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Assessment of the Potential Association between Glutathione S-Transferase Polymorphisms and Colorectal Cancer: A Systematic Review, Meta- and Pooled Analysis

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Abstract

Background: Colorectal cancer is the second leading cause of death from cancer in Canada. Many studies have examined associations between *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms and colorectal cancer but with conflicting results. The objective of this study was to examine the totality of evidence for association between these polymorphisms and risk for colorectal cancer or adenoma.
Methods: A systematic review, meta- and pooled analysis were conducted.

Results: The meta- and pooled analysis suggest weak significant associations between *GSTM1* (ORpooled=1.11; 95%CI: 1.02,1.23), *GSTT1* (ORpooled=1.22; 95%CI: 1.10,1.35), and with presence of null variants of each and colorectal cancer, but with heterogeneity between studies. No association between *GSTP1* and colorectal cancer, or between any of the polymorphisms and colorectal adenoma, was observed. Significant multiplicative GST gene-gene interactions or gene-environment (smoking) interaction effects were not observed.

Conclusion: Evidence to date shows weak associations between *GSTM1* and *GSTT1* and colorectal cancer.

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Chapter 1: Introduction

Colorectal Cancer

In Canada in 2007, it is estimated that 20,800 new cases of colorectal cancer will be diagnosed and that 8,700 deaths will occur (1). An individual has a 1 in 16 chance of developing colorectal cancer over their lifetime and it is estimated that 1 in 28 will die from it (2). According to the Canadian Cancer Society, overall colorectal cancer is the second leading cause of death from cancer. It is the second leading cause of cancer related deaths in men, after lung cancer and the third leading cause of cancer death in women, after lung and breast cancer. Colorectal cancer is rare in individuals under 40 years of age and most common in individuals over the age of 70 (3).

Colorectal cancer develops in either the colon or rectum, both of which are parts of the digestive system. After food is swallowed it travels through the esophagus to the stomach. Once food is partially broken down it is sent to the small intestine where digestion continues. The small intestine joins the large intestine in the lower right abdomen. The first and longest part of the large intestine is the colon which is a muscular tube that is approximately five feet long. This is where water and mineral nutrients are absorbed from food. Waste left from this digestive process travels into the rectum, which is the final six inches of the large intestine. The small and large intestines are sometimes referred to as the small and large bowel. The colon is composed of four sections. The first section is the ascending colon which begins where the small intestine attaches to the colon and extends upward on the right side of a person's abdomen. The second section is the transverse colon which crosses the body from the right to left side. The third section is the descending colon which continues downward on the left side. Finally the fourth section is the sigmoid colon due to its s-shape. The sigmoid colon joins the rectum which in turn joins the anus.

Colorectal cancer typically develops slowly over many years. It usually begins as a noncancerous polyp which left untreated may develop into cancer. The adenoma-carcinoma sequence is a fundamental theory which has emerged in the last few years which postulates that colorectal cancer arises from pre-existing adenomatous polyps which are the result of the disruption of normal mechanisms which regulate epithelial renewal; as these lesions progress, genetic changes in tumour suppressor genes and oncogenes accumulate (4). Although approximately 40 percent of the Western population will develop adenomas, only a small proportion of all adenomatous ploys progress to malignancy (5). The adenoma-carcinoma sequence provides an opportunity for early detection and ultimately the prevention of colorectal cancer by the removal of these precursor polyps.

Adenocarcinomas which evolve from the lining of the large intestine make up more than 95% of colorectal cancers (6). Cancer that has developed in the large intestine may begin to grow through the lining and into the wall of the colon or rectum, giving it the opportunity to metastasize by further growing into blood vessels or lymph nodes. The stage of cancer is used to describe the extent to which the cancer has spread. Typically three stages are used to describe the progression of colorectal cancer growth. Local cancers are cancers which have grown into the wall of the colon and rectum but have not invaded nearby tissues. Regional cancers have spread through the wall of the colon or rectum and have invaded nearby tissues or have spread to nearby lymph nodes. Distant cancers have spread to other parts of the body such as the liver and lung. Tumor location is thought to play a role in colorectal cancer. Colon cancer of the left (distal) or right (proximal) of the splenic flexure and rectal cancer exhibit differences in terms of incidence based on geographic region, age, and gender (7). The differences in epidemiological and clinical characteristics of colorectal cancer based on anatomical site suggests that these types of cancer may be the result of different genetic pathways of carcinogenesis and may represent different disease entities (8) .

Substantial differences in rates of colorectal cancer are observed between ethnic groups and geographical area suggesting that variations in environmental, lifestyle and genetic factors may play a role in the development of colorectal cancer. Inherited genetic abnormalities such as Familial Adenomatous Polyposis (FAP) syndromes, and Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are believed to account for approximately 5%-10% of all colorectal cancers (9). Excluding these syndromes, there remains a 2-fold increased risk for colorectal cancer in first degree relatives of colorectal cancer patients (10, 11). It is therefore quite likely that additional genes and genetic factors remain undiscovered. Table 1 summarizes the established and probable risk factors of colorectal cancer. Established risk factors include age, personal history of polyps or inflammatory bowel disease, obesity, physical inactivity, heavy alcohol consumption, and possibly smoking and consumption of meat. Reduced risk is associated with nonsteroidal anti-inflammatory drug use, hormone replacement therapy, and diets high in calcium and folate, although some of these associations remain controversial. In the majority of cases, colorectal cancer develops slowly over time. It is believed that colorectal adenomatous polyps are a precursor to colorectal cancer. Early detection and successful removal of these polyps may prevent the development of colorectal cancer (12, 13).

Table 1: Colorectal cancer risk factors

Risk Factor	Risk [14]
Genetic factors (FAP and HNPCC)	5-10%
Age	Risk is correlated with increasing age; particularly after the age of 50.
Personal history of polyps or inflammatory bowel disease	Particularly greater risk if fist cancer was diagnosed before the age of 60.
Obesity	Obesity and physical activity may account for 25-30% of cancer
Physical Activity	30-50% risk reduction with highest level of physical activity
Heavy alcohol consumption	Individuals who drink 30g/d appear to have a slightly increased risk; greatest risk among those who drink 45g/d or more.
Smoking	Inconsistent results. May be the result of a long induction period and differences in genetics, smoking patterns and environmental exposures.
Consumption of red meat	High consumption of red and processed meats may increase risk; association remains controversial
Consumption of fibre	Approximately a 30% reduction in risk compared to those who consume the least amount of fibre ($\leq$12 g/d)
Calcium and vitamin D	Consumption may decrease risk; still under debate
NSAIDs	Approximately a 25% reduction in risk after ~ 20 years of continuous use
Hormone replacement therapy (HRT)	Approximately 30-40% decreased risk
Folate	May have a small protective effect; remains controversial

Polycyclic aromatic hydrocarbons (PAHs), found in tobacco smoke and in cooked and processed meats, have been of considerable interest in the aetiology of colorectal neoplasia. Tobacco smoking has consistently been found to be associated with an increased risk for adenomas and hyperplastic polyps (15-18). In recent studies, long term smokers have been found to be at an elevated risk of colorectal cancer following an induction period of 35-40 year (19), although it has been suggested that this association could be due to inadequate control of confounding (15). Heterocyclic amines are generated during the cooking of red meats at high temperatures, and increased consumption of well-done red meat has been associated with increased risk of colorectal neoplasia in some studies (20, 21). The relation between vegetables and fruit and colorectal neoplasia is complex (22). Case control studies have consistently demonstrated an inverse association between the intake of vegetables, including cruciferous vegetables, and risk of colorectal cancer (23). However this

association is less consistent in cohort studies which are less susceptible to bias (22, 24-26). While this heterogeneity may reflect the challenges observational studies encounter in terms of studying associations between diet and disease (12), it also is possible that differences in the gene pools of the populations studied may contribute to differences in the results.

Glutathione S-Transferases (GSTs)

Glutathione S-transferases (GSTs) play a central role in the detoxification of carcinogens by catalysing the conjugation of glutathione to potentially genotoxic compounds, including PAHs (27). As a result, the null GST genotype, which is associated with no enzyme activity, would be expected to increase the risk for the development of cancer. However GSTs also conjugate isothiocyanates, which are potent inducers of enzymes that detoxify environmental mutagens, thereby diverting the isothiocyanates from the enzyme induction pathway to excretion (27, 28). Compounds present in brassica and allium vegetables can induce the GST detoxification system (29). It would therefore be expected that individuals with low GST activity would metabolize these compounds at a slower rate, thereby promoting their protective effects. As a result the null genotype would be expected to decrease the risk for the development of cancer. These two opposing potential mechanisms suggest that the role of GSTs and cancer risk is complex. GSTs also modulate the induction of the enzymes and proteins important for cellular functions such as DNA repair (30). Thus, GSTs modulate exposures thought to be important in the aetiology of colorectal neoplasia.

In humans, seven classes of cytosolic GSTs have been described (31). Each class consists of one or more isoenzymes where substrate activity may be overlapping or distinct. In terms of cancer susceptibility the *GSTM1*, *GSTT1*, and *GSTP1* have gained the most attention. The polymorphisms of *GSTM1*, *GSTT1* and *GSTP1* and their association with a variety of cancers have been investigated including colorectal, lung, head and neck, gall bladder, breast and ovarian cancer. In terms of colorectal cancer susceptibility and the potential association with GST polymorphisms, studies to date have primarily focused on the role of GSTμ (GSTM), GSTθ (GSTT), and GSTπ (GSTP) polymorphisms. Both *GSTM1* (32) (at low levels) and *GSTT1* (33) are expressed in the colon. In contrast to all the other classes of GSTs, *GSTT1* is expressed in erythrocytes (34). A substantial proportion of the population display a genetic variant in which no enzyme activity can be detected (null deletion) (35, 36). Homozygosity for deletion variants that occur at the *GSTM1* and *GSTT1* loci are associated with reduced, or no conjugation activity (27). This variation in activity may be important in modifying cancer risk. Evidence is lacking as to whether heterozygosity for either

deletion variant affects gene function. *GSTP1* has two polymorphisms: I105V and A114V. The Val allele encoded enzyme is associated with lower activity and is therefore thought to result in less effective capability of detoxification. It is therefore postulated that individuals carrying the Val allele are at a greater risk for developing cancer than among those carrying the Ile genotype.

There are other enzymes involved in the detoxification/activation pathway which are known to be polymorphic. It has been hypothesized that these genes may interact to affect disease susceptibility (37). In terms of colorectal cancer susceptibility, interactions between GST and NAT polymorphisms (38) and GST and *CYP1A1* gene activities have been most commonly postulated. Polymorphic NAT genes are thought to affect cancer susceptibility by influencing an individual's response to carcinogens. It has been proposed that NAT enzymes play a role in the activation of a variety of aromatic amines and heterocyclic aromatic amines found in tobacco smoke or well-done meats. Cytochrome P-450s (CYPs) are phase I metabolizing enzymes which metabolize environmental contaminants such as chemical carcinogens and mutagens by oxidizing the substrate. *CYP1A1* plays a key role in metabolizing PAHs. Increased activity of this enzyme is associated with increased carcinogen activation and therefore increased cancer risk. As yet, there appears to be little work investigating the joint effects between GST variants and these other polymorphisms. The few studies that have investigated the potential for interaction have lacked statistical power to detect interactions.

Several meta- and pooled analyses have been conducted examining the potential role of GSTs on cancer risk. The following summarizes some of the studies that have been conducted, the results observed and the challenges encountered when conducting meta- and pooled analyses. Engel et al. (2002) conducted a HuGE review examining the association between *GSTM1* and bladder cancer risk (39). The meta-analysis which included 17 studies resulted in a summary odds ratio of 1.44 (95%CI:1.23,1.68) for *GSTM1* and bladder cancer. Similar results were obtained for the pooled analysis which included 10 studies. A multiplicative interaction between *GSTM1*, smoking and bladder cancer was not observed. A HuGE-GSEC review was conducted by Kellen et al. (2007) which examined the association between *GSTP1* Ile105Val polymorphism and bladder cancer risk. The meta-analysis included 16 studies and a significant association was observed (OR=1.44; 95%CI:1.17,1.77). However, the pooled analysis included eight studies and a non-significant association was observed. No evidence of interaction between *GSTP1* and smoking was observed. Lai et al. (2005) conducted a meta-analysis to examine the association between GSTs and risk of adult brain tumors. Overall no significant association was observed between *GSTM1*, *GSTT1* or

GSTP1 and brain tumor risk. Vogl et al. (2004) conducted a pooled analysis examining the association between *GSTM1*, *GSTT1* and *GSTP1* and breast cancer risk. None of the GSTs examined were associated with breast cancer risk. In addition none of the GSTs were found to interact statistically with smoking or reproductive history to modify breast cancer risk. Ye et al. (2003) conducted a meta-analysis examining the association between *GSTM1* and colon cancer risk (40). Eighteen case-control studies were included in the analysis and a non-significant result was obtained. Boccia et al. (2006) conducted a meta-analysis and pooled analysis examining the association between *GSTT1* and gastric cancer risk. The meta-analysis included 18 studies and a non-significant association was observed. However the subgroup analysis based on high and low quality resulted in a significant association for high quality studies (OR=1.23; 95%CI:1.04,1.45). A significant interaction between *GSTT1*, smoking and gastric cancer was not observed. Seven studies reported sufficient information to examine the joint effect of *GSTT1* and *GSTM1* deletion mutations on gastric cancer risk; resulting in a significant association compared to those who were heterozygous (OR=1.95; 95%CI:1.42,2.67). Houlston et al. (1999) conducted a meta-analysis examining the association between *GSTM1* and lung cancer risk. Eighteen studies were included in the analysis which resulted in a weak but statistically significant association (OR=1.13; 95%CI:1.04,1.25). Raimondi et al. (2006) examined the association between *GSTT1* and lung cancer by conducting a HuGE-GSEC review. The meta-analysis and pooled analysis included 34 studies. The meta-analysis showed a significant association for Asians (OR=1.28; 95%CI:1.10,1.49); but not overall. In the pooled analysis there was no significant overall association and there was no evidence of interaction between *GSTT1* and smoking in relation to lung cancer. Another meta-analysis examining the association between GSTs (*GSTM1*, *GSTT1*, *GSTP1* and *GSTM3*) and lung cancer risk was conducted by Ye et al. (2006). This meta-analysis included 130 studies. Both *GSTM1* (OR=1.18; 95%CI:1.14,1.23) and *GSTT1* (OR=1.09; 95%CI:1.02,1.16) were found to be statistically associated with lung cancer risk. However when the analysis was restricted to only the larger studies, the associations were no longer statistically significant. Ntais et al. (2005) conducted a meta-analysis examining the association between *GSTM1*, *GSTT1* and *GSTP1* and prostate cancer risk. The meta-analysis included 12 studies. No significant interactions were observed between the GSTs examined and prostate cancer risk. It can be seen that heterogeneity across studies in terms of sample size, power and ethnicity are important factors contributing to the inconsistency of results. In addition many of the meta- and pooled analyses highlighted the limited information collected by the individual studies on environmental exposures which are known to be important risk factors for cancer.

Due to the fact that a systematic review was conducted a detailed literature review on GSTs and colorectal neoplasia is not presented here. Due to the considerable focus on these three GSTs, it was believed that sufficient evidence was available to investigate the potential association of *GSTM1*, *GSTT1* and *GSTP1* and colorectal cancer susceptibility. Therefore the objective of this study was to examine the potential association of GSTμ, GSTθ, and GSTπ polymorphisms and colorectal cancer.

1.1 Research Question

Is there an association between glutathione S-transferase *GSTM1*, *GSTT1*, or *GSTP1* polymorphisms and colorectal cancer?

1.2 Value of Study

A Human Genome Epidemiologic (HuGE) review of *GSTM1* and *GSTT1* and colorectal cancer was published in 2000 (27). HuGE reviews focus on human genetic variations at one or more loci, describe what is known about the frequency of these variants in different populations, identify diseases that are associated with these variants and summarize the magnitude of risks and associated risk factors. These reviews identify gaps in existing epidemiologic and clinical knowledge thereby stimulating further research in these areas. At the time the study was published, there was only one study of colorectal adenomas and *GSTM1*. The relationship between colorectal cancer and the *GSTM1* and *GSTT1* variants have been included in meta-analyses which aimed to cover all genetic polymorphisms investigated in relation to colorectal cancer (41-44) and there has been a specific meta-analysis on colorectal cancer and *GSTM1* (40). There has also been one pooled analysis done on GSEC (International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens) data on colorectal cancer, *GSTM1* and tobacco smoking (45). The results of this study did not support an association between *GSTM1* null genotype and colorectal cancer (OR=0.92; 95%CI: 0.73,1.14), nor did it find any evidence of interaction between smoking and *GSTM1* genotype on colorectal cancer risk (p=0.97). There has been an increasing interest in the association between cancer susceptibility and the inheritance of genetic variants responsible for the levels of metabolizing enzymes. Genes of low penetrance may carry a low independent risk. However, if they are highly prevalent within certain populations, their ultimate contribution to total cancer burden may be high (46). To date, evidence concerning the association between colorectal cancer susceptibility and GST polymorphisms has been conflicting. The associations observed from individual studies have been weak and inconsistent. This may be due to the small sample size used in many of the

studies resulting in the inability to detect modest effects. As a result, it was anticipated that a combined analysis may provide not only insight into the true relationship between GSTs and colorectal cancer but provide sufficient statistical power to properly assess potential interactive effects.

1.3 Objectives

The primary objectives are to:

1. Determine whether or not there is an association between *GSTM1* and (a) colorectal cancer (b) colon cancer (c) rectal cancer (d) colorectal adenomatous polyps.

2. Determine whether or not there is an association between *GSTT1* and (a) colorectal cancer (b) colon cancer (c) rectal cancer (d) colorectal adenomatous polyps.

3. Determine whether or not there is an association between *GSTP1* and (a) colorectal cancer (b) colon cancer (c) rectal cancer (d) colorectal adenomatous polyps.

4. Determine whether or not there is an interaction on the risk of developing colorectal cancer and polyps between *GSTM1*, *GSTT1* or *GSTP1* polymorphisms and meat intake, isothiocyanate intake or tobacco smoking.

5. Determine whether or not there is an interaction on the risk of developing colorectal cancer and polyps between *GSTM1*, *GSTT1* or *GSTP1* polymorphisms and NAT polymorphisms or *CYP1A1* polymorphisms.

1.4 Ethics Approval

The thesis was approved by the University of Ottawa Health Sciences and Science Research Ethics Board (H 03-06-06).

