 34.3 years (±11.5); 37% male	207	20	15.0, 26.4
Esteller et al. (39), 1997	Spain (Barcelona)	Women free of clinical or histologic malignancy, randomly selected from those attending an annual gynecologic cancer screening program in a Barcelona hospital; age range, 44–76 years	60	20	10.8, 32.3
Warholm et al. (83), 1995	Sweden	Subjects recruited from patients attending a migraine clinic ($n = 41$), and welders ($n = 129$); no further details on where the remaining 100 subjects were recruited; 67% male	270	10	6.4, 13.8
Warwick et al. (84), 1994	UK* (North Staffordshire)	Women with menorrhagia who had undergone a hysterectomy and who had normal cervical cytology; mean age, 43 years	167	16	10.9, 22.6
Duncan et al. (47), 1995	UK (North Staffordshire)	Hospitalized subjects without clinical or histologic evidence of malignant or inflammatory disease; mean age, 59.1 years (±18.6)	266	18	13.3, 22.8
Elexpuru-Camiruaga et al., (48), 1995; Deakin et al. (49), 1996	UK (North Staffordshire)	Hospitalized subjects without malignancy or inflammatory pathologies	509	18	15.2, 22.1
Hand et al. (51), 1996; Yengi et al. (52), 1996	UK (North Staffordshire)	Subjects with tension headaches (25%), inguinal hernias (about 25%), varicose veins (about 25%), benign breast lumps (about 15%) or hiatus hernias (about 10%)	284	20	15.3, 24.8
Sarhanis et al. (53), 1996	UK (North Staffordshire)	Unrelated white women undergoing hysterectomy and bilateral salpingoophorectomy for benign disease ($n = 232$) or with benign breast lumps or mild iron deficiency ($n = 93$)	325	19	14.7, 23.4
Heagerty et al. (55), 1996	UK (Staffordshire, Cornwall, and Hampshire)	White hospital in- and outpatients with a variety of nonmalignant and noninflammatory diseases; mean age, 70 years; 47% male	484	19	15.2, 22.4
		North America			
Nelson et al. (80), 1995	US	"Caucasian" construction carpenters participating in a voluntary health screening	257	24	18.7, 29.4
Chen CL et al. (59), 1996	US	Healthy volunteers — American Blacks	203	24	18.4, 30.6
		Healthy volunteers — American Whites	213	15	10.5, 20.5
Gertig et al. (58), 1998	US	Sample of subjects not diagnosed with colorectal cancer in the Physicians' Health Study, matched with cases of colorectal cancer on year of birth and smoking history	220	23	17.8, 29.3
Abdel-Rahman et al. (8), 1996	US (Galveston-Houston area, TX)	Healthy volunteers participating in a case-control study of lung cancer	80	15	8.0, 24.7
Nelson et al. (80), 1995	US (Houston, TX)	Healthy controls participating in a case-control study of lung cancer, recruited through community centers, churches, cancer screening programs, and hospital employees — African Americans	119	22	14.8, 30.4
		Healthy controls participating in a case-control study of lung cancer, recruited through community centers, churches, cancer screening programs, and hospital employees — Mexican Americans	72	10	4.0, 19.0

Table continues

TABLE 3. Continued

Study (reference no.) and year	Area of study	Type of subjects		No. of subjects	Frequency (%)	95% CI
Helzlsouer et al. (63), 1998	US (Maryland)	White women selected from a research specimen blood bank, matched to breast cancer cases on age, menopausal status, time from last menstrual period, and date of blood donation		112	21	14.2, 30.2
Bailey et al. (65), 1998	US (Nashville, TN)	"Caucasian" and African-American women hospitalized for reasons other than cancer, matched by age and race to breast cancer cases	"Caucasian"	162	27	20.5, 34.7
			African American	59	29	17.8, 42.1
Nelson et al. (80), 1995	US (New England)	"Caucasian" friends and spouses of lung cancer patients selected as controls in a case-control study of lung cancer		185	16	10.8, 21.7
Chen H et al. (68), 1996	US (North Carolina)	White patients without a history of cancer recruited in urology clinics with age and sex distributions similar to those of bladder cancer cases		190	16	10.9, 21.8
Wiencke et al. (85), 1995	US (San Francisco, CA, and Houston, TX)	Healthy employees of medical center ($n = 38$) and oil refining company ($n = 40$); mean age, 43 years (±8.2); 47% male		78	15	8.2, 25.3
Trizna et al. (73), 1995	US (Texas)	Blood donors and relatives of head and neck cancer patients, matched with cases of head and neck cancer on age, sex, and ethnicity		42	36	21.5, 52.0
Kelsey et al. (74), 1997	US (Texas)	Convenience sample without a history of cancer recruited from community centers, a cancer screening program, churches, and employee groups, frequency matched with lung cancer cases on gender, ethnicity, and age	Total	278	17	12.4, 21.4
			Mexican Americans	146	12	6.9, 18.0
			African Americans	132	22	15.2, 30.0
Trizna et al. (75), 1998	US (Texas)	Blood donors matched on age (±5 years), sex, and race to cases of glioma; average age of cases, 43.2 years; about 87% non-Hispanic whites, about 13% African Americans, about 4% Hispanic		90	30	20.8, 40.6
		Oceania				
Butler et al. (77), 1997	Australia (Adelaide)	White blood donors		200	19	13.8, 25.1
Chenevix-Trench et al. (78), 1995	Australia (Queensland)	Unselected controls and geriatric patients (without cancer or family history of cancer) participating in a case-control study of colorectal cancer	All	148	16	10.1, 22.4
			Unselected controls	94	19	11.8, 28.6
			Geriatric patients	54	9	3.1, 20.3