Chapter 2: Methods

The objectives of this thesis were accomplished by carrying out:

1. Systematic review
2. Meta-analysis of studies
3. Pooled analysis of individual data

2.1 Systematic Review

A systematic review examining the relationship between *GSTM1, GSTT1* and *GSTP1* polymorphisms and colorectal cancer was performed. A systematic review is defined by the Cochrane Collaboration as "a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review." The review also investigated the potential role of GST polymorphisms and meat intake, isothiocyanate intake, and tobacco smoking in terms of colorectal cancer/adenoma susceptibility. In addition the potential interactive joint effect of GST polymorphisms, NAT polymorphisms and *CYP1A1* polymorphisms and colorectal cancer/adenoma susceptibility were considered.

The search strategy employed in a systematic review should be comprehensive and exhaustive, using clear and reproducible eligibility criteria (47). The search strategy was based on that developed from the previous HuGE review conducted on GST polymorphisms and colorectal cancer and customized for the databases searched. The following three databases were chosen: Medline, EMBASE and Scopus. The search included the MeSH heading "glutathione transferase", the text words "GST" and "glutathione S transferase", and MeSH headings and text words relevant to colorectal cancer or polyps (Appendix A).

Medline was chosen since it is the most commonly used database. It is known for it's broad coverage of scientific literature and contains more that 12 million bibliographic records from more than 4,800 journals worldwide. Embase is a major biomedical and pharmaceutical database indexing over 3,500 international journals. It has greater coverage of European and non-English language publications. Depending on the topic of interest, the overlap between Medline and Embase is estimated to be between 10% to 87% (48). Finally, the database Scopus which is a comprehensive

database for scientific, technical and medical information containing 12,900 journal titles from 4,000 publishers was searched.

Inclusion & Exclusion Criteria:

The primary objective of the study was to investigate the association of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms and colorectal cancer susceptibility (including adenomas). The following inclusion and exclusion criteria were used to screen potential studies:

1. The primary objective of the study must be to investigate the association of GST polymorphisms and either colon, rectal, or colorectal cancer and/or adenomas.
2. Study must be either a case-control or cohort study.
3. Studies whose primary objective was to investigate the association of GST polymorphisms and survival, outcome or treatment effectiveness were excluded.
4. No language restrictions were imposed.

Once all studies were identified by the three search databases, the references were loaded into Refworks and duplicate studies were identified and removed (49). Trialstat's software SRS was used to review and identify studies for final inclusion in the systematic review. SRS is a web-based software designed to perform transparent, reproducible and auditable reviews (50). SRS was used to electronically review and screen the abstracts of all the studies identified by the search strategy to determine eligibility. SRS was also used to track included and excluded studies. Each reference was reviewed individually by examining title and abstract. The following criteria were used to determine study eligibility:

(1) Is the primary goal of the study to investigate *GSTM1*, *GSTT1* or *GSTP1* polymorphisms?
(2) Is the type of cancer being studied colorectal cancer (including colon cancer, rectal cancer, and adenomatous polyps)?
(3) Was a case-control or cohort study conducted to investigate the association between gene polymorphisms and susceptibility to cancer?

It is recommended that more than one reviewer independently review all the studies identified by the systematic review search strategy. By having more than one reviewer, relevant studies are less likely

to be missed and subjectivity of study selection is reduced (47). A 10% random sample of references was therefore reviewed by JL in SRS. The agreement rate was 100%.

In an attempt to identify grey literature, the CDC National Office of Public Health Genomics (NOPHG) medical literature search was searched, and conference abstracts were reviewed. A manual review of the references of all retrieved articles was conducted.

Search Results

The literature search identified 459 potentially relevant articles. The initial examination of titles and abstracts resulted in the identification of 234 duplicate articles. Table 2 summarizes the number of articles identified by the search database used.

Table 2: Search database, number and percent of articles identified and retained for the systematic review

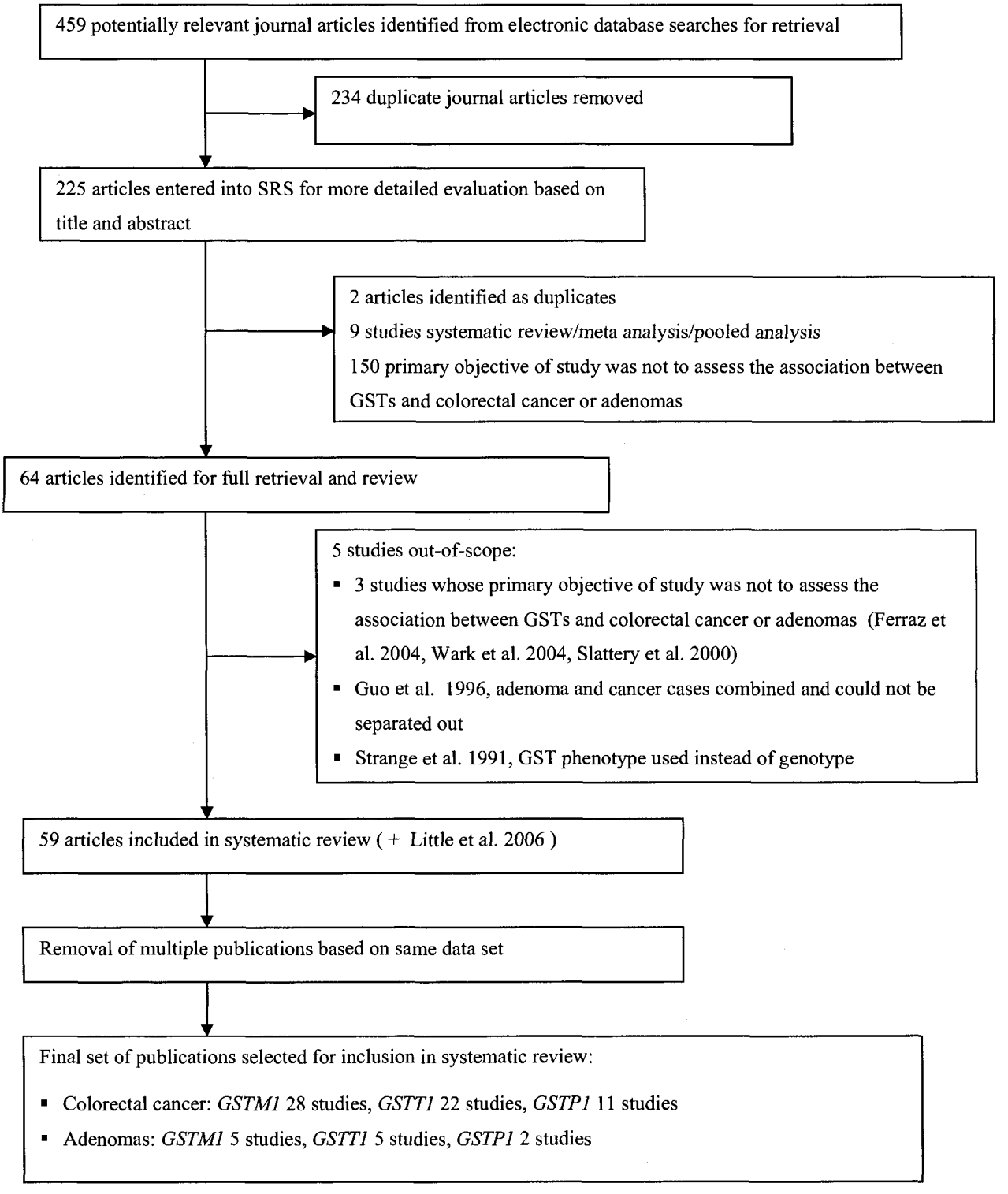
Search Database	# of Articles Identified	# of Articles Retained for Review	% of Articles Retained
Medline Only	24	6	25%
Embase Only	47	0	0%
Scopus Only	12	1	8%
Medline and Embase	25	7	28%
Medline and Scopus	9	2	22%
Embase and Scopus	17	2	12%
Medline, Embase & Scopus	91	34	37%
TOTAL	**225**	**52**	**23%**

The search results revealed that searching more than one database was necessary in order to capture all relevant studies. Medline was the most inclusive database. Considerable overlap between the three databases used was observed. For the purposes of this review, the same results would have been obtained had EMBASE not been searched.

The title and abstract information for the remaining 225 journal articles were imported into SRS for further review. Nine studies were identified as review or articles conducting a meta-analysis and/or pooled analysis and were excluded. Two articles were identified as additional duplicate articles. Finally, 150 articles were identified for exclusion based on the fact that they were either not case-control studies or the primary purpose of the study was not the examination of the association between colorectal adenoma or colorectal cancer and GSTs. The results of the review process

resulted in 59 articles being retrieved for full review. During the full article review, five articles were removed due to the fact that the study design was not a case-control study where the primary objective was to investigate susceptibility to either colorectal cancer or adenoma. Another article was also added to the review but was in press at time of search. The references for all 60 articles were reviewed to ensure that additional studies were not eligible for inclusion into the study. Multiple publications based on the same study population were identified. When this situation arose, the publication with the most inclusive recruitment period and number of subjects was used as the main reference. Publications that referenced another publication when describing the study design and subject recruitment were retrieved and relevant information was abstracted. Two studies were published in the Chinese language (Chen et al. 2004 and Zhu Y et al. 2002). Information was pulled from the English abstracts and a Chinese speaking colleague helped extract the information required for the analysis.

Figure 1: Search Strategy Results



2.2 Meta-Analysis

2.2.1 Overview of Meta-Analysis Technique

Meta-analysis is defined by the Cochrane Collaboration as "the use of statistical techniques in a systematic review to integrate the results of included studies". Meta-analysis is a technique which employs statistical methods to combine the results from a number of individual studies attempting to answer the same question in order to summarize the evidence relating to a particular issue.

Meta-analysis enables individuals to make sense of the vast range of literature, often with conflicting results, which is spread not only over many journals generated over many years but conducted in very diverse settings (51). To date, the metabolic gene polymorphisms studied for cancer risk have shown low penetrance and odds ratios generally between 1.5-2.0 (52). The study of low penetrance genes is challenging due to the lack of adequate statistical power to detect an association due to sample size limitations individual studies often encounter. As a result, meta-analysis is a technique that is particularly useful in the study of cancer susceptibility of low penetrance genes. Main advantages of meta-analyses include increasing power, computing a more accurate estimate of risk and reducing the potential of false negative results (53). Disadvantages of conducting a meta-analysis of published studies include the possibility that all studies conducted may not have been published thereby potentially biasing results. In addition it is not always possible to compare the results of individual studies directly. The information collected across the individual studies must be collected in a uniform and consistent manner. Another disadvantage is that subgroup analysis is not always possible with this statistical method. Finally due to the fact that the study (instead of the subject) is the statistical unit, meta-analyses are subject to ecological bias.

The quality of any meta-analysis is based on the underlying systematic review. A comprehensive systematic review identifies all relevant studies (published and unpublished) addressing the same scientific question, assesses the quality of each study identified based on design and implementation of the study and combines the results or findings of each study in an unbiased method. The meta-analysis provides an overall summary estimate in an attempt to provide a clearer picture and more precise estimate of effect size. Meta-analyses are subject to many potential biases. The strategy of any good meta-analysis is to acknowledge and minimize potential bias and heterogeneity as much as possible. Bias may be introduced through publication bias in which individual studies reporting statistically significant findings are more likely to be published than those which find no treatment effect (54) . In addition these studies are more likely to be published in the English language journals

which in turn are more likely to be indexed in various scientific databases and therefore these studies are more likely to be cited by studies conducted later (55). Failure to identify all relevant studies may bias the results of a meta-analysis. Also of issue is whether unpublished studies should be included in the meta-analysis due to questions concerning the quality of such unpublished studies. In addition the quality of studies published should also be assessed and a decision as to whether they should be included should be made. If possible, quality assessment should be conducted using a validated quality assessment tool in order to ensure that the assessment is conducted in an unbiased manner. By doing so, the quality of each individual study is assessed using explicit and objective criteria. Mixing the results of many individual studies leads to heterogeneity. Heterogeneity in meta-analysis is unavoidable. The issue is whether the degree of heterogeneity compromises the results and conclusions of the meta-analysis. For instance the cases may differ in terms of ethnicity, age, or disease severity, the primary outcome examined may differ across studies or studies may be conducted in distinct settings where other factors may influence study results.

In order to be included in the meta-analysis, two criteria needed to be met. Firstly, either an odds ratio (crude or adjusted) had to be reported or sufficient information provided in order to compute an odds ratio. Secondly a minimum of four studies had to be available to compute an overall summary estimate. As a result, a meta-analysis was not conducted for colon and rectal cancer. In addition the meta-analysis for the *GSTP1* genotype was restricted to I105V and to colorectal cancer. Insufficient information was reported by Lee et al. in order to compute an odds ratio. The study by Gawronska-Szklarz et al. stratified their case group results into cases with HNPCC, suspected of HNPCC, sporadic CRC and colonic polyps. Only the results for cases with CRC were included in the meta-analysis.

2.2.2 Data Abstraction

The following information was abstracted from each study: publication date, location of study, type of cancer, recruitment period, brief description of cases and controls (number, recruitment method, matching etc.), demographics of cases and controls (ethnicity, age, and gender), subgroup analysis, crude and adjusted odds ratios and corresponding 95% confidence intervals, variables used for adjustment and exposures assessed. To take account of concern about population stratification, ethnic group specific data was abstracted where possible. In instances where there was more than

one publication based on related data sets the study with the greatest number of subjects was included.

2.2.3 Statistical Analysis

The crude OR for all studies was computed if sufficient information was reported. In situations where the computed OR was not the same as the reported OR it was assumed that the reported OR had been adjusted. Use of adjusted odds ratios is challenging since the adjustments made are not always comparable across studies. The choice of variables collected in each study is not only dependent on the primary objective of the study but also the subjective opinion of the study authors. As a result, combining the results of many studies can be difficult and the validity of doing so questionable. In addition, there is debate about the appropriateness of adjustment for gene-disease associations. The choice of which OR to use is also controversial. Many meta-analyses use the crude OR from each study since they should be comparable. It however has been argued that the adjusted OR should be used since it is the best estimate of the effect. In order to account for this controversy both the crude OR and adjusted OR (or crude OR if not available) and the 95% CI was computed separately for *GSTM1*, *GSTT1* and *GSTP1 1105V* and *GSTP1 A114V*.

Statistical Model

Two statistical methods can be used to compute the combined estimate in a meta-analysis. The fixed effects model assumes that the underlying true exposure effect in each study is the same and that any observed differences across studies are due to random error, whereas the random effects model incorporates not only variation within studies but variation between studies (56). As a result, the random effects model allows for heterogeneity between the effects of different studies. In general, the random effects model generates wider confidence intervals than the fixed effects since it incorporates a between study component of variation. The random effects model was used to compute the combined estimate due to its conservative nature. Cumulative meta-analysis was also conducted in order to evaluate how the summary estimate changed with time. The cumulative meta-analysis estimate is computed by sequentially pooling studies one at a time based on publication date. A cumulative meta-analysis can be used to determine the robustness of the estimate over time as well as determine the point in time when the pooled result reached statistical significance (57).

Heterogeneity

Heterogeneity is an important and challenging issue in meta-analysis that must be considered. Studies are likely to differ in many important aspects such as study design, inclusion and exclusion of subjects, exposures measured, outcomes investigated and study quality. The diversity of study characteristics must be considered when combining study results and interpreting the overall combined estimate. Statistical heterogeneity exists when the true effects being measured differ between studies (58). Heterogeneity was addressed by computing the I^2 statistic to test for heterogeneity. Interpretation of I^2 was based on guidelines suggested by Higgins et al. (2003): I^2=0% no heterogeneity, I^2=25% low heterogeneity, I^2=50% moderate heterogeneity and I^2=75% high heterogeneity.

Heterogeneity was also examined by performing meta-regression analysis. Meta-regression is a statistical technique which examines the relationship between one or more study-level characteristics and the size of the effect observed in each study included (59). The following study characteristics were considered: year of publication, number of cases, language, type of controls and geographic region were considered. The number of cases was dichotomized based on the distribution of cases included in the meta-analysis. Type of control was categorized into population based, healthy, hospital and other. Geographic region was used as a proxy for ethnic group. Grouping ethnicity into meaningful categories is not only challenging but divisive. In an attempt to be as conservative as is possible, ethnicity was grouped according to the continent in which the study was conducted. It is acknowledged that this grouping is not ideal however due to the limited reporting in addition to its subjective and contentious nature it was deemed the most appropriate for this study. Type of control used was also considered as a potential source of heterogeneity. Bias may result if the controls are not generated from the same source population as cases. Type of control was categorized into the following categories: population based, healthy, hospital and other. Sample size was also considered as a source of heterogeneity. It has been hypothesized that underpowered studies due to a low sample size may be responsible for inconsistent results. Studies which employ small sample sizes may lack the power to detect a significant association. The distribution of case sample size was examined across studies for each of the GST polymorphisms and size was categorized into two categories based on the distribution. The categories used for *GSTM1* and *GSTT1* were $\leq$150 and >150 whereas $\leq$200 and >200 was used for *GSTP1*. A subgroup analysis based on language English and Chinese was also conducted.

Sensitivity analyses were also conducted to address potential heterogeneity. A sensitivity analysis enables the assessment of the impact on the overall combined estimate of including studies whose inclusion in the meta-analysis is questionable. The study by van der Hel et al. (2003) included women subjects only. As a result, a combined estimate was computed with and without this study. To address the issue of including crude odds ratios when an adjusted odds ratio was not computed or reported, a sensitivity analysis was conducted to examine the effect of including publications reporting a crude odds ratio from those reporting an adjusted odds ratio.

Subgroup analyses were conducted when sufficient information was reported. If at least four studies reported sufficient information in order to compute odds ratios for each subgroup, a combined estimate was computed. The effects of sex, tumor site and smoking status were examined. Tumor site was categorized into proximal and distal. It has been suggested that proximal and distal tumors in colorectal cancer may differ in etiology (60, 61). Smoking status was categorized into two categories: non-smokers and smokers.