* *GSTT1*, glutathione *S*-transferase *T1* gene; CI, confidence interval; UK, United Kingdom.

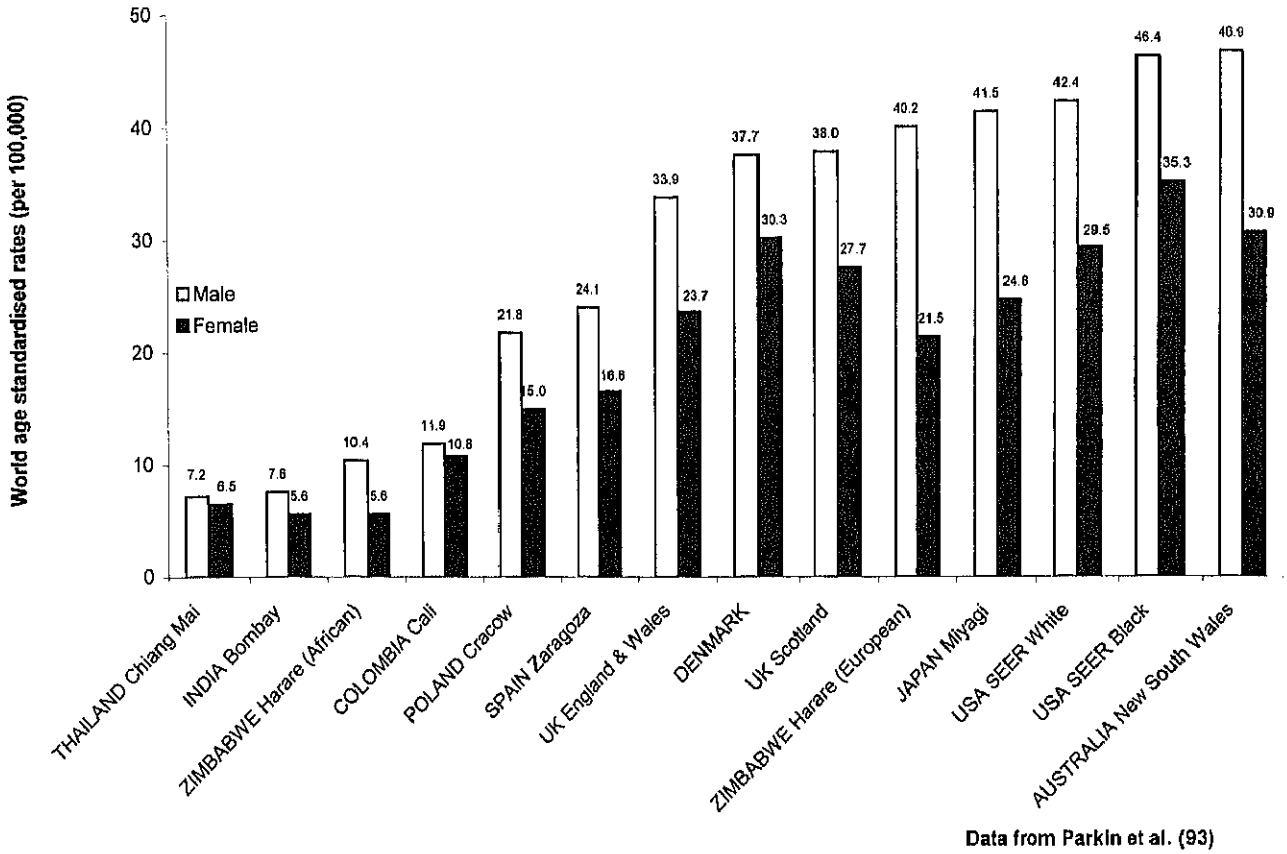

FIGURE 1. World age-standardized incidence rates (per 100,00 people) for colorectal cancer from selected population-based cancer registries for the period 1988–1992).