Publication Bias

Publication bias is another important issue that should be considered in a meta-analysis. Publication bias is a type of bias which is part of a family of biases termed reporting bias (47). Reporting bias includes the tendency for studies with statistically significant or positive findings to be submitted and published (publication bias), more likely to be published more rapidly (lag-time bias), more likely to be published in the English language (language bias) and more likely to be cited more often (citation bias). Publication bias or unpublished non-significant findings may result in an artificial inflation of the magnitude of the combined overall estimate. Publication bias may be tested through graphical or statistical tests. Publication bias was assessed using the Begg's and Egger's test and their corresponding funnel plots. The Begg's test plots the effect size estimates and their variances. If there is no publication bias the plot should look like and inverted funnel. Random error is thought to be greater in smaller studies and therefore will be more widely spread around the mean effect resulting in a funnel shape. If publication bias is present, the left hand corner of the graph will be lacking thereby creating asymmetry in the funnel shape (62). Asymmetry in the Egger's plot is identified by assessing whether or not the intercept deviates significantly from zero in a regression of the standardized effect estimates versus their precision (62) . Due to the subjectivity associated with the visual interpretation of these plots, conclusions were drawn from the output of the statistical tests.

Other factors including heterogeneity, chance, choice of effect measure and choice of precision measure were considered in addition to publication bias as other potential causes of asymmetry (63). The influence of each study was examined by conducting influence analysis in which each study is excluded one at a time and the influence of the study on the overall magnitude of the summary estimate is assessed.

STATA version 9.0 was used to conduct the meta-analysis.

2.3 Pooled Analysis

Pooled analyses were conducted on GSEC data using unconditional logistic regression methods. Logistic regression is a regression method used to describe the relationship between an outcome variable and an independent variable (or set of independent variables), where the outcome variable is discrete, taking on two or more possible values. Logistic regression is different from linear regression models in that the outcome variable is binary or dichotomous. In particular, the conditional mean of the regression equation must be formulated to be bounded between zero and one and the binomial distribution describes the distribution of errors and will be the statistical distribution upon which the analysis is based (not the normal distribution) (64).

The pooled analysis was conducted using data at the individual subject level. This method offers many advantages over the meta-analysis of the results of studies. Advantages include increased statistical power, standardization of definitions of cases and variables, better control of confounding, and consistent determination of subgroup effects and the opportunity to examine gene-gene and gene-environmental interactions (65-67). A disadvantage of this method is that not all studies identified for inclusion in the analysis participated in GSEC. As a result, inclusion bias is a concern. In addition, the analysis is limited to variables collected across studies.

Approval to conduct an updated HuGE Review by the GSEC Advisory Members was granted. GSEC is an international venture whose primary objectives include gathering and analyzing existing information on metabolic gene polymorphisms, intermediate end points and/or cancer risk; establishing and coordinating a data bank; and fostering the collaboration among cancer researchers in this field. The ultimate goal of this project is the publication of consistent findings, leading to an accelerated understanding of genetic susceptibility to environmental carcinogens (68).

GSEC is a collaborative project whose objective is to enable investigators to analyze all of the available information on polymorphisms in genes that metabolize environmental carcinogens by compiling original data from both published and unpublished studies worldwide. In order to create the database, individual investigators who have conducted case-control studies on phase I and phase II metabolic gene polymorphisms were contacted and invited to participate by submitting all original data on all subjects included in his/her study. All data is to be submitted without individual identifiers to ensure confidentiality. The invitation to participate, instruction sheets outlining the variables and format required as well as a questionnaire to collect information pertaining to laboratory methods used to analyze the genotype, study design, response rate, and inclusion criteria is submitted to each investigator by letter. Data received is coded into a standardized manner. Regular reviews of the published literature are performed every six months and authors of new publications are invited to participate. The data collected are available to all those who have submitted data and investigators may submit proposals for use of the data. In addition, investigators are given the opportunity to participate in the analysis and are included among the authors for all studies that have been accepted by the Advisory Committee to the study. A detailed description of the GSEC study design is available elsewhere (52).

In order to obtain the data for the pooled analysis, the systematic review was performed and eligible studies were identified. A list of eligible studies was complied and sent to GSEC. GSEC contacted the identified study authors and invited them to participate in the study. Investigators were provided with a list of variables that GSEC was interested in receiving. Variables of interest included: age, sex, race, genes studied, genotyping methods, source of DNA, genotype blinding, type of cancer(s) studied, type of controls, geographical area where subjects were recruited, response rates, description of exposure assessment (i.e. smoking, alcohol consumption, diet, use of medications, occupational exposures) and tumor site, tumor stage, tumor histology and family history of cancer. Investigators were also encouraged to send the entire data file for their study. Data from each study was sent in the original format and re-coding was performed by GSEC. Continual correspondence was made with GSEC once the initial data set was received from GSEC. A few additional updates and edits were received and the data file was therefore updated. Once the final data set was established, various quality checks were conducted. For instance, the umber of cases and controls per study was compared to the number of subjects reported in each publication.

All analyses were conducted separately for colorectal adenomas and colorectal cancers. Adjustment for age, sex and study were performed. The overall significance of the model was assessed by

examining the likelihood ratio test. The likelihood ratio test compares the likelihood of the full model (contains all the predictor variables) with the likelihood of the null model (contains the intercept only). If the likelihood ratio test is significant it suggests that the predictors contribute significantly to the model (contribute to the prediction of the outcome). Two criteria or assumptions must be met when fitting a logistic model. The first criterion assumes that the observations are independent from each other. This assumption was met using the GSEC data. The second criterion is that any predictor that is measured on the continuous scale is assumed to have a linear relationship with the outcome. Testing of whether continuous variables were linear in the logit was conducted by plotting the continuous variable against the logit of the outcome and examining the relationship between the two variables. The only continuous variable used in the models considered was age. All the plots indicated that the assumption of linearity was reasonably met.

All studies included in the pooled analysis provided information on age and sex however some subjects had to be excluded due to missing information. As a result, pooled estimates of the odds ratio were computed using a data set where age, sex and the genotype under study were available for all subjects. This same data set was used to compute both the crude and adjusted ORs for comparison purposes. As a result the number of cases and controls reported in the various study publications may not be the same as the number included in the pooled analysis. The pooled analysis included 13 studies were for *GSTM1* and *GSTT1*, eight studies for *GSTP1* I105V and two studies for *GSTP1* A114V. Originally the data from the GSEC database was limited to one study that investigated colorectal adenomas. In order to increase the number of studies available for the pooled analysis, direct contact was made with various investigators to further encourage them to participate in the current study. Participation by Moore et al. (2005) was agreed and the data was provided to us. This data from this study is from the Prostate, Lung, Colorectal and Ovarian Screening Trial (PLCO) which is a large scale clinical trial designed to determine whether certain types of screening tests reduce death from cancer (69). As a result, two studies were included in the analysis of colorectal adenomas and the analysis was restricted to *GSTM1* and *GSTT1*. The study by van der Hel et al. 2003 included women subjects only. As a result, a combined estimate was computed with and without this study.

One of the objectives of this study was to examine whether or not there was an association between *GSTM1*, *GSTT1*, or *GSTP1* polymorphisms, colorectal cancer or adenoma susceptibility and the following exposures: meat intake, isothiocyanate intake and tobacco smoking. Due to the variability of information collected across studies, a limited number of the variables were included in the pooled

analysis. Information concerning diet was not available from the GSEC database. As a result the only exposure examined in the pooled analysis was tobacco smoking. Not all studies included collected information concerning tobacco smoking. In addition, information concerning tobacco smoking was not collected in a consistent manner across the studies in which information was available. Potential variables included in the GSEC database included smoking status (never, ex, current, ever and ex+never), years smoked and cigarettes smoked per day. The variable most consistently available was smoking status. Smoking status was categorized into 'never' or 'ever' for the pooled analysis. Table 3 summarizes the distribution of the GSEC variables used by cases and controls.

Table 3: Distribution of GSEC Factors by Cases and Controls

Factor	Cases (n=4070)	%	Controls (n=6817)	%
Sex (13 studies)				
Males	2166	53%	2938	43%
Females	1902	47%	3832	56%
Missing	2	0%	47	1%
Age (13 studies)				
Mean	63.9	100%	58.1	93%
Missing	3	0%	462	7%
Ethnicity (13 studies)				
Caucasian	3094	76%	4799	70%
African-American	34	1%	87	1%
Asian	942	23%	1931	28%
Height (6 studies)				
Mean	164.9	41%	163.1	53%
Missing	2409	59%	3175	47%
Weight (6 studies)				
Mean	69.3	40%	66.9	53%
Missing	2432	60%	3188	47%
Smoking status (11 studies)				
Never	1611	40%	3267	48%
Ever	1461	36%	2249	33%
Missing	998	25%	1301	19%
***GSTM1* (13 studies)**				
Null	2047	50%	3176	47%
Functional	1751	43%	3142	46%
Missing	272	7%	499	7%
***GSTT1* (13 studies)**				
Null	1129	28%	1715	25%
Functional	2701	66%	4716	69%
Missing	240	6%	386	6%
***GSTP1* I105V (8 studies)**				
Ile/Ile	1450	36%	2274	33%
Ile/Val or Val/Val	1419	35%	1918	28%
Missing	1201	30%	2625	39%
***GSTP1* I114V (2 studies)**				
Ile/Ile	688	17%	752	11%
Ile/Val or Val/Val	121	3%	143	2%
Missing	3261	80%	5922	87%

In terms of the *CYP1A1* variant three studies examined *CYP1A1**2A (m1), two studies examined *CYP1A1**2C (m2), and only one study examined *CYP1A1**4 (m4). Table 4 summarizes the number of cases and controls for each variant. Due to the limited number of studies that examined *CYP1A1* variants, potential interactions with GST variants were not evaluated.

Table 4: Number of cases and controls for *CYP1A1* variants included in GSEC data set

CYP1A1	Cases (n=4070)	%	Controls (n=6817)	%
***CYP1A1* (m1) (3 studies)**				
wt/wt	342	8%	554	8%
wt/m1	67	2%	117	2%
m1/m1	5	0%	4	0%
Missing	3656	90%	6142	90%
***CYP1A1* (m2) (2 studies)**				
wt/wt	75	2%	337	5%
wt/m2	2	0%	22	0%
m2/m2	1	0%	2	0%
Missing	3993	98%	6457	95%
***CYP1A1* (m4) (1 study)**				
wt/wt	235	6%	351	5%
wt/m4	15	0%	40	1%
m4/m4	0	0%	0	0%
Missing	3820	94%	6426	94%

In terms of the NAT variants, Table 5 summarizes the number of studies that provided data for the NAT variants and the number of cases and controls for each variant. Due to the limited number of studies that examined the NAT variants, potential interactions with GST polymorphisms were not evaluated.

Table 5: Number of cases and controls for NAT variants included in GSEC data set

NAT	Cases (n=4070)	%	Controls (n=6817)	%
***NAT1*3* (2 studies)**				
0	335	8%	874	13%
1	48	1%	99	1%
2	48	1%	10	0%
Not available	3687	91%	5834	86%
***NAT1*4* (3 studies)**				
0	172	4%	300	4%
1	125	3%	394	6%
2	172	4%	594	9%
Not available	3601	88%	5529	81%
***NAT1*10* (2 studies)**				
0	189	5%	647	9%
1	111	3%	356	5%
2	27	1%	91	1%
Not available	3743	92%	5723	84%
***NAT1*11* (2 studies)**				
0	317	8%	1065	16%
1	8	0%	27	0%
2	2	0%	2	0%
Not available	3743	92%	5723	84%
***NAT2*4* (3 studies)**				
0	468	11%	1086	16%
1	171	4%	306	4%
2	39	1%	83	1%
Not available	3392	83%	5342	78%
***NAT2*5* (2 studies)**				
0	144	4%	240	4%
1	185	5%	258	4%
2	83	2%	110	2%
Not available	3658	90%	6209	91%
***NAT2*6* (2 studies)**				
0	263	6%	373	5%
1	127	3%	192	3%
2	22	1%	43	1%
Not available	3658	90%	6209	91%
***NAT2*7* (2 studies)**				
0	393	10%	589	9%
1	17	0%	18	0%
2	2	0%	1	0%
Not available	3658	90%	6209	91%

Joint effects were tested by computing ORs for the following combinations of genotypes 1) *GSTM1, GSTT1* and *GSTP1*; 2) combined GST: *GSTM1* and *GSTT1* (both null/one GST null/neither GST null); 3) *GSTM1, GSTT1* and *GSTP1* and smoking. The change in deviance (-2*log likelihood) between the main effects model and the model that included an interaction term was computed as a test for interaction.

The pooled analyses were conducted using SAS 9.0.

2.4 Comparison of Combined Overall Estimate between Meta- and Pooled-Analyses

Results between the meta-analyses and the pooled analyses were compared. A challenge in comparing the combined estimates from the two procedures was the fact that not all studies participated in GSEC and therefore the data for several studies was not available for the pooled analyses. In addition several studies did not report an odds ratio or provide sufficient information to compute an odds ratio for the main effects under investigation. In order to do a direct comparison, a data set was created where only studies which participated in GSEC and reported an odds ratio or provided sufficient information to compute an odds ratio were included.

2.5 Investigation of Joint Effects: GSTs, Diet, Smoking, *CYP1A1* and NATs

The examination of the joint effects of *GSTM1*, *GSTT1*, *GSTP1* and diet, smoking, *CYP1A1*, *NAT1*, *NAT2* and colorectal adenoma and cancer risk was challenging. Not only did the genotypes investigated across studies vary in terms of genotypes examined, they also varied in terms of the variants investigated. In addition the exposures considered varied as did the type of information individual studies collected. It was therefore deemed inappropriate to combine the results of the studies included in terms of diet (isothiocyanate and meat intake), *CYP1A1*, *NAT1* and *NAT2* at either the study or individual subject level. Sufficient information however was reported on smoking to perform a subgroup meta-analysis and to be included in the pooled analysis.

GSTs and Diet

Several studies examined the interactive effects of GSTs and diet on the susceptibility to colorectal cancer and adenomas. Most studies used self-administered diet questionnaires to collect information regarding how often various food items were eaten. In terms of diet, information on vegetable intake and meat intake were most commonly the focus. The types of meat and vegetables considered varied across studies. As an example, one study may have focused (or reported results) only on broccoli or red meat intake whereas others looked at a broad spectrum of vegetables and/or meat (i.e. poultry, beef, lamb, pork etc.). In addition how the variables were categorized also varied across studies. For instance some studies categorized intake of vegetables into categories of 'high' and 'low', others categorized intake into quartiles and others treated intake as a continuous variable. The amount of detail reported also varied greatly across studies. For instance, many studies simply reported that there was no evidence of an interactive effect of genotypes and diet on colorectal risk. However, the actual details of the analyses performed were not reported. As a result, combining results across studies to perform a meta-analysis was not feasible. In addition the GSEC database did not contain information concerning diet. As a result, the examination of the interactive effects of GSTs, diet and colorectal cancer and adenoma risk were limited to the systematic review (see Chapter 3 for detailed summaries of the individual studies).

GSTs and Smoking

The potential interactive effect of GSTs, smoking and colorectal cancer risk or adenoma risk was investigated by several studies. As a result, subgroup analyses were conducted when sufficient information was reported. In terms of the meta-analysis, if at least four studies reported sufficient information in order to compute an odds ratio, a combined estimate was computed. Combining the smoking information across studies was challenging due to the variety of ways in which smoking was collected. For instance some studies classified individuals as 'smokers' and 'non-smokers' or 'non-smokers', 'former smokers' and 'smokers', whereas others collected detailed information concerning the number of cigarettes smoked per day, age when individuals started smoking, number of years smoked etc. In order to combine the smoking information, smoking status was categorized into two categories: 'non-smokers' and 'smokers'. Sufficient information was also provided in the GSEC database in order to examine the multiplicative effect of smoking, GSTs and colorectal cancer and adenoma risk. No significant interactions among any of the GSTs, smoking and colorectal cancer or colorectal adenoma risk were observed.

GSTs and *CYP1A1*

A limited number of studies examined the combined effects of GSTs and *CYP1A1* and colorectal adenomas or cancer. Only one study examined the combined effects of GSTs, *CYP1A1* and colorectal adenoma risk (Inoue et al. 2001) and another four studies examined colorectal cancer risk. The variants of *CYP1A1* investigated however varied across studies. In addition the reporting of results was limited. Some studies reported the results of combined 'high risk' genotype groupings whereas others provided more detailed results. As a result a meta-analysis could not be performed. Although information on *CYP1A1* was provided in the GSEC database, only a limited number of studies examined (or provided) information on *CYP1A1*. As a result it was decided that the information was not sufficient to conduct a pooled analysis that would produce meaningful results. Due to limited information the investigation of the combined effects of GSTs, *CYP1A1* and colorectal adenoma and cancer risk was limited to the systematic review (see Chapter 3).

GSTs, *NAT1* and *NAT2*

Similarly the number of studies that examined the combined effects of GSTs and *NAT1* or *NAT2* and colorectal adenomas or cancer was limited. A single study examined the combined effects of GSTs, *NAT2*, smoking and colon cancer and rectal cancer separately. Another two studies examined *NAT2* and colorectal cancer, on of which also examined *NAT2*. The GSEC database also had a limited number of studies that examined NATs. As a result, the investigation of the combined effects of GSTs, *NAT1*, *NAT2* and colorectal adenoma and cancer risk was restricted to the systematic review.

Chapter 3: Individual Study Summaries

The individual study summaries are separated into four categories based on cancer type investigated: colorectal adenomas, colorectal cancer, colon cancer and rectal cancer. Each summary is then presented by GST genotype(s) studied and by study date.

3.1 Colorectal Adenomas

GSTM1 and *GSTT1*

Inoue H et al. (2000) examined the association between *GSTM1*, *GSTT1* and colorectal adenomas in Japanese men. Cases were composed of men receiving pre-retirement health examination at SDF Fukuoka Hospital and SDF Kumamoto Hospital with histologically confirmed colorectal adenomas without in situ or invasive carcinoma. Controls were recruited from the same population and had a normal colonoscopy. A significant association between the *GSTM1* genotype and colorectal adenomas was not observed. Stratified analysis by location, smoking or adenoma size did not reveal any significant differences in terms of the *GSTM1* genotype. The association between the *GSTM1* non-null genotype and cigarette smoking tended to be slightly stronger than for those with the null genotype, however the interaction between *GSTM1* and smoking was not significant (p=0.35). A significant association between the *GSTT1* genotype and colorectal adenomas was not observed, nor was the interaction between the *GSTT1* genotype and smoking significant (p=0.60). Inoue et al. (2004) extended the analysis to examine the relationship between *CYP1A1 Msp*I and *GSTM1* on the risk of colorectal adnenomas, with an emphasis on the potential interaction with smoking cigarettes. A significant interaction was not observed between *GSTM1*, *CYP1A1*, smoking and colorectal adenoma risk.

Gunter MJ et al. (2005) examined the association between GST polymorphisms and risk of colorectal adenoma. Cases were part of the UK Flexible Sigmoidoscopy Screening Trial, a randomized controlled trial of 368,583 participants from 14 geographic areas. Cases were individuals with histologically confirmed adenoma of the distal bowel from 3 centers: Leeds, Norwich, and Portsmouth (n=918). Age and sex matched individuals with a negative flexible sigmoidoscopy result served as controls (n=981). A significant association between *GSTM1* genotype or *GSTT1* genotype and colorectal adenoma risk was not observed. Stratification by gender revealed no association between gender, genotype and adenoma risk. Evidence of a multiplicative

interaction between diet and smoking, genotype and adenoma was not observed for either *GSTM1* or *GSTT1*.

GSTM1, GSTT1 and GSTP1

Lin et al. (1998) examined the effect of *GSTM1* null and *GSTT1* null genotypes, broccoli intake and colorectal adenoma risk. A case-control study of subjects 50-74 years of age, undergoing a screening sigmoidoscopy at one of two Southern California Kaiser Permanente Medical Centers during 1991-1993 was conducted. Cases (n=459) had a first time diagnosis of one or more histologically confirmed adenomas, whereas controls (n=507) were recruited from the same source population and had no polyp detected. A significant association between the *GSTM1* null genotype or *GSTT1* null genotype and colorectal adenomas was not observed. To asses the interaction between *GSTM1* and cruciferous vegetable intake, vegetable intake was categorized into quartiles. Overall, increasing intake of cruciferous vegetables resulted in decreased risk of colorectal adenomas, particularly for broccoli. The interaction reached statistical significance for broccoli ($p_{interaction}$=0.01) but not for cruciferous vegetables combined ($p_{interaction}$=0.26). The highest protective effect was seen in individuals in the highest intake quartile with an OR of 0.36 (95%CI: 0.19, 0.68). The protective effect of broccoli intake was observed only for individuals with the *GSTM1* null genotype (p_{trend}=0.001 for *GSTM1* null vs. 0.43 for *GSTM1* non-null). Similar results were obtained when cruciferous vegetable intake was treated as a continuous variable (broccoli intake: p_{trend}=0.02 $p_{interaction}$=0.3; cruciferous vegetables combined p_{trend}=0.38 $p_{interaction}$=0.16). In terms of *GSTT1*, decreased risk of colorectal adenoma was observed with increasing broccoli and cruciferous vegetable intake for individuals with the *GSTT1* null genotype (broccoli: p_{trend}=0.0006; cruciferous vegetable intake p_{trend}=0.003) however the interaction was not significant (broccoli: $p_{interaction}$=0.14; cruciferous vegetable intake p_{trend}=0.45). Analysis was also conducted examining *GSTM1* and *GSTT1* genotype combinations where sample size permitted. Among individuals with both the *GSTT1* and *GSTM1* genotypes, the OR comparing the highest to lowest quartile of broccoli intake was 0.43 (95%CI: 0.29, 0.64). Subjects with at least one null genotype of either *GSTM1* or *GSTT1* compared with subjects with both non-null genotypes showed the lowest prevalence of colorectal adenomas among subjects with high broccoli intake and at least one null genotype (OR 0.41; 95%CI:0.24,0.70). The highest prevalence of colorectal adenomas was observed among individuals with no broccoli intake and both non-null genotypes (OR 1.93; 95%CI: 1.03,3.64). In a 2001 publication, Lin et al. extended their analysis concerning the association between *GSTM1*, *GSTT1*, broccoli intake and colorectal adenomas to include *GSTP1*. Only an abstract form of the published

results could be identified. The odds ratio for the effects of the *GSTP1* I105V variant on adenoma prevalence across four levels of broccoli intake was computed. The authors reported that a significant difference was not detected between subjects who were homozygous for the ILE105 and subjects with the VAL105 mutation. A significant interaction between *GSTP1* and *GSTM1* was not observed. The ORs were not reported and could not be computed due to insufficient reporting of information.

Moore LE et al. (2005) examined advanced colorectal adenoma risk and *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms. Cases were randomly selected from the Prostate, Lung, Colorectal, and Ovarian Trial (10 screening centers throughout the US) with at least one advanced colorectal adenoma in the distal colon (n=772). Controls were also randomly selected from participants of the same study with a negative sigmoidoscopy screening (n=777). Controls were frequency matched to cases by gender and ethnicity. The relationship between colorectal adenoma risk, GST polymorphisms and smoking was of primary interest in this study. A PCR based method which determined the number of active alleles carried by the subjects was used. This study combined the alleles for the *GSTM1* genotype differently than the other studies conducted. The results demonstrated that the risk of colorectal adenoma was lower in subjects carrying at least one *GSTM1* null allele (OR=0.6; 95%CI: 0.4, 0.9) in comparison with those carrying two active *GSTM1* alleles. When heterozygotes were grouped with those carrying two active alleles as is the usual grouping used with PCR fragment analysis, a significant difference in risk was not observed. A significant difference in risk was not observed for adenoma size, smoking status or broccoli intake and *GSTM1* genotype. In terms of *GSTT1*, a significant association between the *GSTT1* null genotype and colorectal adenoma risk was not observed. A significant difference in risk was not observed for adenoma size and *GSTT1* genotype. The *GSTT1* null genotype was significantly associated with colorectal adenoma risk among smokers (OR=1.4; 95%CI: 1.1, 1.9) but not among never smokers. The gene-environment interaction however was only significant when the +/- and the -/- allele groups were combined ($P_{interaction}$=0.05). Broccoli intake and adenoma risk was also examined. Individuals with the *GSTT1* null genotype (-/-) were at greater risk of adenoma risk (OR 1.7; 95%CI:1.13,2.7) when compared to the *GSTT1* (+/+) allele group. A significant association between the *GSTP1* genotype and colorectal adenoma risk was not observed for either *GSTP1* I105V or *GSTP1* A114V. The *GSTP1* GG genotype at codon 105 was found to be significantly associated with large adenomas (>1 cm) in comparison with small adenomas (P_{trend}<0.006). A significant difference in risk was not observed for smoking status and *GSTP1* genotype.

Tijhuis MJ et al. (2005) conducted a case-control study in the Dutch population in order to explore the hypothesis that lower GST activity (*GSTM1*, *GSTT1*, and *GSTP1*) and higher cruciferous vegetable intake is associated with lower colorectal adenoma risk. Cases were composed of individuals with least one histologically confirmed colorectal adenomatous polyp ever in their life and were recruited among patients undergoing endoscopy at the outpatient clinics of eight hospitals in the Netherlands (n=746). Controls were recruited from the same population and had no history of any type of polyps (n=698). The main effect for the *GSTM1*, *GSTT1* or *GSTP1* (I105V) genotype and colorectal adenomas was not reported. Cruciferous vegetable intake was analyzed by dichotomizing intake into high/low consumption, grouping intake into tertiles and treating intake as a continuous variable. The *GSTM1* and *GSTT1* genotypes did not modify the association between cruciferous vegetable intake and colorectal adenoma risk. Overall a significant, positive association was observed between the low activity *GSTP1* genotype (GG genotype), high cruciferous vegetable intake and colorectal adenoma risk (OR 1.94; 95%CI: 1.02,3.69). It should be noted that this positive association was dependent on the categorization of the vegetable intake variable. The following GST genotype combinations were also considered: *GSTP1/GSTA1*, *GSTA1/GSTM1*, and *GSTP1/GSTT1*. A statistically significant interaction between cruciferous vegetable consumption, adenoma risk, and the combination of *GSTP1* and *GSTA1* genotypes was observed (OR 1.87; 95%CI:1.23,2.84, p for interaction 0.033). These results are contradictory to the authors' hypothesis that low capacity GST genotypes would benefit from higher consumption of cruciferous vegetables compared to the low consumption group. Approximately half of all cases did not have a history of polyps (n=394). Analysis restricted to these cases was also conducted. A similar pattern of results were observed and were more pronounced for *GSTP1* and *GSTA1* genotypes. The study by Timersma et al. (2004) conducted an analysis using the same study population. They investigated the effect of combinations of genes (*GSTM1*, *GSTT1*, *NAT1*, *NAT2* and *SULT1A1*) on the risk of colorectal adenomas. The only significant interaction observed was between *GSTT1* and *SULT1A1*. The association of meat and gravy consumption on adenoma risk was also considered. Consumption was assessed using a habitual consumption questionnaire which collected information regarding 16 meat types (frequency and portion) and gravy. No differences in risk were observed between combinations of *GSTM1* or *GSTT1* variants and consumption of meat or gravy on adenoma risk. Tiemersma et al. (2004) also published a study based on the same study population. In this publication the effect of *SULT1A1*, *EPHX*, *NAT1*, *NAT2*, *GSTM1* and *GSTT1*, smoking and colorectal adenoma risk was investigated. A significant interaction between either *GSTM1* or *GSTT1*, smoking and adenoma risk was not observed.

3.2 Colorectal Cancer

GSTM1

Zhong et al. (1993) conducted a case-control study in the United Kingdom examining the relationship between the *GSTM1* genetic polymorphism and susceptibility to bladder, breast and colon cancer. Controls were recruited from three different sources: Clinical Chemistry Department at the Royal Hallamshire Hospital in Sheffield, the Royal Infirmary in Edinburgh and a group of volunteers in ICRF Clare Hall Laboratories. A significant association between the *GSTM1* null genotype and colorectal cancer was observed (OR=1.8, 95%CI: 1.2, 2.6). It was observed that more than 70% of individuals with a tumor in the proximal colon were *GSTM1* nulled; translating into an ~2 fold increase in colon cancer risk.

Gawronska-Szklarz B et al. (1999) examined the association between the *GSTM1* genotype and colorectal cancer and colonic polyps in Poland. Cases with colorectal cancer were separated into three categories: patients from families with HNPCC (n=17), patients with a high risk of HNPCC and suspected of having HNPCC (n=25) and patients with sporadic colorectal cancer (n=28). Controls were healthy volunteers selected based on age and sex (n=147). No further information concerning recruitment was provided for either the cases or controls. A borderline significant association between the *GSTM1* null genotype and sporadic colorectal cancer was observed (OR=2.53, 95%CI: 0.98,6.74).

Kiss et al. (2000) conducted a study to investigate the association between *CYP1A1*, *CYP2E1*, *GSTM1* polymorphisms and colorectal cancer risk. The study was conducted in Hungary. Cases were histologically confirmed (n=163) and healthy volunteers matched by age and sex were used as controls (n=163). A significant association between *GSTM1* and colorectal cancer risk was not observed. Combined analysis of *GSTM1*, *CYP1A1* and *CYP2E1* revealed that individuals carrying all three 'high risk' (*GSTM1* null, *CYP1A1* Val, *CYP2E1* c2*)* alleles were at a significantly higher risk of colorectal cancer than subjects with no 'high risk' alleles (OR=4.62; 95%CI: 1.23,25.68).

Tiemersma EW et al. (2002) conducted a study to investigate the possible interplay between meat consumption or tobacco smoking, *NAT1*, *NAT2* and *GSTM1* polymorphisms and colorectal cancer risk on the Dutch population. Their focus was on potential gene-environment interactions. They conducted a nested case-control study from the Prospective Monitoring Project on CVD Risk

Factors. Each year a random sample of men and women, aged 20-59 years of age, were selected from municipal registries and invited to participate. From the resulting database, all incident cancer cases were included (n=102). A random sample of individuals from the database with the same distribution of gender, age and center served as the controls (n=537). A significant association between *GSTM1* and colorectal cancer risk was not observed. Meat consumption was categorized into: 0-1, 1-4, and 4+ times per month. Red meat, poultry, fish, sausage, and sandwich filling consumption were considered. Frequent consumption of poultry was associated with a decreased risk in the presence of the *GSTM1* null gene (p=0.01). Cigarette smoking exposure was also examined. Smoking was categorized into smoking status (never, former, current), smoking duration in years (0, 1-25, >25), and cigarettes smoked per day (, 1-14, >14). No significant association between *GSTM1* genotype and smoking was observed.

Luchtenborg et al. (2005) examined the association between APC Mutations, hMLH1 expression and *GSTM1* and *GSTT1* polymorphisms in sporadic colorectal cancer. The primary analysis used tumor tissue to examine various associations. Data from 1989 to 1994 from the Prospective Netherlands Cohort Study on Diet and Cancer which was initiated in September 1986 was used. The study population was derived from 204 municipal population registries throughout the Netherlands. Incident cases were identified by monitoring of the entire cohort for cancer occurrence through annual record linkage to the Netherlands Cancer Registry and the Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA). A secondary case-only analysis was performed to examine the interaction between cigarette smoking and the *GSTM1* and *GSTT1* genotypes. No statistically significant interactions between either *GSTM1* or *GSTT1* and smoking were observed. A borderline significant association between the *GSTM1* null genotype and smoking frequency (increment of five cigarettes/day) was observed (OR=1.07; 95%CI:1.00,1.14; p=0.06).

GSTT1

Rajagopal et al. (2005) conducted a study to examine the association between the *GSTT1* null genotype and colorectal cancer risk. This study was built on the previous study conducted by Deakin et al. (1996). Northern European Caucasian patients with operative and histological confirmation of adenocarcinoma of the colon and rectum were recruited from the University Hospital of North Staffordshire. Patients were recruited during three time periods 1990-1994, 1996-1998 and 1998-2000 and were followed up for 2-15 years. Only patients with sporadic CRC undergoing potentially curative surgery were included in the study. Unrelated controls were recruited from the same hospital. A significant association between the *GSTT1* null genotype and colorectal cancer was

observed (OR=1.65; 95%CI:1.22,2.24). A significant association between the *GSTT1* null genotype and sex, tumor site, tumor differentiation, TNM classification and tumor margin morphology and colorectal cancer was not observed. Stratification by age revealed that the *GSTT1* null genotype was more common in female patients in comparison with males in the younger (< 70 years) age group (p=0.017).

GSTP1

Harris et al. (1998) examined *GSTP1* and colorectal cancer susceptibility. Study subjects were composed of 131 colorectal cancer cases (as well as 184 lung cancer cases) and 199 controls from the Canberra Blood Transfusion Service. The colorectal cancer cases are an extension of the Chenevix-Trench et al. (1995) study. No significant association between *GSTP1* I105V or *GSTP1* A114V and colorectal cancer susceptibility was observed.

Katoh et al. (1999) conducted a study in Japan examining *GSTP1* and susceptibility to smoking related epithelial cancer including oral (n=83), lung (n=47), gastric (n=140), colorectal (n=103) and urothelial (n=106) cancer. Cases were histologically confirmed. The control group was composed of individuals who had visited local medical clinics (n=122). No significant association between *GSTP1* and colorectal cancer was observed. A significant association between *GSTP1*, smoking and colorectal cancer risk was not observed.

GSTM1 and *GSTT1*

Chenevix-Trench et al. (1995) conducted a case-control study in Australia examining the association between *GSTM1* and *GSTT1*, susceptibility to colorectal cancer and age of onset. A significant relative risk of colorectal cancer associated with the *GSTM1* null genotype was not observed. A significant change in the distribution of *GSTM1* was not observed when stratification by age group (less than 70 years of age vs. 70 years and older) or tumor site (proximal vs. distal) was conducted. A significant difference in the distribution of the *GSTT1* genotype was observed when stratified by age. The *GSTT1* null genotype was significantly more common in individuals diagnosed before the age of 70 years than those diagnosed at an older age (p=0.028). This finding suggests that the *GSTT1* genotype may be associated with age of onset for colorectal cancer.

Deakin et al. (1996) conducted a case-control study examining *GSTT1* genotypes and susceptibility to cancer and interactions with *GSTM1* in lung, oral, gastric and colorectal cancers in the United Kingdom. A significant association between the *GSTM1* null genotype and colorectal cancer was not observed.

Gertig et al. (1998) conducted a nested case-control study to examine the association between *GSTM1*, *GSTT1* and colorectal cancer. The potential interaction of genotype with smoking history was also examined. This study was part of the Physicians' Health Study in the United States where participants were white males. A significant association between colorectal cancer risk and either the *GSTM1* or *GSTT1* null genotype was not observed. Differences in risk were not observed when the analysis was stratified by age (≤60 years vs. ›60 years) or tumor site (proximal vs. distal). An increased risk between the *GSTM1* or *GSTT1* null genotype and current smoking, pack years of smoking and age at smoking was not observed. A combined effect of *GSTM1* and *GSTT1* null genotypes and colorectal cancer was not observed. In addition effect modification by age was not observed for the combined *GSTM1/T1* null genotype and colorectal cancer.

Lee et al. (1998) examined the association between *GSTM1, GSTT1, NAT1* and *NAT2* polymorphisms and colorectal cancer in Singapore. A significant association between the *GSTM1* null genotype and colorectal cancer was not observed. A significant association between site (54.1% right, 48.3% left and 40.2% recto/sigmoid) and histological differentiation of tumor was reported (66.7% poorly differentiated, 63.6% mucinous cancers, 41% moderately differentiated and 42.9% well differentiated). A significant association between the *GSTT1* null genotype and colorectal cancer was not observed. An inverse association to that observed for the *GSTM1* null genotype and tumor site was reported for the *GSTT1* null genotype (48.6% right and 56.4% recto/sigmoid).

Abdel-Rahman SZ et al. (1999) conducted a case-control study examining the association between *GSTM1* and *GSTT1* and colorectal cancer susceptibility in Egypt. Cases were composed of newly diagnosed colorectal cancer patients recruited from three cancer hospitals in Egypt (n=83). Controls were healthy friends of other cancer patients, matched to cases on age (n=63). A significant association between *GSTM1* or *GSTT1* null genotype and colorectal cancer was not observed. A significant change in the distribution of *GSTM1* or *GSTT1* was not observed when stratification by age group (less than 40 years of age vs. 40 years and older) was conducted. Significant differences in the distribution of the *GSTM1* or *GSTT1* genotype in relation to site (right, left, transverse or rectal), urban/rural residents and gender was not observed.

Zhang H et al. (1999) conducted a study where the *GSTM1* and *GSTT1* genotypes were compared in patients with colorectal cancer (n=99) and healthy controls (n=109). Tumor samples were obtained from patients with primary colorectal adenocarcinoma diagnosed at the Department of Pathology, Linkoping University Hospital and Norrkiping Central Hospital between 1989 and 1997. Control subjects were randomly selected from a healthy control group of residents in South-Eastern Sweden. A significant difference in frequency of *GSTM1* null genotype between cancer cases and controls was not observed (p=0.33). In comparison with the controls, the frequency of the *GSTT1* null genotype in cases was significantly increased (p<0.0001).