RR of colon cancer associated with the *GSTM1* null genotype for men and women combined was 0.9 (95 percent CI: 0.8, 1.1). When the analysis was stratified by age (<67 years, ≥67 years), the relative risks were not substantially different. When proximal and distal tumors were considered separately, the crude RRs for both genders combined associated with the *GSTM1* null genotype were 1.0 (95 percent CI: 0.8, 1.1) and 0.9 (95 percent CI: 0.8, 1.1) respectively.

In the one study of colorectal adenomatous polyps, from the United States (114), 446 cases were matched with 488 controls who did not have colorectal adenomas on sex, age, date of sigmoidoscopy, and center. The RR associated with the *GSTM1* null genotype was 0.9 (95 percent CI: 0.7, 1.1) (adjusted for the matching factors). When the analysis was stratified by ethnic group, the RR for whites was 1.0 (95 percent CI: 0.7, 1.4), that for Hispanics (not Blacks) was 0.8 (95 percent CI: 0.4, 1.7), that for Blacks was 0.6 (95 percent CI: 0.3, 1.4), and that for Asians and Pacific Islanders was 0.4 (95 percent CI: 0.2, 1.1). Cases and controls were identified after sigmoidoscopy. Only the left colon is accessible to sigmoidoscopy, so it is possible that some controls harbored tumors in the rest of their colon. The effect of this would be to bias the relative risks toward the null.

GSTT1 and colorectal cancer

Six of the studies described above also reported *GSTT1* genotype (table 5) (14, 23, 49, 58, 77, 78). Two assessed exposure and are discussed in the Interactions section of this paper (14, 58).

The results of these studies are inconsistent. In two studies (49, 77), the *GSTT1* null genotype was associated with a statistically significant increase in the risk of colorectal cancer, while in the other four, no noteworthy associations were apparent.

Chenevix-Trench et al. (78) reported an RR for colorectal cancer of 0.9 (95 percent CI: 0.4, 1.7) associated with the *GSTT1* null genotype when the unselected and the geriatric controls were considered together. However, when the analysis was repeated using the different control groups separately, the RRs were 0.7

TABLE 4. Summary of studies of colorectal lesions and *GSTM1** (studies published in 1993–1998)

Study (reference no.) and year	Place of study; recruitment period	Type of cases	No.	Type of controls	No.	% of controls *GSTM1* null	RR* for null vs. other genotypes	95% CI*	Adjustment	Subgroup analysis reported	Exposure assessment
Zhong et al. (54), 1993	UK* (Sheffield, Edinburgh, and Potters Bar); period not stated	"Caucasian" colorectal cancer patients from one hospital in Edinburgh	196	"Caucasian" subjects from a clinical chemistry department in Sheffield and an Edinburgh hospital, and volunteers from Potters Bar	225	42	1.8	1.2, 2.6	None	Position of tumor	None
Chenevix-Trench et al. (78), 1995	Australia (Queensland); period not stated	Patients with colorectal adenocarcinoma	132	"Unselected" subjects (n = 100; source not stated) and geriatric patients (n = 100) without cancer or a family history of cancer	200	51 (no difference between geriatric and unselected controls)	0.9	0.6, 1.4	None	Position of tumor, age	None
Deakin et al. (49), 1996	UK (North Staffordshire); cases and controls, 1990–1994	Unrelated English "Caucasian" patients with colorectal cancer recruited from one hospital; 51% male; mean age, 66 years	252	Hospitalized English "Caucasian" subjects without malignancy or inflammatory pathologies; recruited in the same hospital as cases; 48% male; mean age, 70 years	577	55	1.0	0.7, 1.3	None	Position of tumor	None
Katoh et al. (14), 1996	Japan (Kitakyushu City); cases, 1991–1995; controls, 1993–1995	Consecutive patients with colorectal adenocarcinoma diagnosed in two hospitals and one medical center; 65% male; mean age, 64.4 years	103	Subjects who had visited local medical centers for regular health checkups; no gastrointestinal symptoms and no current or prevous diagnosis of cancer; 57% male; mean age, 61.9 years	126	44	1.5	0.9, 2.6	None	Position of tumor	Medical, residential, occupational, and smoking histories assessed by interview
Butler et al. (77), 1997 (reported in abstract only)	Australia (Adelaide); period not stated	While adults with sporadic colorectal cancer; source not stated	219	White blood donors	200	54	1.0	0.7, 1.4	None	None	None

Gertig et al. (58), 1998	US (nested case-control study in Physicians' Health Study); cases, 1982–1996	Cases with colorectal cancer from those randomized in the Physicians' Health Study; physicians excluded from randomization if they had a history of MI*, stroke, transient ischemic attack, cancer, renal or liver disease, peptic ulcer, or gout	212	Sample of subjects not diagnosed with colorectal cancer in the Physicians' Health Study (same exclusion criteria as listed for cases); matched to cases on year of birth and smoking history	221	53	1.0	0.7, 1.5	Body mass index, physical activity, alcohol use	Position of tumor, age, and smoking	Smoking history, alcohol intake, diet, frequency of meat intake, physical activity, and other diseases
Lee et al. (23), 1998	Singapore; period not stated	Chinese colorectal carcinoma patients recruited from a surgical department	300	Chinese patients with no history of neoplasms obtained from clinical chemistry department	183	49	0.8	0.5, 1.1	None	Position of tumor and tumor histology	None
Slattery et al. (69), 1998†	US (North Carolina, Utah, Minnesota); cases, 1991–1994	Cases of colon cancer aged 30–79 years, English speaking, and mentally competent. Those with tumors of the rectosigmoid junction or rectum and those with familial adenomatous polyposis, ulcerative colitis, or Crohn's disease were excluded	1,567	Controls randomly selected to meet age/sex distribution of cases from medical care program lists, drivers license lists, social security lists, and random digit dialing	1,889	55	0.9	0.8, 1.1‡	—‡	Age at diagnosis, position of tumor, and smoking	Physical activity, diet, weight, height, family history of cancer, medical and reproductive history, and tobacco use
Lin et al. (114), 1995§	US, California; cases and controls, 1991–1993	Cases and controls selected from those undergoing sigmoidoscopy drawn from two medical centers; age 50–74 years; no history of inflammatory bowel disease, familial polyposis, bowel surgery, or severe gastrointestinal symptoms				Not stated	0.9	0.7, 1.1	Date of sigmoidoscopy, age, gender, and center	Ethnic group	Smoking, therapeutic drug use, physical activity, height, weight, family history of cancer, and diet
		Patients with a first-time diagnosis of one or more colorectal adenomas confirmed by histology (participation rate, 71%)	446	Subjects free from polyps, matched on gender, age (±5 years), date of sigmoidoscopy (±3 months), and center (participation rate, 71%)	488						

* *GSTM1*, glutathione *S*-transferase *M1* gene; RR, relative risk; CI, confidence interval; UK, United Kingdom; MI, myocardial infarction.

† The recent paper by Kampmann et al. (104) provides further results for the same study population.

‡ The result presented here is for men and women combined and is unadjusted. However, the authors present the relative risk for men (RR = 1.0, 95 percent CI: 0.8, 1.2) and women (RR = 0.9, 95 percent CI: 0.7, 1.1) separately, adjusted for age, energy intake, body mass index, long-term physical activity, dietary fiber, and usual number of cigarettes smoked.

§ The paper by Lin et al. (5) provides further results for the same study population. This more recent paper has an additional 13 cases and 19 controls.

TABLE 5. Summary of studies of colorectal lesions and *GSTT1 (studies published 1993–1998)**

Study (reference no.) and year	Place of study; recruitment period	Type of cases	No.	Type of controls	No.	% of controls *GSTT1* null	RR* for null vs. other genotypes	95% CI*	Adjustment	Subgroup analysis reported	Exposure assessment
Chenevix-Trench et al. (78), 1995	Australia (Queensland); period not stated	Patients with colorectal adenocarcinoma	125	Unselected subjects ($n = 94$; source not stated) and geriatric patients without cancer or family history of cancer ($n = 54$)	94 54	19 9	0.7 1.5	0.3, 1.4 0.6, 4.3	None None	Position of tumor; age	None
Deakin et al. (49), 1996	UK* (North Staffordshire); cases and controls, 1990–1994	Unrelated English "Caucasian" patients with colorectal cancer recruited from one hospital	211	Hospitalized English "Caucasian" subjects without malignancy or inflammatory pathologies; recruited in the same hospital as cases	509	18	1.9	1.3, 2.7	None	Position of tumor	None
Katoh et al. (14), 1996	Japan (Kitakyusko City); cases, 1991–1995; controls, 1993–1995	Consecutive patients with colorectal adenocarcinoma diagnosed in two hospitals and one medical center; 65% male; mean age, 64.4 years	103	Subjects who had visited local medical centers for regular health checkups; no gastrointestinal symptoms and no current or previous diagnosis of cancer; 57% male; mean age, 61.9 years	126	44	1.2	0.7, 2.0	None	Position of tumor	Medical, residential, occupational, and smoking history assessed by interview
Butler et al. (77), 1997 (reported in abstract only)	Australia (Adelaide); period not stated	White adults with sporadic colorectal cancer; source not stated	219	White blood donors	200	19	3.4	2.1, 5.4	None	None	None
Gertig et al. (58), 1998	US (nested case-control study in Physicians' Health Study); cases, 1982—1996	Cases with colorectal cancer from those randomized in the Physicians' Health Study; physicians excluded from randomization if they had history of myocardial infarction, stroke, transient ischemic attack, cancer, renal or liver disease, peptic ulcer, or gout	212	Sample of subjects not diagnosed with colorectal cancer in the Physicians' Health Study (same exclusion criteria as listed for cases); matched on year of birth and smoking history	221	23	0.8	0.5, 1.2	Body mass index, physical activity, alcohol use	Position of tumor, age, and smoking	Smoking history, alcohol intake, diet, frequency of meat intake, physical activity, and other diseases