Butler WJ et al. (2001) conducted a case-control study to examine metabolic genotypes and colorectal cancer risk in Australia. Cases were white adults with sporadic colorectal cancer (n=219). White individuals attending a blood donation bank were recruited for controls (n=200). Several polymorphisms were considered individually including *NAT1, NAT2, CYP1A1, CYP2E1, GSTM1* and *GSTT1*. Overall a significant association was not observed between any of the genotypes alone. A significant association was observed between *GSTT1* and colorectal cancer (OR=2.18, 95%CI: 1.38,3.43). However once an adjustment was made for age, this association failed to reach significance (OR=1.91, 95%CI: 0.99,3.70). In addition a significant association was not observed between any combination of genotypes and colorectal cancer. Stratification by age, gender and site of cancer did not reveal significant differences. In addition excess risk by combining fast acetylators *NAT1* and *NAT2* or for both *GSTM1* and *GSTT1* was not observed. Stratification by age, gender and site of cancer did not reveal significant differences.

Saadat I and Saadat M. (2001) conducted a case-control study in Iran to examine *GSTM1* and *GSTT1* genotypes and gastric and colorectal cancer risk. Cases consisted of pathologically confirmed primary colorectal cancer (n=46). Healthy blood donors matched to the patients according to age and gender served as the control group (n=131). A significant association was not observed between *GSTM1, GSTT1* or *GSTM1* and *GSTT1* in combination and colorectal cancer. Differences were not observed between sex groups.

Laso et al. (2002) conducted a hospital based case-control study in order to examine the association between *GSTM1* and *GSTT1* and colorectal cancer susceptibility. Cases were composed of consecutive colorectal cancer patients undergoing surgery at the University of Barcelona Hospital Clinic in Spain (n=247). Consecutively recruited patients presenting to the Trauma Service of the

Hospital during the same recruitment time period as cases served as controls (n=296). A significant association between the *GSTM1* genotype and colorectal cancer was not observed however a borderline significant association between the *GSTT1* genotype and colorectal cancer was observed (OR=1.68; 95%CI:1.0,2.82). Stratification by gender did not reveal any significant differences for either *GSTM1* or *GSTT1*. Stratification of the *GSTT1* null genotype by smoking status revealed an increased colorectal cancer risk for individuals in the smoking strata than in the non-smoker strata (OR=2.5; 95%CI:1.04,6.1). The presence of both the *GSTM1* and *GSTT1* null genotypes did not significantly increase colorectal cancer risk. Stage, differentiation or tumor location and genotype were not associated with colorectal cancer risk.

Sgambato A et al. (2002) performed a case-control study to evaluate the distribution of the *GSTM1* and *GSTT1* polymorphisms and their association with various cancers, including colorectal cancer, in the Basilicata population. Consecutive, unselected patients admitted at the Division of Oncology at the Centro di Riferimento Oncologico of Basilicata in Southern Italy served as cases (n=110). Controls were healthy subjects visiting the hospital for routine blood tests with no personal and/or family history of cancer (n=100). A significant difference in the distribution of the *GSTM1* null genotype among cases compared to controls was observed (p=0.02). Thirty-two out of 44 (73%) colorectal cancer patients were *GSTM1* null compared to 53% of the control population. Seven cancer cases were both *GSTM1* and *GSTT1* null and six of these cases were colorectal cancer cases. The small number of *GSTT1* null individuals in this study limited the analyses that could be conducted for this gene. Overall the frequency of the *GSTT1* null genotype was greater in the control (18%) than in the case population (9%). However, in terms of colorectal cancer the pattern observed was the inverse, eight (80%) colorectal cancer patients were *GSTT1* null compared to 18% of the control population. Seven patients were both *GSTM1* and *GSTT1* null and six of these cases were colorectal cancer cases.

Zhu Y et al. (2002) evaluated the association between the polymorphisms *GSTM1* and *GSTT1* and colorectal adenocarcinoma susceptibility. This article was published in the Chinese language. This Chinese case-control study was composed of 104 cases of sporadic colorectal cancer and 101 healthy controls. The difference in frequency of the *GSTM1* null genotype for cases and controls did not reach statistical significance. Stratification by tumor location (proximal vs. distal) or age (<60 years of age vs. ≥60 years of age) did not reveal any significant differences. Overall a significant difference in the frequency of the *GSTT1* null genotype for cases and controls was not observed. The *GSTT1* null genotype was more prevalent in distal colorectal cancers in comparison to proximal

cancers ($p<0.05$). In addition the *GSTT1* null genotype was more frequent among the older cases relative to the younger cases ($p<0.05$). The association between the *GSTT1* genotype, smoking and colorectal cancer was investigated. The difference in frequency of the *GSTT1* null genotype between heavy smoking cases and heavy smoking controls reached statistical significance ($p=0.021$). The frequency of the *GSTT1* null genotype was also significantly more frequent in the heavy smoking case group and distal SCRAC ($p=0.011$), in the younger cases ($p=0.03$) and in the poorly differentiated SCRAC case group ($p=0.045$) when compared to heavy smoking controls. Cases carrying both the *GSTM1* and *GSTT1* null genotypes were at a significantly greater risk for colorectal cancer (OR=4.33, 95%CI: 1.56,12.04) than those carrying both the non-null genotypes.

Nascimento H et al. (2003) examined the association between *GSTM1*, *GSTT1*, and colorectal cancer risk in Brazil. Cases were consecutive patients with histologically confirmed sporadic colorectal adenocarcinoma who were treated at the University Hospital of the State University of Campinas (n=102). Controls were blood donors from the same hospital (n=300). The individual effect of *GSTM1* and *GSTT1* genotypes and the combined null genotypes was examined. The *GSTM1* null and *GSTT1* null genotypes were not significantly associated with colorectal cancer individually or in combination. Stratification by age, gender, ethnic origin, smoking status, tumor location, stage or grade of differentiation of the tumor did not result in a significant difference in the frequency of the *GSTM1* or *GSTT1* null genotype. However, stratification by age, gender, ethnic origin, smoking status, tumor location, stage or grade of differentiation of the tumor revealed a significant difference in terms of age and sporadic colorectal carcinoma. The frequency of the *GSTT1* null genotype was significantly greater in cases diagnosed before the age of 60 years than those diagnosed after the age of 60 years ($p=0.0008$).

van der Hel OL et al. (2003) investigated the combined effect of smoking and the genetic polymorphisms of the metabolic genes *NAT1*, *GSTM1* and *GSTT1* and colorectal cancer risk. Cases were participants of a population-based screening program for early detection of breast cancer (Diagnosticsh Onderzoek Mammacarcinoom project, 1974). A subcohort of 1000 randomly selected women from the source population served as controls. This is the only study that used urine samples for DNA retrieval. A significant increase in colorectal cancer risk among those with the *GSTM1* null or *GSTT1* null genotype was not observed. Nor was a significant interaction between *GSTM1* or *GSTT1*, smoking and colorectal cancer risk observed. Women were also categorized into groups of increasing number of putative risk genotypes. No effect on cancer risk was observed. The potential

modifying effect of smoking and number of putative risk genotypes was not evaluated due to inadequate sample size.

Chen K et al. (2004) evaluated the associations between *GSTM1*, *GSTT1,* smoking and colorectal cancer susceptibility. This study was published in the Chinese language. A case-control study of 126 patients and 343 healthy controls was conducted. Among individuals with the *GSTT1* null genotype, individuals with the *GSTM1* null genotype had a significantly increased risk of rectal cancer compared to those with the *GSTM1* non-null genotype (OR=9.74, 95%CI: 1.13,83.85). Note that the confidence interval for this odds ratio is quite large. Current smoking and the *GSTT1* null genotype was associated with colon cancer (OR=4.55 95%CI: 1.14,18.17) and rectal cancer (OR=4.6, 95%CI: 1.11,19.11).

Little et al. (2006) conducted a population based case-control study in Scotland to examine the association between *CYP1A1*, *GSTM1* and *GSTT1* and colorectal cancer risk. Cases were composed of individuals with histologically confirmed invasive tumors of the colon or rectum identified from the database of the pathology laboratory (n=264). Controls were selected from the Grampian Community Health Index (list of everyone registered with a GP) and were matched to cases by age and sex (n=408). A significant association between the *GSTM1* or *GSTT1* genotype and colorectal cancer was not observed. Evidence for an interaction between the *GSTM1* or *GSTT1* genotype and the *CYP1A1* genotype, smoking, total meat intake or green leafy vegetable intake was not obtained.

GSTM1 and *GSTP1*

Landi S et al. (2005) examined various phase I and phase II metabolism gene polymorphisms and colorectal cancer risk. Newly diagnosed patients with colorectal adenocarcioma attending a University Hospital in Barcelona, Spain were used as cases (n=359). Individuals living in the same area and representative of the general population, randomly enrolled among patients admitted to the same hospital during the same period of time with a diagnosis of disease other than cancer served as controls (n=320). All subjects were Caucasian and born in Spain. A significant association between *GSTM1 or GSTP1* and colorectal cancer was not observed.

GSTM1, *GSTT1* and *GSTP1*

Yoshioka M et al. (1999) carried out a case-control study examining the association between *GSTM1*, *GSTT1*, *GSTP1*, *NAT1* and *NAT2* polymorphisms and colorectal cancer susceptibility. Cases were histologically confirmed consecutive patients attending the University of Occupational and Environmental Health Hospital, Mitsubishi Chemical Hospital, and Kitakyushu Medical Center in Japan between September 1991 and June 1995 (n=106). The control group was composed of individuals who had visited local medical clinics in Kitakyushu City between September 1993 and April 1995 for regular medical check-ups with no current of previous diagnosis of cancer (n=100). A significant association between *GSTM1*, *GSTT1* or *GSTP1* genotypes and colorectal cancer was not observed. A significant association between genotype, smoking and cancer risk was not observed. An examination of the effect of various genotype combinations revealed a significant association between *GSTM1* and *GSTP1*. Using the following groupings and comparison, *GSTM1* present and *GSTP1* A/A vs. other combinations (*GSTM1* present/*GSTP1* with G, *GSTM1* null/*GSTP1* A/A and *GSTM1* null/*GSTP1* with G) a significant association with colorectal cancer was observed (OR=2.15, 95%CI: 1.21,3.79).

Welfare M et al. (1999) examined the relationship between *GSTM1*, *GSTT1* and *GSTP1* polymorphisms and susceptibility to colorectal cancer. A matched pair case-control study was conducted in England (178 matched pairs). This design was chosen in order to minimize biases due to age, sex, and area of residence. Cases with histologically confirmed colorectal cancer diagnosed at the Newcastle and North Tyneside health district between December 1994 and September 1995 were invited to participate. Community controls matched on age and sex were recruited for each case, identified from GP records. A significant effect of *GSTM1* on colorectal cancer susceptibility was not observed. Subgroup analysis by age, sex, stage or site did not reveal significant differences. A significant effect of *GSTT1* on colorectal cancer susceptibility was not observed. Stratified analysis by age revealed that the frequency of the *GSTT1* null genotype decreased with increasing age (less than 65 years of age, 65-75, and older than 75 years of age) however, this trend did not reach statistical significance (p=0.143). Stratification by Dukes' stage revealed that the *GSTT1* null genotype was more common in Dukes stage A tumors than in more advanced tumors (OR=3.36; 95%CI:1.23, 9.15). Analysis of combinations of genotypes revealed a significant interaction between *GSTT1* null genotype and *NAT1* slow acetylator genotype (OR=2.33, 95%CI: 1.10,5.00) and *GSTT1* null and *NAT2* slow acetylator genotype (OR=2.24, 95%CI: 1.00,4.99; p=0.049) and colorectal cancer risk. In terms of *GSTP1* I105V or A114V, a significant association was not observed.

Loktionov A et al. (2001) examined *GSTM1*, *GSTM3*, *GSTT1* and *GSTP1* gene polymorphisms in colorectal cancer patients in the UK. Patients with histologically confirmed colorectal cancer treated at the Department of Survery, Norwich and Norfolk Health Care NHS Trust served as cases (n=206). Controls were participants of the UK Flixible Sigmoidoscopy Screening Trial subjected to a flexible sigmoidoscopy at the same department, who had a normal test result (n=345). A significant association between the *GSTM1*, *GSTT1* or *GSTP1* genotype and colorectal cancer was not observed. A strong linkage between the *GSTM1**A and *GSTM3**B alleles was observed.

Sachse C et al. (2002) conducted a case-control study in the United Kingdom in order to investigate the effect of several dietary factors including fruit, vegetable and meat consumption on colorectal cancer risk in relation to genotype. Cases were incident colorectal cancer patients recruited at either Ninewells Hospital, Dundee, Perth Royal Infirmary, Leeds General Infirmary, St. Jame's Hospital, Leeds or York District Hospital (n=490). Healthy population based individuals (GP controls), recruited by age, sex and general practitioner matching of incident cases served as controls (n=593). The unmatched analysis revealed a significant association between the *GSTM1* null genotype and colorectal cancer susceptibility (OR=1.33, 95%CI: 1.04,1.69). Similar results were obtained when a matched analysis was conducted (OR=1.53, 95%CI: 1.16,2.02). A significant association between *GSTT1* or *GSTP1* genotype and colorectal cancer susceptibility was not observed. The joint effect of inheriting the following genotype combinations was considered: *GSTM1* null/*NAT2* slow, *GSTM1* null/*GSTT1* null and *GSTM1* null and *CYP1A1*2B*. No evidence of significant interactions was observed. Turner et al. (2004) extended the analysis by examining vegetable, fruit and meat consumption and potential risk modifying genes in relation to colorectal cancer risk. Six summary variables representing monthly number of servings of vegetable, fruit and meat consumption were created: overall meat consumption, red meat consumption, vegetable consumption, fruit consumption, total fruit and vegetable consumption and cruciferous vegetable consumption. All dietary factors were grouped into quartiles with the exception of vegetable consumption which was grouped into tertiles. Both categorical and quantitative variables were used in the analysis. Matched analysis was conducted. The interaction between red meat and *GSTP1* rare homozygotes reached nominal significance in the full model (p=0.02). An association between *GSTT1* and red meat consumption and colorectal cancer risk was observed. The OR increased from 1.3 in those with the deficient phenotype to 2.8 in the fast phenotype. However the significance of the association was not significant in either the full or simple model (p=0.18, p=0.06). A significant interaction between *GSTT1* and vegetable intake was observed, particularly for cruciferous vegetables (p=0.003). Results

suggest a protective effect for those with *GSTT1* null genotype and potentially a protective effect for those with the intermediate genotype.

Seow A et al. (2002) used data from the Singapore Chinese Health Study to examine the association between dietary ITC and its interaction with *GSTM1*, *GSTT1* and *GSTP1* genotypes and colorectal cancer. Cases (n=213) and controls (n=1194) were participants of the Singapore Chinese Health Study which is a population based, prospective investigation of diet and cancer risk. Incident colorectal cases were identified through the population based Singapore Cancer Registry. Overall there was no association between *GSTM1*, *GSTT1* or *GSTP1* genotypes and colorectal cancer risk. Similar results were obtained for both colon and rectal cancer. Individuals with both *GSTM1* null, *GSTT1* null and high dietary ITC intake had a significantly lower risk of developing colorectal cancer (OR 0.43; 95%CI:0.20,0.96). When this group was stratified by colon and rectal cancer this protective effect was observed for colon cancer cases only (OR 0.31; 95%CI:0.12,0.84). Note the sample size was too small to investigate the combined null genotype effect of *GSTM1*, *GSTT1* and *GSTP1*.

Kiss I et al. (2004) conducted a case-control study in order to evaluate the role of *GSTM1*, *GSTT1*, *GSTP1*, *NAT1*, and *NAT2* polymorphisms in colorectal cancer susceptibility. Cases were histologically confirmed colorectal patients from the Central Hospital of the Ministry of Internal Affairs and from the area of Baranya and Vas County, Hungary. Cancer-free controls from the same regions including non cancer patients from in- or out-patient wards and volunteers from the health status examination study were matched to cases according to sex and age. The *GSTM1* null genotype was significantly associated with an increased colorectal cancer risk (OR=1.48, 95%CI: 1.15,1.92). Neither *GSTT1*or *GSTP1* genotypes were significantly associated with an increased colorectal cancer risk. The joint effect of allelic combinations was also examined by creating groups of increasing number of high risk alleles. A strengthening effect of the *GSTM1* and *NAT2* alleles was reported. Significantly more cases possessed both high-risk alleles than the controls (OR=2.39, 95%CI: 1.75,3.26). Significant results of the paired analysis of *GSTT1-GSTP1, GSTT1-NAT1* and *GSTP1-NAT1* were not obtained. The triple combination of *GSTM1-NAT1-NAT2* revealed the greatest difference when comparing the simultaneous presence of the three high risk allele group (OR=3.28, 95%CI: 2.06,5.23). A significant difference in the distribution of cases possessing 4 or 5 high risk alleles relative to the control group was observed (OR=3.69, 95%CI: 2.33,5.86).

van der Logt EM et al. (2004) conducted a case-control study in the Netherlands to determine whether genetic polymorphisms in detoxification enzymes predispose individuals to the development of colorectal cancer. Isoenzymes of both the GST and UGT family were identified for study. Colorectal cancer patients were recruited from the Departments of Gastroenterology and General Surgery, University Medical Centre St. Radboud, Nijmegen and served as cases (n=371). Controls were recruited from the local newspaper (n=415). Both cases and controls were of Caucasian origin. The *GSTM1* null genotype was not significantly associated with colorectal cancer risk. Analyses were conducted to examine the role of tumor location and stage. The *GSTM1* present genotype was significantly associated with a reduced risk for Dukes A/B colorectal cancer (OR=0.47; 95%CI:0.25,0.83). Neither the *GSTT1* nor *GSTP1* genotypes were significantly associated with colorectal cancer risk. Analyses further stratified by tumor location and stage did not result in any significant differences.