Lee et al. (23), 1998	Singapore; period not stated	Chinese colorectal carcinoma patients recruited from a surgical department	300	Chinese patients obtained from the clinical chemistry department with no history of neoplasms	183	Not stated†	Position of tumor and tumor histology	None

* *GSTT1*, glutathione *S*-transferase *T1* gene; RR, relative risk; CI, confidence interval; UK, United Kingdom.
† The frequency of *GSTT1* null individuals was similar in cases and controls.

(95 percent CI: 0.3, 1.4) for unselected controls and 1.5 (95 percent CI: 0.6, 4.3) for geriatric controls. This reflects the different proportions of individuals carrying the *GSTT1* null genotype in each of the control groups (19 percent in the unselected controls and 9 percent in the geriatric controls) and illustrates the potential for selection bias to distort associations between chronic diseases and genetic polymorphisms. With the unselected and geriatric control groups combined, the RRs associated with the *GSTT1* null genotype were 0.4 (95 percent CI: 0.0, 1.5) for proximal tumors and 1.0 (95 percent CI: 0.5, 2.1) for distal tumors. In those cases who were diagnosed before age 70 years, 21 percent were homozygous for the *GSTT1* null genotype, and in those diagnosed at age 70 or older, 7 percent carried this genotype.

Deakin et al. (49) reported an RR of 1.9 (95 percent CI: 1.3, 2.7) associated with null genotype. They found increased relative risks for each of the tumor subsites reported: for right-sided tumors, RR = 1.5 (95 percent CI: 0.8, 2.7); for left-sided tumors, RR = 2.3 (95 percent CI: 1.3, 4.2); and for tumors of the rectum, RR = 1.9 (95 percent CI: 1.1, 3.2).

Katoh et al. (14) found an RR of 1.2 (95 percent CI: 0.7, 2.0) for colorectal cancer, and Butler et al. (77) reported an RR of 3.4 (95 percent CI: 2.1, 5.4) associated with the *GSTT1* null genotype. Neither of these studies presented relative risks in relation to tumor subsite.

Gertig et al. (58) reported an RR of colorectal cancer associated with the *GSTT1* null genotype of 0.8 (95 percent CI: 0.5, 1.2), adjusted for body mass index, physical activity, and alcohol use. For proximal tumors, the adjusted RR was 0.9 (95 percent CI: 0.5, 1.7), and for distal tumors, it was 0.6 (95 percent CI: 0.3, 1.2). In men aged less than 60 years, the RR associated with *GSTT1* null genotype was 0.5 (95 percent CI: 0.2, 1.0), and in those aged 60 years or older, it was 0.9 (95 percent CI: 0.5, 1.7). It is not clear whether the age-stratified relative risks were adjusted.

Lee et al. (23) stated that the frequency of the *GSTT1* null genotype was similar in both cases and controls and that tumor histology had no effect on the frequency of the null genotype. However, insufficient information was presented for a relative risk to be calculated.

Comment on the studies on *GSTM1* and *GSTT1* and colorectal cancer

It is difficult to assess how far selection and participation biases may account for the inconsistencies in the results. Most studies involved hospital-based case series, and most of the control groups were not population based. This has implications for the generaliz-

ability of the study results. The potential problems of selecting controls who do not represent the population from which cases arose is demonstrated by the divergence in relative risks obtained for the *GSTT1* null genotype when the different control groups were analyzed in the study by Chenevix-Trench et al. (78). Most of the studies were not large; five included fewer than 250 cases. The smaller studies are likely to have limited statistical power, particularly for subgroup analyses. Two of the studies were undertaken in Asian populations; the others were in predominantly white populations. There is little information available for other ethnic groups. It is unclear whether any of the established risk factors for colorectal cancer are associated with the *GSTM1* or *GSTT1* genotype. The studies made little attempt to adjust for potential confounders.

The findings of these studies require confirmation in other populations.

GSTM1 and other cancers

In a recent review, Rebbeck (6) suggests that there is evidence from case-control studies that *GSTM1* is involved in the etiology of both lung and bladder cancers, although not all studies have shown this. While some studies of other cancer sites have shown an association with *GSTM1*, these findings have not been confirmed.

GSTT1 and other cancers

There have been fewer case-control studies of *GSTT1*. Statistically significant associations have been reported for astrocytoma, meningioma, and myelodysplasia, but these have not been confirmed (6).

INTERACTIONS

Because the GST enzymes have detoxifying activity, it would be expected that, rather than affecting the risk of cancer per se, they would modify risk in relation to exposure to potential carcinogens. The enzymes play a major role in the detoxification of polycyclic aromatic hydrocarbons found in tobacco smoke and in cooked and processed meats. In four studies of colorectal lesions and *GSTM1* (14, 58, 69, 114) and two of *GSTT1* (14, 58), exposure to tobacco smoke was considered. Meat consumption was considered in relation to *GSTM1* in two studies (58, 104) and in relation to *GSTT1* in one (58). Consumption of broccoli, the richest source of isothiocyanates that induce enzymes that detoxify environmental mutagens, was considered in a study of *GSTM1* and colorectal adenomas (5). Three studies have considered GST gene-gene interactions and colorectal cancer (23, 58, 69).

The limited statistical power of small studies to detect associations between genotype and disease is particularly important with regard to effect modification. To give adequate statistical power to detect a multiplicative interaction, very large sample sizes (in some circumstances, thousands of cases) may be required (115).

GSTM1 and smoking

Little evidence of interaction between *GSTM1* genotype, tobacco exposure, and colorectal cancer was found in the three studies (14, 58, 69). However, the one polyp study (114) suggests that the *GSTM1* genotype may modify the association between smoking and disease.

Lin et al. (114) report the effect of cigarette smoking and *GSTM1* on adenoma risk. With a reference group of subjects who were never smokers and were *GSTM1* positive, significantly increased adenoma risk was seen both in current smokers who were *GSTM1* positive (RR = 1.7, 95 percent CI: 1.0, 2.9) and in current smokers who were *GSTM1* null (RR = 2.1, 95 percent CI: 1.1, 3.8). When this analysis was restricted to adenomas greater than 1 cm in size, the RRs were 1.3 (95 percent CI: 0.6, 2.9) and 2.5 (95 percent CI: 1.1, 5.5).

Katoh et al. (14) reported that *GSTM1* did not influence risk differently in subjects classified by smoking status (smoker or nonsmoker) or extent of tobacco exposure (pack-years).

Gertig et al. (58) investigated the joint effect of *GSTM1* and cigarette smoking status at entry into the Physicians' Health Study on subsequent risk of colorectal cancer. The RR associated with the *GSTM1* null genotype was 1.1 (95 percent CI: 0.6, 2.1) in never smokers, 1.0 (95 percent CI: 0.6, 1.6) in past smokers, and 1.2 (95 percent CI: 0.3, 4.2) in current smokers. There was no significant interaction between pack-years of smoking at baseline and *GSTM1* genotype.

In the study of Slattery et al. (69), those who smoked more than one pack per day were at approximately 40 percent increased risk of colon cancer. No interaction was observed in either men or women between *GSTM1* genotype and any of the following categories of tobacco exposure: smoking status, usual number of cigarettes smoked per day, pack-years of cigarettes smoked, age started smoking cigarettes, and years since stopping smoking cigarettes.

GSTT1 and smoking

Katoh et al. (14) reported that smoking had no effect on the risk associated with *GSTT1* genotypes.

Gertig et al. (58) reported RRs of colorectal cancer associated with the *GSTT1* null genotype of 0.8 (95 percent CI: 0.4, 1.8) in those who were never smokers at the time of enrollment, 0.5 (95 percent CI: 0.3, 1.1) in past smokers, and 1.1 (95 percent CI: 0.3, 4.7) in current smokers. There was no interaction between pack-years of smoking and *GSTT1* genotype.

GSTM1 and meat intake

In the study by Gertig et al. (58), men who were homozygous for *GSTM1* null who consumed more than one serving of red meat per day were at slightly lower risk compared with men who were not homozygous for *GSTM1* null who consumed less than 0.5 servings per day (RR = 0.8, 95 percent CI: 0.4, 2.0).

Kampmann et al. (104) reported associations between *GSTM1* genotype and various measures of meat consumption in the subjects investigated by Slattery et al. (69). There was no evidence that *GSTM1* genotype modified the relative risks associated with amount of 1) red meat, 2) processed meat, or 3) poultry consumed; 4) frequency of fried, broiled, baked, or barbecued red meat; 5) preferred "doneness" of red meat; 6) frequency of use of red meat drippings; 7) frequency of use of white meat drippings; or 8) red meat mutagen index. *GSTM1* genotype modified risks associated with frequency of consumption of fried, broiled, baked, or barbecued white meat; white meat mutagen index; and total meat mutagen index. Unexpectedly, the strongest positive associations were observed among those who were *GSTM1* positive.