Ates NA et al. (2005) investigated the risk for developing colorectal cancer and *GSTM1*, *GSTT1* and *GSTP1* genotypes in Turkey. The case-control study included all cases (n=181) of newly diagnosed colorectal cancer consecutively hospitalized in the surgery departments of Mersin and Kocaeli University hospital (in and out patients). Cases were recruited from September 2001 through April 2004. Unrelated healthy individuals recruited from two hospitals in Turkey served as controls (n=204). Current and lifetime smoking status and occupational risk factors for colorectal cancer were assessed for all subjects. The *GSTM1* null genotype was associated with an increased risk of developing colorectal cancer (OR=1.62; 95%CI:1.06,2.46). A statistically significant association between smoking and the *GSTM1* null genotype was observed (OR=2.06; 95%CI:1.19,3.57). The *GSTM1* null genotype was also associated with an increased risk of a transverse or rectal tumor (OR=1.86; 95%CI:1.15,3.00). No effect for stratification by age was observed. The *GSTT1* null genotype was associated with an increased risk of developing colorectal cancer (OR=1.64; 95%CI:1.10,2.59). A statistically significant association between the *GSTT1* null genotype and a history of cigarette smoking was observed (OR=2.44; 95%CI:1.24,4.81). The *GSTT1* null genotype was also associated with an increased risk of a transverse or rectal tumor (OR=1.70; 95%CI:1.02,2.84). No effect for the *GSTT1* genotype and stratification by age was observed. A significant departure from the Hardy-weinberg equilibrium was noted for the control population (p=0.002) but not for cases. A significant association between the *GSTP1* genotype and colorectal cancer risk was not observed. Among smokers, the frequencies of the *GSTP1* heterozygous Ile/Val or homozygous Val/Val genotypes did not differ significantly among cases and controls. No association between the *GSTP1* genotype, colorectal cancer risk and either tumor location or age was

observed. Genotype combinations and colorectal cancer susceptibility were also examined. The reference group for the analyses was composed of individuals with all three putative low risk genotypes (*GSTM1* present, *GSTT1* present and *GSTP1* Ile/Val or Val/Val alleles combined). Results indicated that the presence of at least one putative high risk genotype was associated with an increased risk of colorectal cancer. This risk increased as the number of putative high risk genotypes increased. The combined *GSTM1* null, *GSTT1* present and the *GSTP1* Ile/Val or Val/Val genotypes was associated with a significant increase for colorectal cancer (OR=2.34; 95%CI:1.15,4.77). The combined *GSTM1* null, *GSTT1* null and the *GSTP1* Ile/Val or Val/Val genotypes was also associated with a significant increase for colorectal cancer (OR=2.69; 95%CI:1.02,7.11).

Yeh C et al. (2005) conducted a hospital based case-control study in order to examine the role of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in colorectal cancer and their combined effects with meat consumption, cigarette-smoking and vegetable/fruit intake. Cases were newly diagnosed histologically confirmed colorectal adenocarcinoma cancer cases from the Chang Gung Memorial Hospital (n=727). Controls were recruited from the Physical Check-Up Department for comprehensive health examinations (including colonoscopies) and were matched by age and sex (n=747). A significant association was not observed for the *GSTM1* genotype and colorectal cancer risk. Stratified analysis by gender, age, and colon/rectal cancer did not reveal any significant differences. The *GSTT1* null genotype was moderately associated with colorectal cancer risk (p=0.06). The *GSTT1* null genotype was significantly associated with colorectal cancer risk in men (OR=1.45; 95%CI:1.07,1.97). Males with the *GSTT1* null genotype were found to be at a significant risk for rectal cancer (OR=1.55; 95%CI:1.08,2.23) but not colon cancer. Men diagnosed before the age of 60 with the *GSTT1* null genotype were also found to be at a greater risk (OR=2.03; 95%CI:1.29,3.21). The *GSTP1* genotype was moderately associated with colorectal cancer risk in men with the G allele in comparison with those with the A/A genotype (OR=1.36; 95%CI:0.98,189). Males with the *GSTP1* with the G allele in comparison with those with the A/A genotype were found to be at a significant risk for rectal cancer (OR=1.48; 95%CI:1.00,2.18) but not colon cancer. The combined effect of *GSTT1* and *GSTP1* genotypes was examined. Men with either or both the *GSTT1* null genotype and the *GSTP1* G allele genotypes were at higher risk (OR=1.42; 95%CI:1.01,1.99; OR=1.91; 95%CI:1.21,3.02 respectively) of colorectal cancer in comparison with men with both the *GSTT1* present and *GSTP1* A/A genotypes (P_{trend}<0.01). In terms of vegetable intake, a protective effect of high vegetable/fruit intake was observed in men with the *GSTT1* present genotype compared with those with the *GSTT1* null genotype and low consumption (OR 0.32 95%CI: 0.21, 0.50). A protective effect in men was also observed for the *GSTP1* A/A genotype and high vegetable/fruit

consumption (OR 0.40 95%CI: 0.25, 0.64). In women, the strongest protective effect of high vegetable consumption was observed in women with the *GSTT1* null or the *GSTP1* with G allele genotypes. The combined effect of smoking, vegetable/fruit consumption and the *GSTT1* and *GSTP1* genotypes was also examined. There were not enough women who smoked. As a result, the effect of smoking was only examined in men. The enhanced protective effect of vegetable/fruit consumption and the *GSTT1* present or *GSTP1* A/A genotypes was consistent among smokers and non-smokers. However non-smokers appeared to benefit more than their smoking counterparts. A significant interaction between meat intake and GST polymorphisms was not observed.

3.3 Colon Cancer

Kampman E et al. (1999) conducted a case-control study examining meat consumption, genetic susceptibility and colon cancer risk in the United States. The analysis was part of the Diet, Activity and Reproduction Study of Colon Cancer. Participants were between 30 and 79 years of age, area residents, able to speak English, with no history of colorectal cancer, FAP, ulcerative colitis, or Crohn's disease and were recruited from the Kaiser Permanente Medical Care Program of Northern California. Cases were composed of incident primary colon carcinoma patients diagnosed between October 1991 and September 1994 (n=1542). Controls were randomly selected from membership lists (social security lists and driver's licence lists) and Random digit dialling and were frequency matched to cases by sex and age (n=1860). A significant association between colon cancer risk and the *GSTM1* gene variant was not reported for either men or women. Overall the study results suggest little association between meat consumption and colon cancer risk. A significant association between *GSTM1* and colon cancer was not observed. In addition there was no significant association between the *GSTM1* genotype and meat consumption or preparation risk and colon cancer risk. In terms of red meat, no significant differences were observed between *GSTM1* genotypes and colon cancer risk. Alternatively, significant differences were observed with white meat consumption. The *GSTM1* non-null genotype and frequency of fried, broiled, baked or barbequed white meat (white meat prepared at high temperatures) was associated with increased colon cancer risk when comparing low to high intake categories (OR 1.4; 95%CI:1.1, 1.18). The *GSTM1* non-null genotype and the white meat mutagen index was also significantly associated with an increased colon cancer risk (OR 1.4; 95%CI:1.1, 1.19) when comparing low vs. high categories. Finally the total meat mutagen index was positively associated with colon cancer risk regardless of *GSTM1* genotype. Several other publications used the same study population. Slattery et al. (1998) examined *NAT2*, *GSTM1*,

cigarette smoking and colon cancer risk. Neither *GSTM1* nor *NAT2* were significantly associated with colon cancer risk. In addition, no significant interaction between either *GSTM1*or *NAT2*, cigarette smoking and colorectal cancer was observed. Slattery et al. (2000) examined the interplay between dietary inducers of GST and the *GSTM1* genotype and colon cancer risk. In particular, the association between cruciferous vegetables, coffee, *GSTM1* genotype and colon cancer was explored. The dietary inducers considered included: broccoli, brussel sprouts, cabbage, cauliflower and greens (kale, mustard, turnip). Overall, no association among cruciferous vegetable intake or coffee intake and colon cancer was observed. In terms of the *GSTM1* genotype, no interaction between any of the dietary inducers, *GSTM1,* and colon cancer risk was observed. Age-specific analyses revealed that *GSTM1* and high intake of cruciferous vegetables was associated with a decreased risk of colon cancer among those diagnosed at a younger age (<55 years of age). Among younger individuals, this association was observed among both the *GSTM1* null and non-null genotypes, however this association was most pronounced for those with the *GSTM1* null genotype and high levels of cruciferous vegetable intake compared to those with no intake (cruciferous vegetables: OR 0.23; 95%CI: 0.10, 0.54; broccoli: OR 0.30; 95%CI: 0.13, 0.70). For individuals with the functional *GSTM1* genotype, the protective effect of cruciferous vegetable consumption varied little by level of intake (OR 0.74 95%CI: 0.30, 1.79 for no intake; OR 0.44 95%CI: 0.21, 0.92 for <4 servings per week; and OR 0.44 95%CI: 0.19, 0.99 for ≥4 servings per week). An association between non-smokers, high levels of coffee consumption, the *GSTM1* non-null genotype and colon cancer risk was observed (OR 1.78; 95%CI: 1.02, 3.08). Interestingly, the inverse association was observed among smokers, high levels of broccoli and coffee intake, and the *GSTM1* functional genotype, where a decreased risk of colon cancer was observed (ORs 0.62; 95%CI: 0.37, 1.01 and 0.63; 95%CI: 0.38, 1.02). Further analysis revealed that the most consistent associations were confined to smokers. Among individuals who were less than 65 years of age, smoked and had the *GSTM1* non-null genotype, a protective effect was observed regardless of the level of cruciferous vegetable consumption (OR 0.45; 95%CI: 0.20, 1.01). High level of cruciferous vegetable intake only marginally impacted colon cancer risk (OR 0.36; 95%CI: 0.17, 0.76). This protective effect was not observed in older individuals. Slattery et al. (2000) examined the effect of Western diet, family history of colorectal cancer, *NAT2*, *GSTM1* and colon cancer risk. In particular the effect of age (≤55yrs, 56-66yrs, ≥67yrs) in conjunction with a Western dietary pattern in relation to genetic susceptibility was explored. Overall, increased Western dietary pattern consumption and colon cancer risk was associated. This association was particularly strong for subjects in the ≤55 years of age group (OR 3.1; 95%CI: 1.6, 6.2). In terms of the *GSTM1* genotype, a slight association between Western diet consumption and the *GSTM1* functional genotype, particularly for those diagnosed at

younger ages, was observed. However this association was not significant and it was proposed that the association observed was more likely due to the Western diet rather than the *GSTM1* non-null genotype. Slattery et al. (2002) evaluated the associations between diet and lifestyle factors that may be modified by the genetic variants of *GSTM1* and *NAT2* and their potential influence of genetic alterations in tumors. *GSTM1* was not significantly associated with any tumor alteration. Individuals with the *GSTM1* functional genotype were at a slightly higher risk of having a p53 transversion mutation if they smoked, however this interaction was not statistically significant.

Ye Z and Parry JM (2002) evaluated the association of *CYP1A1*, *GSTM1* and *GSTT1* in colon cancer and specific colon sites. Cases were histologically confirmed colon cancer patients from Singleton Hospital in Swansea, United Kingdom (n=41). Unrelated healthy individuals served as controls (n=82). A significant association between the *GSTM1* genotype and colon cancer was not observed. Stratification by distal or proximal tumor did not reveal any significant differences.

3.4 Rectal Cancer

Slattery ML et al. (2003) conducted a population based case-control study to examine the association between active and passive cigarette smoking, *GSTM1* and *NAT2* and rectal cancer risk. Cases with a first primary tumor in the rectosigmoid junction or rectum were identified in Northern California by the Surveillance Epidemiology and End Results Cancer Registries and Utah using a rapid-reporting system (May 1997-May 2001). An on-line pathology reporting system was searched for rapid case-ascertainment for rectal cancer cases at Kaiser Permanente Northern California Cancer Registry (n=952). Controls were randomly selected from various membership lists (i.e. social security, drivers licence) and frequency matched to cases by sex and age (n=1205). The *GSTM1* genotype was not associated with rectal cancer in either men or women. A borderline significant interaction among individuals who smoked and had the *GSTM1* null genotype compared with those who never smoked and were *GSTM1* present was observed (OR=2.0; 95%CI: 1.2, 3.3; p=0.08). Other studies were also published based on the same study population. Murtaugh et al. (2004) examined the association of rectal cancer, meat consumption and preparation, *GSTM1* and *NAT2* in both men and women. The authors hypothesized that the *NAT2*-imputed phenotype and the *GSTM1* genotype may interact with meat consumption or preparation to increase rectal cancer risk. Overall, no significant association between red meat, processed meat or poultry and rectal cancer risk was observed in either men or women. In men, the *GSTM1* null genotype and well doneness of red meat, as well as high levels of the red meat mutagen index, were associated with increased rectal cancer risk (OR 1.72 95%CI: 1.04,

2.83; OR 1.62 95%CI: 1.03, 2.53). The results for women and meat consumption were unexpected. Frequency of red meat drippings use was associated with a decreased risk in rectal cancer risk in women with the *GSTM1* null genotype (OR 0.50; 95%CI: 0.29, 0.86). In addition, women with the *GSTM1* functional genotype who consumed the smallest amount of poultry were at increased risk for rectal cancer (OR 1.87; 95%CI: 1.10, 3.19). Murtaugh et al. (2004) examined the association between *CYP1A1* in combination with *GSTM1*, meat consumption patterns and preparation and colorectal cancer risk in men and women. The association of red meat consumption and colorectal cancer risk was not significantly modified by the combination of *CYP1A1* and *GSTM1* genotypes. In women, an increased risk was associated with red meat mutagen index, any *CYP1A1* variant and the *GSTM1* present genotype and colorectal cancer risk (OR 1.98; 95%CI: 1.06, 3.67). In addition a significant interaction among *GSTM1*, *CYP1A1* variant and the white meat mutagen index was observed in women. The *GSTM1* present genotype, *CYP1A1* variant genotype and low white meat mutagen index was associated with an increased risk of colorectal cancer ($p_{interaction}<0.05$). In men, increased frequency of white meat drippings, any *CYP1A1* variant and the *GSTM1* present genotype resulted in a decreased risk for colorectal cancer (OR 0.53; 95%CI: 0.31, 0.92). The interaction between high white meat mutagen index, *CYP1A1* genotype and *GSTM1* genotype indicated that among men there is an increased cancer risk with high white meat mutagen index and any combination of *CYP1A1* genotypes and *GSTM1* genotypes except for the any *CYP1A1* variant genotypes and *GSTM1* present where there is neither an increase nor decrease in colorectal cancer risk ($p_{interaction}<0.05$).

3.5 Colon and Rectal Cancer

A few publications were based on both the colon study population of Kampman et al. (1999) and the rectal cancer population of Slattery et al. (2003). The two case groups cannot be combined together to represent colorectal cancer since they were recruited at different time periods. Slattery et al. (2004) examined *CYP1A1*, cigarette smoking and colon and rectal cancer. In addition combinations of *GSTM1*, *CYP1A1* and *NAT2* were examined. An increased risk in colon cancer was observed for individuals having *GSTM1* present, *CYP1A1*Wt (wild type) and the rapid-acetylator *NAT2* imputed phenotype (OR=1.7; 95%CI: 1.2,2.3). Murtaugh et al. (2005) studied whether *CYP1A1* genotype alters the association of meat consumption patterns and preparation with risk of colorectal cancer in men and women. Once again various gene combinations were examined among *GSTM1*, *CYP1A1* and *NAT2*. In women, a 2-fold increase in risk was associated with a high red meat mutagen index, any *CYP1A1* variant and *GSTM1* present (OR=1.98; 95%CI: 1.06,3.67).

Chapter 4: Quality Assessment

4.1 Overview

Study quality is an essential component in the meta-analytic process. The primary source of potential bias in any meta-analysis is bias that is a result of the underlying studies that contribute to the overall combined estimate (70). Studies of poor quality should be identified and either excluded from the overall estimate or a sensitivity analysis should be conducted to examine the influence studies of poor quality may have on the overall estimate. The process of assessing study quality was extremely challenging due to the inadequate reporting of several studies. Many studies did not report sufficient information in terms of recruitment and characteristics of the study participants, inclusion and exclusion criteria, participation rates, blinding, genotyping methods and quality control. Due to the inadequacy of reporting it was very difficult to assess whether or not cases and controls were recruited from the same source population, the internal validity and generalizability of the study and potential biases that the study may have encountered.

The process of assessing study quality is complex. Although advances have been made, unlike the scales and checklists developed for randomized trials, those which have been developed for observational studies have not been fully validated and are still in the developmental phase (71, 71). Adding to the challenge is the fact that many of these checklists do not address the various issues that are specific to gene-disease association studies. For instance genotyping methods, sample collection and storage, population stratification and analytic issues (such as multiple testing, potential data-dredging and subgroup analysis) which are all issues which critically impact study quality in gene-disease association studies, are not typically addressed in existing scales (72). Adding to the debate is the fact that quality scores that are the product of quality assessment scales are often thought to be arbitrary, unreliable and hard to interpret (73). As a result, currently there is no gold standard when selecting a tool in which to assess the quality of observational studies.

The Black and Down's checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions was selected as the method for evaluating individual study quality (74). This checklist was chosen due to the fact that it was the only known instrument which has been validated and is appropriate for observational studies. The checklist was developed with the objective of developing a checklist that was valid, reliable and appropriate for addressing both randomized and non-randomized studies. Additionally the checklist was devised to

provide an overall score as well as providing sub-scores addressing quality of reporting, internal validity, power and external validity. It is acknowledged that there are limitations with the checklist chosen. Future development and revisions are probable. In addition more rigorous testing is required.

4.2 Results

The Black and Down's checklist is composed of a total of twenty-seven items broken down into five themes: reporting, external validity, bias, confounding and power. The last item (Question 27) required the computation of post hoc power calculations. It has been argued that power should not be computed once the data has been collected (75). As a result, this last item was not included in the quality assessment. In addition, another eight of the 27 items were deemed as not applicable to gene-disease association studies (30%) and were excluded. These questions addressed issues of adverse events, treatment administration characteristics, blinding of subjects to treatment, length of follow up between intervention and outcome, compliance, randomization to intervention groups, concealment of intervention randomization and losses to follow up (Q8, Q9, Q13, Q14, Q17, Q19, Q23 and Q24). The following scoring system was used Yes=1, No=0 for all questions with the exception of the question concerning the distribution of principal confounders where Yes=2, Partially met=1, and No=0. As a result the scoring system was rated out of a total of 16 points. In total, 30 studies were included in the quality assessment. All of the studies reviewed used the case-control design, four of which were of a nested case control design. The quality scores ranged from 9 to 16, with a mean score of 13. A random sub-set of 20% the studies was reviewed by JL. The Kappa agreement rate was 70%.

The reporting on items such as stating the objectives/aim/ hypothesis of the study, main outcomes and exposures to be measured and main findings was generally well conducted. However, the quality assessment highlighted several areas requiring improvement in terms of reporting. The reporting in terms of subject characteristics, distributions of principal confounders by case/control status, participation rates, recruitment methods, comparison of participants from non-participants, statistical adjustments and blinding varied considerably across studies.

Considerable inconsistency in the detail of reporting in terms of subject selection was observed. With regard to cases, the majority of studies provided a case definition and described where the cases were recruited. However not all studies reported a clear and complete definition or provided

inclusion/exclusion criteria. The lack of information on cases made it difficult to assess the comparability of cases across the studies reviewed. For instance, there was substantial variation in the amount of details provided for such issues as whether or not the study included prevalent cases, histological confirmation, method of recruitment (i.e. consecutive cases or selective recruiting) and recruitment period.

In terms of controls, the information provided was more often minimal and incomplete. Often there was no discussion as to how controls were recruited, description of control characteristics was not provided and recruitment period was not specified. Selection of appropriate controls in a case-control study is critical to study validity. In order to assess this issue, it is imperative that the reader be able to determine whether or not the controls were selected from the same source population as cases. Controls should be comparable to cases with the exception of the disease under study and the frequency of the exposure under investigation. In order to minimize selection bias or confounding, it is essential that the controls selected represent individuals who could have been selected as cases had they developed the disease under study (76). In addition the same inclusion/exclusion criteria used for cases should be used for controls. Reporting on this issue was incomplete for several studies. For instance, some studies reported that "healthy controls" were used but did not report where they were recruited. Five studies did not report any information concerning where the controls were recruited. Of the studies that reported sufficient information concerning the recruitment of the controls, the most common sources of recruitment were controls who were part of a screening study (i.e. had a negative sigmoidoscopy), hospitals/medical centers, GP offices, blood donor banks, or membership lists such as drivers license lists. Use of hospital controls offers several advantages including convenience, potentially better recall and comparability between cases and controls with respect to the factors that led the individuals to use the facility (such as socioeconomic status). A disadvantage however is that the frequency of exposure may be different from the source population which in turn may lead to biased results. Population controls on the other hand are believed to be representative of the source population in terms of exposure. Potential disadvantages include low rates of participation and measurement bias due to poor recall. Population based controls were used in less than 20% of the studies. Due to the importance of case ascertainment and control recruitment and inconsistency in reporting, it may be useful to separate out this item into two items in the checklist.

The distribution of characteristics which may potentially confound the relationship between disease and exposure such as age, ethnicity, gender, socioeconomic status, occupational status or lifestyle

behaviours should be reported for cases and controls so that similarity can be assessed. The majority of studies reported the distribution of cases and controls with respect to such potential confounders as age and gender. However there was considerable variation in the confounders chosen for consideration and the reporting of the distribution of these potential confounders by case/control status. Often the rationale for the confounders chosen was not reported. Ethnicity is particularly important for assessing the quality of gene-disease association studies due to the potential effect of population stratification.

The issue of whether or not the subjects who were asked to participate were representative of the entire population from which they were recruited was poorly reported. Only one study broached this issue. The reporting indicated that the population included for study was representative of the general population but did not provide an explanation or rationale for how they came up with this conclusion. Participation rates by case/control status were also poorly addressed. Differential participation between cases and controls may result in bias. Non-participation may result in various ways: refusal to participate, refusal or inability to provide a biological specimen or insufficient or incomplete data. Improvement in this area is required. It was often not always clear whether the number of cases and controls included in the analysis was the same as what was initially identified for inclusion into the study. A flow diagram or clear and simple documentation of the number of cases and controls from initial sampling, recruitment and participation to data analysis is essential for data quality assessment. In terms of recruitment period, only 50% of the studies evaluated reported the recruitment period for both the cases and the controls.

In terms of the statistical analysis, all studies reported 95% confidence intervals and actual probability values when necessary. However, in a few studies, it was not always clear whether or not the odds ratio reported was a crude or adjusted odds ratio. Often the distribution of genotype by case/control status was not reported so that a crude OR could be computed. In addition the adjustments made for potential confounding varied considerably across studies. An issue of particular concern in gene-disease association studies is that of a tendency to report only a selected subset of analyses when multiple analyses have been conducted. Genetic studies are able to investigate numerous potential genetic markers which is not always so with environmental exposures. Genomic scans for instance permit the investigation of a large number of markers. The result of such studies is the conduct of multiple comparisons which is vulnerable to false-positive findings. Adjustment for multiple comparisons may address the statistical significance of the findings however it does not address the issue of selective reporting of findings. It was often not

feasible to determine whether the analysis conducted had been planned during study conception or much later in the process. Another issue of particular relevance in gene-disease association studies is that of Hardy-Weinberg equilibrium (HWE). The issue as to whether studies which have not assessed HWE, have not met the HWE assumption or cannot assess HWE, should or should not be included in the meta-analysis is still under debate (77). It is believed that an important cause of deviation from HWE is genotyping error. For those studies which investigated genotypes in which HWE could be assessed only half reported whether or not departures were observed. Sample size of a study also contributes to the quality of the results. A potential explanation for the inconsistency of gene-disease association results observed is thought to be the result of under-powered studies. It is important that effect size estimation and power calculations be performed during the study design phase. Reporting on this issue was extremely poor. Only one study explicitly stated computing sample size requirements during the design phase and only nine studies addressed the issue of power.

The analytic validity of genotyping should be addressed. The reporting of genotyping and issues surrounding genotyping is required when assessing the study quality of gene-disease association studies. Reporting in this area was not well conducted. For instance, source of the DNA, genotyping method, processing information, storage method and period should be reported. The success rates for both cases and controls should be reported. In terms of quality control measures such as blinding, re-analysis of random samples, analysis of replicate samples, etc. should be performed and reported. Only 27% of the studies reviewed reported that an attempt was made to blind those measuring the main outcomes. Additionally, only 20% of studies reported any type of quality control measures implemented such as degree of reproducibility between quality control replicates, success rate in extracting DNA by study group, etc.

Finally, the issue of language bias should be addressed. Two Chinese studies were identified that were not published in the English language. Studies conducted in non-English speaking countries have the option of publishing in either English language journals, which are usually indexed in major international bibliographic databases, or in domestic journals, many of which are not indexed. Evidence stemming from the literature of randomized clinical trials suggests that the decision to publish in international versus domestic journals may be influenced by the direction of the results (significant vs. non-significant) (78). There appears to be a tendency to publish significant results in international journals and non-significant results in local journals. This issue is of particular relevance to the area of genetic epidemiology. There are an overwhelming number of studies being conducted throughout the world examining potential associations between the millions of

polymorphisms in the human genome and diseases, treatment outcomes, and survival. Selective publication of these results may obscure the true relationship between the polymorphisms under investigation and the outcome of interest. Another issue that is likely to be impacted by language bias is ethnicity. Different allele frequencies associated with ethnic background may in turn be associated with the outcome under study. If studies are not being published in international journals the complete picture may not be obtained.

4.3 Discussion

Overall, the checklist chosen had considerable limitations for use in the quality evaluation of gene-disease association studies. Several questions included in the checklist were not applicable to gene-disease association studies. For instance questions regarding intervention, adverse events and compliance were not applicable. In addition, there were quality issues relevant to gene-disease association studies that were not included in the checklist. Sanderson et al. (2007) conducted a systematic review to identify tools that are available to assess the quality and susceptibility to bias in observational studies in epidemiology. Although they identified a total of 86 tools, none were recommended for use. Areas of concern raised included variation in terms of the number and nature of items, ranges of scores and the levels of development of the various tools available to date. Until a tool is designed and developed specifically for use in assessing the quality of observational studies and rigourous testing is conducted, the authors suggest the use of a transparent checklist approach that "concentrates on the few, principal, potential sources of bias in a study's findings". Due to the limitations, concerns and usefulness that has been raised for quality scales, the individual scores for each item and study were not presented.

The lack of information reported by the majority of studies reviewed made quality assessment extremely challenging. As a result, a decision was made to include all the studies in the meta-analysis. This decision was made due to the fact that for several studies the information provided was not sufficient to make an adequate judgement about the quality of the study in question.

The lack of reporting on issues critical to quality assessment was evident. Awareness on this issue must continue to be encouraged so that the quality of observational study reporting can meet that of clinical trial reporting. Although progress has been made, it is evident that there is still much work to be done.

Chapter 5: *GSTM1*

5.1 Introduction

Overall, 36 studies examining the association between the *GSTM1* genotype and either colon, rectal, colorectal or adenomas were identified.

Table 6: Number of studies examining *GSTM1* by type of cancer

GSTM1	# of Studies
Colorectal cancer	28
Colorectal adenomas	5
Colon cancer	2
Rectal cancer	1

Due to the limited number of studies examining the association between *GSTM1* and colon cancer or rectal cancer, the meta-analysis and pooled analysis were restricted to colorectal adenomas and colorectal cancer. The systematic review identified two studies which examined colon cancer risk and *GSTM1* polymorphism (Kampman et al. 1999 and Ye Z and Parry 2002). A significant association between *GSTM1* null and colon cancer risk was not observed. One study examining the association between rectal cancer and *GSTM1* polymorphism was identified (Slattery ML et al. 2003). A significant association between *GSTM1* null and rectal cancer risk was not observed.

5.2 Colorectal Adenomas and *GSTM1*

5.2.1 Systematic Review Results: Colorectal Adenomas and *GSTM1*

A total of five studies examining the association between *GSTM1* and colorectal adenomas were identified. Only one of the studies found a significant association between *GSTM1* and colorectal adenomas (Moore et al. 2005). This study used a novel PCR based method which enabled the determination of the number of active alleles carried by each subject. Risk of colorectal adenoma was found to be lower in subjects carrying at least one *GSTM1* null allele (OR=0.6; 95%CI: 0.4, 0.9) in comparison with those carrying two active *GSTM1* alleles. When heterozygotes were grouped with those carrying two active alleles as is the usual grouping used with PCR fragment analysis, a significant difference in risk was not observed. Table 7 presents the results and summarizes the study characteristics of the individual studies identified by the systematic review. The results are presented by region and date of publication.

Table 7: Summary of Studies of Colorectal Adenomas and *GSTM1* by Region and Date of Publication

Authors	Year	Region & Country	Study Period	Description of Cases	# of Cases	Description of Controls	# of Controls	% of controls *GSTM1* null	OR (95% CI)	Adjustments	Subgroup Analysis Reported
Inoue et al.	2000	Asia Japan	Jan95-Dec96	Men receiving pre-retirement health examination at SDF Fukuoka Hospital and SDF Kumamoto Hospital[1]	205	Men receiving pre-retirement health examination at SDF Fukuoka Hospital and SDF Kumamoto Hospital with normal study of colonoscopy	220	55.9	Crude OR: 0.9 (0.6, 1.3) Adjusted OR: 0.9 (0.6, 1.4)	Hospital, rank in SDF, cigarette-years, alcohol use, BMI	Location, adenoma size, smoking, genotype combinations
Tijhuis MJ et al.	2005	Europe Nether-lands	Jun97-Oct02	Recruited among patients undergoing endoscopy at the outpatient clinics of eight hospitals in the Netherlands.	746	Recruited among patients undergoing endoscopy at the outpatient clinics of eight hospitals in the Netherlands. No history of any type of polyps.	698	54.1	Not reported		Cruciferous vegetable intake

[1] SDF: Self Defence Force

Lin et al.	1998	North America U.S.	01Jan91-25Aug93	Subjects undergoing screening sigmoidoscopy at Kaiser Permanente's Bellflower or Sunset medical centers	459	No polyps of any type at sigmoidoscopy or history of polyps. Individually matched to cases by gender, age, date of sigmoidoscopy and center.	507	49.1	Crude OR: 0.85 (0.65, 1.10)	Date of sigmoidoscopy (6-month intervals), age (5 year intervals), gender and clinic attended	Cruciferous vegetable intake
Moore LE et al.	2005	North America US	Sept. 1993 – Sept. 1999	Randomly selected from the Prostate, Lung, Colorectal, and Ovarian Trial (10 screening centers throughout the US)	772	Randomly selected participants from the Prostate, Lung, Colorectal, and Ovarian Trial with a negative sigmoidoscopy screening. Frequency matched to cases by gender and ethnicity.	777	54.6	0.9 (0.8,1.2)		Smoking, broccoli intake, adenoma size, multiplicity, advanced histological features
Gunter MJ et al.	2005	UK	Not reported	Nested case-control study, UK Flexible Sigmoidoscopy Screening Trial covering 14 geographic areas	768	Age and sex matched individuals with a negative flexible sigmoidoscopy result	814	62.4	Adjusted OR: I.I (0.9, I.4)	Age, sex, center	Gender, Interaction between diet, smoking & genotype

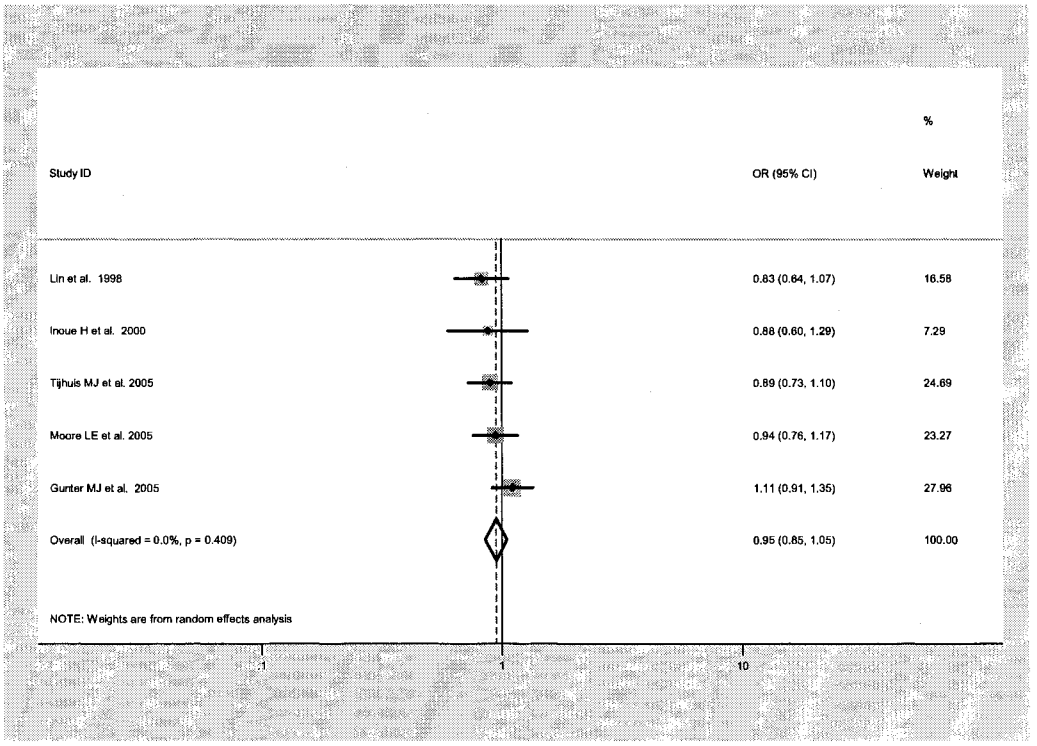
5.2.2 Meta-Analysis Results: Colorectal Adenomas and *GSTM1*

Five studies examining the association between colorectal adenomas and *GSTM1* were included in the meta-analysis. Due to the limited number of studies stratified analyses were not conducted. The estimated odds ratios of the individual studies ranged from 0.82 to 1.11. The combined OR for the five studies was not significant for the random effects model (OR=0.95; 95%CI: 0.85,1.05; I^2=0%; 0%,79%). Using the adjusted odds ratio when reported resulted in a similar combined estimate (OR=0.93; 95%CI: 0.83,1.03; I^2=0%; 0%,66%). All of the studies included in the meta-analysis used population based controls. The study by Inoue et al. (2000) included men only in their study. The studies were conducted in various regions, Japan, Europe, North America and the United Kingdom. All of the studies were published in the English language. In terms of sample size all five of the studies included a minimum of 200 cases and controls.

Table 8: Meta-Analysis Individual Study Crude Odds Ratios, Summary Odds Ratio and 95% Confidence Intervals for Colorectal Adenomas and *GSTM1*

Author(s)	Year	OR	LCI	UCI
Lin et al.	1998	0.83	0.64	1.07
Inoue H et al.	2000	0.88	0.60	1.29
Gunter MJ et al.	2005	1.11	0.91	1.35
Moore LE et al.	2005	0.94	0.76	1.17
Tijhuis MJ et al.	2005	0.89	0.73	1.10
Combined OR		**0.95**	**0.85**	**1.05**

Figure 2: Colorectal Adenomas and *GSTM1*: Odds Ratios and 95% Confidence Intervals



Assessment of Publication Bias

The Begg's test (p=0.33) and the Egger's test (p=0.39) do not provide evidence of publication bias. The Begg's plot is reasonably symmetrical and the intercept does not deviate significantly from zero in the Egger's plot. Results should be interpreted cautiously due to the limited number of studies.

Figure 3: Begg's Funnel Plot: Colorectal Adenomas and *GSTM1*

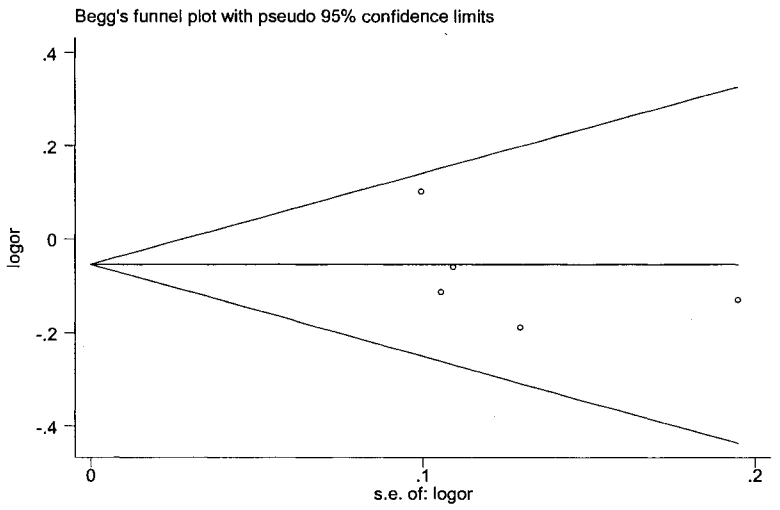
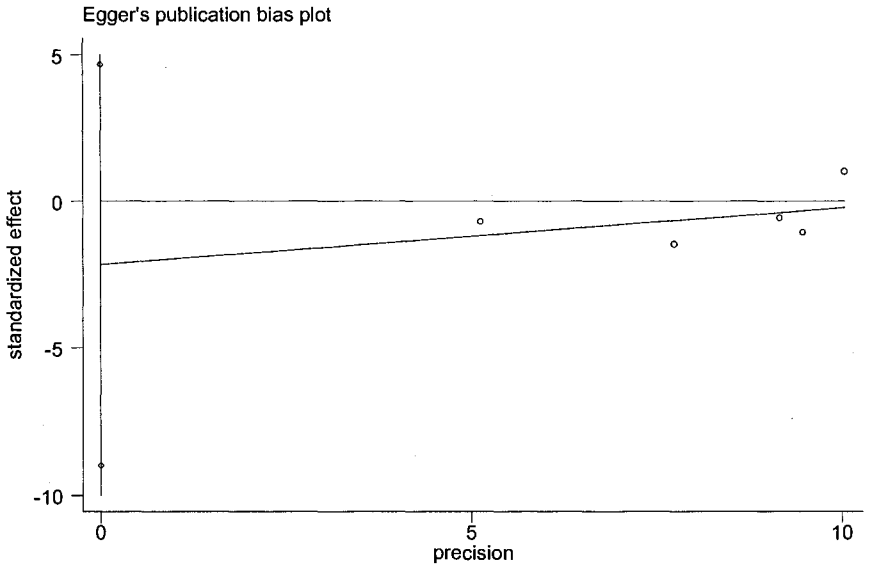


Figure 4: Egger's Publication Bias Plot: Colorectal Adenomas and *GSTM1*



5.2.3 Pooled Analysis Results: Colorectal Adenomas and *GSTM1*

The pooled analysis of *GSTM1* and colorectal adenoma risk was based on two studies and included 1122 controls and 1096 cases. The combined OR was not significant (OR=1.04; 95%CI: 0.87, 1.23).