GSTT1 and meat intake

In the Physicians' Health Study (58), men who were *GSTT1* null homozygous and who consumed more than one serving of red meat daily had a lower risk compared with men who were *GSTT1* nonnull and who consumed less than 0.5 servings daily (RR = 0.4, 95 percent CI: 0.1, 1.4).

GSTM1 and isothiocyanates

Lin et al. (5) postulated that a cancer preventive effect of broccoli would be stronger in *GSTM1* null individuals and investigated this in the subjects studied earlier by Lin et al. (114). Compared with subjects in the lowest quartile of broccoli intake who were *GSTM1* null, those in the highest intake quartile who were null had an RR of 0.36 (95 percent CI: 0.19, 0.68), and those in the highest intake quartile who were *GSTM1* positive had an RR of 0.74 (95 percent CI: 0.40, 0.99); this interaction was statistically significant ($p = 0.01$).

GSTM1, *GSTT1*, and other genes

In the Physicians' Health Study (58), there was no increased risk of colorectal cancer in men who were homozygous null for both *GSTM1* and *GSTT1* compared with those who were homozygous positive for both *GSTM1* and *GSTT1*. By contrast, Lee et al. (23) reported that 35 percent of cases with right-side tumors were *GSTM1* null and *GSTT1* positive compared with 22 percent of the control series.

Slattery et al. (69) considered the possibility of an interaction between *GSTM1* and *N*-acetyltransferase 2 (*NAT2*) genotypes. There was a suggestion that women with the combined *NAT2* intermediate/rapid and *GSTM1*-positive genotypes were at increased risk compared with those with *NAT2* slow/*GSTM1*-positive genotypes (unadjusted RR = 1.5, 95 percent CI: 1.11, 2.05). This was restricted to women older than age 67 years who had proximal tumors. However, the association was weaker and was not statistically significant in men (unadjusted RR = 1.2, 95 percent CI: 0.89, 1.51). There was no strong evidence of any interaction between *NAT2*, *GSTM1*, and smoking in either men or women.

LABORATORY TESTS

For classification of an individual as *GSTM1* null or nonnull (or *GSTT1* null or nonnull), the genotyping procedure detects either the absence or the presence of the *GSTM1* (or the *GSTT1*) gene. Therefore, after the gene has been amplified by polymerase chain reaction methodology, the product need only be visualized. This method cannot, however, distinguish between the *GSTM1*A* and *GSTM1*B* alleles. For this, a restriction digest must be undertaken. This cleaves the DNA into fragments of characteristic sizes, and the different combinations of these fragments correspond to specific alleles.

To ensure that a polymerase chain reaction occurred, a number of quality control procedures should undertaken. Additional "control" primers should be added. These amplify another region of DNA (one that is thought never to be deleted) to confirm that amplification has worked in null individuals. Along with the samples being amplified, a positive and a negative control should be run. The positive control is a sample of DNA known to contain the gene (i.e., not null); both the band representing the gene in question and the control band should be visible for the genotyping to be validated. The negative control allows a check for contamination to be made; if amplification is seen in this control, the samples run at the same time should not be genotyped. In general, the studies present little information on the proportion of subjects for whom the

genotype could be determined or on reproducibility of genotyping.

Much of the polymerase chain reaction work on genotyping has used DNA from blood; however, work involving DNA from mouthwash samples is now being undertaken (116). This development makes polymerase chain reaction methodology even more appropriate for researchers undertaking molecular epidemiology studies, since it enables subjects to be genotyped without the need for invasive sampling.

GSTM1

In two of the nine studies of *GSTM1* and colorectal lesions, no details of the primers used are given (23, 77). Three studies (58, 78, 114) use the same primers to amplify the *GSTM1* gene, although they reference different papers for these methods (64, 89, 117). The primer 5′-CTGCCCTACTTGATTGATGGG-3′ anneals to the 5′ region of exon 4, and the primer 5′-CTGGATTGTAGCAGATCATGC-3′ anneals to the 3′ region of exon 5. They amplify a 273 base-pair product, but use slightly different amplification cycles. Katoh et al. (14) used the method outlined by Bell et al. (67). The primers are 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAATATACGGTGG-3′; and they amplify a 215 base-pair product. The amplification cycles are undertaken at temperatures similar to those in the studies by Brockmöller et al. (89) and Comstock et al. (117), but the time for each stage of the cycle is considerably shorter, and there are fewer total cycles.

Deakin et al. (49) used the methods of Warwick et al. (84) and Fryer et al. (118), in which three primers (5′-GCTTCACGTGTTATGAAGGTTC-3′, 5′-TTGGGAAGGCGTCCAAGCGC-3′, and 5′-TTGGGAAGGCGTCCAAGCAG-3′) are used to amplify DNA in intron 6 and exon 7, and a restriction digest differentiates alleles *GSTM1*A* and *GSTM1*B* (118). Slattery et al. (69) use the method outlined by Zhong et al. (54), in which three primers (*P1* 5′-CGCCATCTTGTGCTACATTGCCCG-3′, *P2* 5′-ATCTTCTCCTCTTCTGTCTC-3′, and *P3* 5′-TTCTGGATTGTAGCAGATCA-3′) are combined in a single polymerase chain reaction. Primers *P1* and *P3* amplify a 230 base-pair product specific to *GSTM1*; primers *P1* and *P2* anneal to either *GSTM1* or *GSTM4* and amplify a 157 base-pair product, thereby acting as the control primers.

In another two studies, explicit mention is made of the use of control primers: Chenevix-Trench et al. (78) used primers for exon 1 of coagulation factor XIII, and Katoh et al. (14) used primers for β-globin. In the methodology of Warwick et al. (84), used by Deakin et al. (49), β-globin is again used as the control primer. In two studies (14, 58), the use of positive and negative controls samples is reported.

GSTT1

In two of the six studies (23, 77), no details on the methods used are given. In the other four (14, 49, 58, 78), the genotyping methods outlined by Pemble et al. (91) were used. The primers used for amplification in this method are TTCCTTACTGGTCCTCACATCTC and TCACCGGATCATGGCCAGCA. In two studies, the use of control primers is described: Chenevix-Trench et al. (78) used primers for glutathione *S*-transferase *P1*, and Katoh et al. (14) used primers for β-globin. In two studies (14, 58), the use of positive and negative controls samples is mentioned.

POPULATION TESTING

To date, there is insufficient evidence implicating either *GSTM1* or *GSTT1* in the etiology of colorectal neoplasms to make population testing an issue.

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[*Appendix follows*]

APPENDIX. Internet sites of interest

Data on disease frequency	
IARC*- Cancer Mondial	http://www-dep.iarc.fr/
SEER*	http://www-seer.ims.nci.nih.gov/
Information on cancer	
Cancer Research Campaign	http://www.crc.org.uk/homepage.html http://www.crc.org.uk/cancer/cancer_intro.html (URL specific to bowel cancer)
American Association of Cancer Research	http://www.aacr.org/ http://www.aacr.org/5000/5000.html (URL specific to bowel cancer)
National Cancer Institute	http://cancernet.nci.nih.gov/
International Union against Cancer	http://www.uicc.ch/
Genetic information	
CDC* Office of Genetics and Disease Prevention—medical literature search	http://www.cdc.gov/genetics/Medical.htm
Public Health Genetics Unit	http://www.medinfo.cam.ac.uk/phgu/
Human Gene Mutation Database	http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html http://www.uwcm.ac.uk/uwcm/mg/search/120020.html (URL specific to *GSTM1*) http://www.uwcm.ac.uk/uwcm/mg/search/371704html (URL specific to *GSTT1*)
OMIM*	http://www.ncbi.nlm.nih.gov/Omim/ http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?138350#TEXT (URL specific to *GSTM1*) http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?600436 (URL specific to *GSTT1*)
GenAtlas	http://bisance.citi2.fr/GENATLAS/ http://bisance.citi2.fr/cgi-bin/detgen?NUMDOS=30579 (URL specific to *GSTM1*) http://bisance.citi2.fr/cgi-bin/detgen?NUMDOS=16041 (URL specific to *GSTT1*)
UniGene	http://www.ncbi.nlm.nih.gov/Schuler/UniGene/ http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hs&CID=154159 (URL specific to *GSTM1*) http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hs&CID=77490 (URL specific to *GSTT1*)
GeneCards	http://bioinfo.weizmann.ac.il/cards/ http://bioinfo.weizmann.ac.il/cards-bin/carddisp?GSTM1&search=gstm1&suff=txt (URL specific to *GSTM1*) http://bioinfo.weizmann.ac.il/cards-bin/carddisp?GSTT1&search=gstt1&suff=txt (URL specific to *GSTT1*)
Links to chromosome-specific databases and other sites	http://cedar.genetics.soton.ac.uk/public_html/links.html

* IARC, International Agency for Research on Cancer; SEER Program, Surveillance, Epidemiology, and End Results Program; CDC, Centers for Disease Control and Prevention; OMIM, Online Mendelian Inheritance in Man.