Table 9: Pooled Analysis Study Specific Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Colorectal Adenomas and *GSTM1*

Study	Year	No. of cases	No. of controls	Crude OR			Adjusted OR		
				OR	LCI	UCI	OR	LCI	UCI
Moore et al.	2005	662	688	0.94	0.76	1.17	0.93	0.75	1.16
Tiemersma et al.	2005	434	434	1.23	0.94	1.60	1.22	0.92	1.63
Combined OR		**1096**	**1122**	**1.04**	**0.88**	**1.23**	**1.04**	**0.87**	**1.23**

Table 10: Model Fit Statistics: Colorectal Adenomas and *GSTM1*

Predictor	Number of Predictors	Wald's χ^2	df	p	eβ (OR)	UCI	LCI	-2log likelihood
Null Model	Intercept		1					3074.50
Reduced Model			2					3049.03
GSTM1	1	0.26	1	0.61	1.04	0.88	1.23	
Full Model	4		5					2947.52
GSTM1		0.18	1	0.67	1.04	0.87	1.23	
Sex		11.10	1	0.0009				
Age		100.51	1	<.0001				
Study		23.35	1	<.0001				
Likelihood Ratio Test			3					101.51

Removal of the Van der Hel study in which all subjects were female, resulted in an adjusted odds ratio of 1.16 (95%CI: 1.06,1.27) .

5.2.4 Comparison of Combined Overall Estimate between Meta- and Pooled-Analyses: Colorectal Adenomas and *GSTM1*

The pooled analysis resulted in an adjusted odds ratio of 1.04 (95%CI: 0.87,1.23). A similar result was obtained for the meta-analysis where only these two studies were considered (OR: 0.92; 95%CI: 0.79,1.06).

5.3 Colorectal Cancer and *GSTM1*

5.3.1 Systematic Review Results: Colorectal Cancer and *GSTM1*

A total of 28 studies examining the association between *GSTM1* and colorectal cancer were identified. The following four of the identified published studies reported a significant association between colorectal cancer risk and the *GSTM1* null genotype: Zhong et al. (1993) reported an increased risk between the *GSTM1* null genotype and colorectal cancer risk (OR=1.8, 95%CI: 1.2,2.6); Sachse et al. (2002) reported a significant increased risk of developing colorectal cancer (OR=1.33, 95%CI: 1.04,1.69); Kiss et al. (2004) also reported a significant colorectal cancer risk (OR=1.48, 95%CI: 1.15,1.92); Finally, Ates et al. (2005) reported an increased risk of developing colorectal cancer in (OR=1.62, 95%CI: 1.06,2.46). Table 11 presents the results and summarizes the study characteristics of the individual studies identified by the systematic review. Results are presented by region and date of publication.

Table 11: Summary of Studies of Colorectal Cancer and *GSTM1* by Region and Date of Publication

Authors	Year	Region & Country	Study Period	Description of Cases	# of Cases	Description of Controls	# of Controls	% of controls *GSTM1* null	OR (95% CI)	Adjustments	Subgroup Analysis Reported
Abdel-Rahman SZ et al.	1999	Africa Egypt	NR	Newly diagnosed patients from 3 cancer hospitals	63	Healthy controls friends of other cancer patients from the same centers, matched on age	45	66.6	Crude OR: 0.56 (0.23,1.31)		Age, rural/urban, site, gender
Lee et al.	1995	Asia Singapore	NR	CRC patients from surgical department	300	Patients with no history of neoplasms from the clinical chemistry department of a local hospital	183	49	0.8 (0.5,1.1)		Tumor site and histology
Yoshioka M et al.	1999	Asia Japan	Sept91-Jun95	Histologically confirmed consecutive cases, presenting at the University of Occupational & Environmental Health Hospital, Mitsubishi Chemical Hospital, & Kitakyushu Medical Center	106	Individuals who had visited local medical clinics in Kitakyushu City (Sept93-Apr95) for regular medical check-ups with no current of previous diagnosis of cancer	100	42	Crude OR: 1.55 (0.89, 2.68)		Genotype combinations, smoking
Seow A et al.	2002	Asia China	Apr93-Dec98	Incident CRC cases who were participants of the Singapore Chinese Health Study (population based, prospective investigation of diet and cancer risk)	213	Participants of the Singapore Chinese Health Study (population based, prospective investigation of diet and cancer risk)	1194	45	Adjusted OR: 1.22 (0.90, 1.67)	Education, BMI, smoking, physical activity, alcohol, saturated fat	Colon vs. Rectal cancer, GST genotype combinations, ITC intake, smoking
Zhu et al.	2002	Asia China		Sporadic CRC patients	104	Healthy controls	101	46.5	Not significant		Age, location

Chen K et al.	2004	Asia China			125	Healthy controls	339	55.5	Not reported	Age, sex	Colon/rectal, smoking, *GSTM1* and *GSTT1* combinations
Yeh C et al.	2005	Asia China	Jan95-Jan99	Incident, histologically confirmed CRC cases, Chang Gung Memorial Hospital	723	Recruited from the Physical Check-Up Department for comprehensive health examinations (incl. colonoscopies) matched by age and sex	733	55.9	Not significant	Sex, age, physical activity, coffee, cigarettes, alcohol, diet	Sex, tumor site, age at diagnosis, vegetable/ fruit consumption, smoking
Chenevix-Trench et al.	1995	Australia	NR	Patients with colorectal adenocarcinoma	132	Unselected subjects (source not stated) and geriatric patients without cancer or family history of cancer	200	51	Crude OR: 0.9 (0.6,1.4)	None	Age, tumor site
Butler et al.	2001	Australia	NR	Queen Elizabeth Hospital, white adults	203	Queen Elizabeth Hospital, white adults attending a voluntary blood donation bank	200	54.0	Crude OR: 0.93 (0.63,1.38) Adjusted OR: 0.86 (0.48,1.54)	Age	Age, gender, site of cancer, combinations of genotypes
Gawronska-Szklarz B et al.	1999	Europe Poland	NR	Patients with colonic polyps, HNPCC, suspected HNPCC, and sporadic CRC	CRC 28 Polyps 27	Healthy volunteers selected with respect to age and sex	145	49.7	Crude ORs: Sporadic CRC : 2.53 (0.98,6.74) Colonic Polyps: 1.72 (0.65,4.37)	None	
Zhang H et al.	1999	Europe Sweden	1989-1997	Tumor samples from patients with primary CRC diagnosed at the Department of Pathology, Linkoping University Hospital & Norrkiping Central Hospital	99	Subjects were randomly selected from a healthy control group of residents in South-Eastern Sweden.	109	50%	Not significant (47% for cases, 50% in controls), OR not reported		

Sgambato A et al.	2002	Europe Italy	Jun01-Dec01	Consecutive, unselected patients admitted at the Division of Oncology at the Centro di Riferimento Oncologico of Basilicata	44	Healthy subjects visiting the hospital for routine blood tests with no personal and/or family history of cancer	100	53	Not reported		
Tiemersma EW et al.	2002	Europe Nether-lands	Jan87-Dec91	Nested case-control study, Netherlands Prospective Monitoring Project on CVD Risk Factors	102	Random sample of controls from database with the same distribution of gender, age and center as case	537	53	Crude OR: 1.2 (0.8, 1.8)		Meat consumption, smoking
van der Hel OL et al.	2003	Europe Nether lands	1987-1996	Netherlands Population-based screening program for early detection of breast cancer	234	Random sample from the source population	765	48.2	Not significant		Smoking
Kiss I et al.	2004	Europe Hungary	NR	Histologically confirmed CRC patients from Central Hospital of the Ministry of Internal Affairs and from the area of Baranya and Vas County	500	Cancer-free controls from the same regions including non cancer patients from in- or out-patient wards and volunteers from the health status examination study. Matched according to sex and age	500	48.4	Crude OR: 1.48 (1.15, 1.92)		Genotype combinations, high risk vs. low risk allele combinations

van der Logt EM et al.	2004	Europe Nether-lands	NR	CRC patients recruited from the Departments of Gastroenterology and General Surgery, University Medical Centre St. Radboud, Nijmegen Netherlands	371	Controls recruited from local newspaper	415	48.9	Crude OR: 1.0 (0.78, 1.4) Adjusted OR: 0.81 (0.54, 1.2)	Age, gender	Location, Dukes staging
Landi S et al.	2005	Europe Spain	Jun96-Dec98	Newly diagnosed patients attending a University Hospital in Barcelona	176	Individuals living in the same area and representative of the general population, randomly enrolled among patients admitted to the same hospital with a diagnosis of disease other than cancer	162	59.3	Not reported		
Luchtenborg M et al.	2005	Europe Nether-lands	1989-1994	Incident cases participating in the Prospective Netherlands Cohort Study on Diet and Cancer, (204 municipals) Nested case-only design		Prospective Netherlands Cohort Study on Diet and Cancer participants			Not reported		
Laso et al.	2002	Europe Spain	1996-1997	Consecutive patients undergoing surgery from the University of Barcelona Hospital Clinic	247	Consecutively recruited patients presenting to the Trauma Service of the Hospital Clinic	296	53	Crude OR: 1.02 (0.7,1.45)		Smoking status, gender, stage (A,B,C,D), differentiation, location
Saadat I and Saadat M.	2001	Middle East Iran	NR	Patients with pathologically confirmed CRC	46	Healthy blood donor matched to cases on age and gender	131	40.5	Crude OR: 1.75 (0.88, 3.49)		Sex, genotype combinations
Ates NA et al.	2005	Middle East Turkey	Sept01 - Apr04	Consecutive CRC patients from sugery departments of Mersin and Kocaeli University hospital (in and out patients)	181	Unrelated healthy controls recruited from two hospitals in Turkey	204	43.1	Adjusted OR: 1.62 (1.06, 2.46)	Age, sex	Age, smoking status , site, genotype combinations

Nascimento H et al.	2003	South America Brazil	Aug99-Aug01	Consecutive cases with histologically confirmed CRC treated at the University Hospital of the State University of Campina	102	Blood donors from the same hospital.	300	44.6	Crude OR: 1.19 (0.74, 1.91) Adjusted: 1.03 (0.96, 1.10)	Age, gender	Age, gender, ethnic origin, smoking status, tumor location, stage, grade of differentiation, genotype combinations
Zhong et al.	1993	UK		Caucasian CRC patients from Royal Infirmary in Edinburgh	196	Caucasian subjects from a Clinical Chemistry dept. in Sheffield, Royal Infirmary in Edinburgh and volunteers in ICRF Clare Hall Laboratories	200	51	Significant association reported (p=0.003), OR not reported	None	Tumor site
Deakin et al.	1996	UK	1990-1994	English white CRC cases who could give informed consent recruited from North Staffordshire Hospital	252	Two control groups: patients with obstructive lung disease; patients suffering from a variety of non-malignant diseases from North Staffordshire Hospital	577	55	Crude OR: 1.0 (0.7, 1.3)	None	Position of tumor
Welfare M et al.	1999	UK	Dec94-Sept95	Case-control study, histologically confirmed CRC patients diagnosed at the Newcastle and North Tyneside health district, England	201	Age and sex matched community controls were recruited for each case, identified from GP records	187	50.8	Adjusted OR: 1.04 (0.67,1.65)	Matched pair design: age, sex, and area of residence	Sex, age group, stage, site of tumor, genotype combinations
Loktionov A et al.	2001	UK	1997-1999	Patients with histologically confirmed CRC treated at the Department of Surgery, Norwich and Norfolk Health Care NHS Trust	206	Participants of the on-going UK Flexible Sigmoidoscopy Screening Trial with a normal result	355	58.6	Crude OR: 1.26 (0.87, 1.83)		Location

Sachse C et al.	2002	UK	Aug97-Feb01	Incident CRC patients recruited at either Ninewells Hospital, Dundee, Perth Royal Infirmary, Leeds General Infirmary, St. Jame's Hospital, Leeds or York District Hospital	490	Healthy population based controls with no history of previous cancer (GP controls), recruited by age, sex, and general practitioner matching of incident cases	593	49.1	Unmatched analysis: 1.33 (1.04, 1.69) Matched analysis: 1.53 (1.16, 2.02)		Genotype combinations
Little et al.	2006	UK	Sept98-Feb00	Population-based case-control study in North-East Scotland. Histologically confirmed CRC cases	264	Selected from the Grampian Community Health Index (list of everyone registered with a GP) matched by age and sex.	408	58	Adjusted OR: 0.89 (0.63, 1.25) Adjusted OR: 1.08 (0.73, 1.61)	Age, sex Age, sex, family history of CRC, aspirin, NSAIDs, physical activity	Green leafy vegetable *GSTM1* x *CYP1A1*, Smoking, total meat intake
Gertig et al.	1998	U.S.	NR	White male cases with CRC from the prospective Physician's Health Study.	212	White male participants not diagnosed with CRC within the prospective Physician's Health Study.	221	53	Adjusted OR: 1.0 (0.7, 1.5)	Age, smoking, BMI, physical activity, alcohol intake	Site, smoking, *GSTM1* and *GSTT1*, age

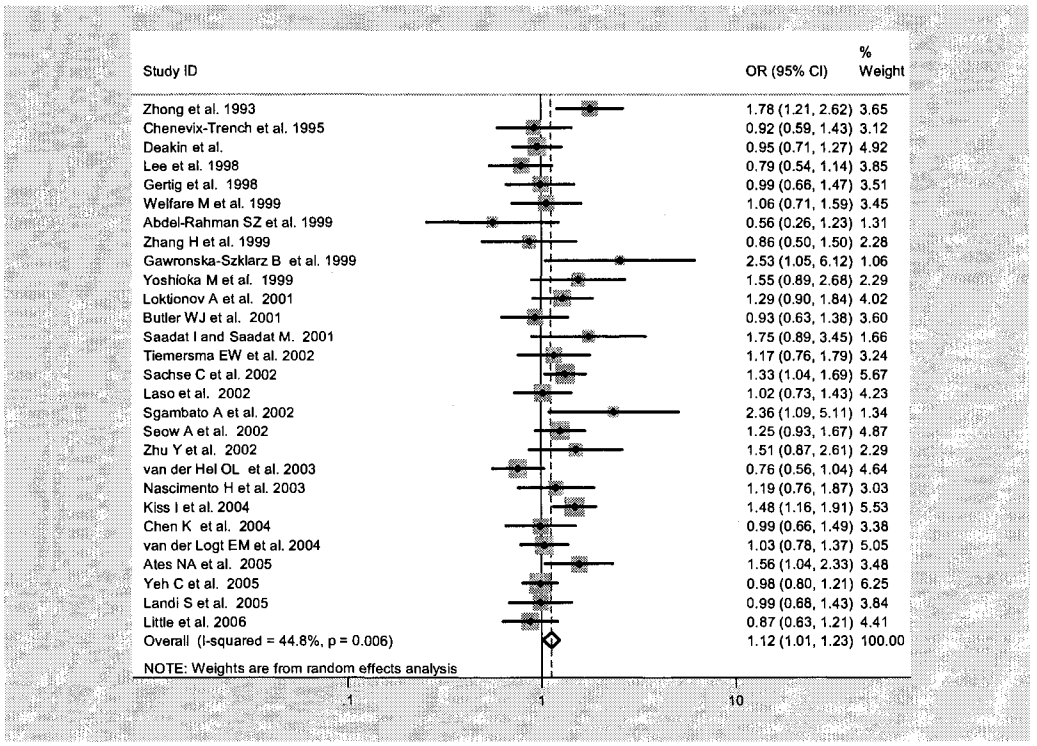
5.3.2 Meta-Analysis Results: Colorectal Cancer and *GSTM1*

A total of 28 studies with a total of 5827 cases and 9418 controls, were deemed eligible for inclusion for the investigation of the potential association between *GSTM1* and colorectal cancer risk. The estimated odds ratios for the individual studies ranged from 0.56 to 2.54. The combined OR for all studies was 1.12 (95%CI: 1.02, 1.23) for the random effects model. The formal test for heterogeneity revealed that there is moderate heterogeneity across the studies (I^2=45%; 14%,65%). Using the adjusted odds ratio, when reported, resulted in a combined summary odds ratio of 1.03 (95%CI: 0.95, 1.12) with an I^2 of 29% (0%,56%). Caution must be exercised when interpreting this result since the combined odds ratio is the result of both adjusted and unadjusted odds ratios. In addition the adjustments performed were not uniform across all studies.

Table 12: Meta-Analysis Study Specific Crude Odds Ratios, Summary Odds Ratio and 95% Confidence Intervals for Colorectal Cancer and *GSTM1*

Author(s)	Year	OR	LCI	UCI
Zhong et al.	1993	1.78	1.21	2.63
Chenevix-Trench et al.	1995	0.92	0.59	1.43
Deakin et al.	1996	0.95	0.72	1.27
Gertig et al.	1998	0.99	0.66	1.47
Lee et al.	1998	0.79	0.54	1.14
Abdel-Rahman SZ et al.	1999	0.56	0.26	1.23
Gawronska-Szklarz B et al.	1999	2.54	1.05	6.12
Welfare M et al.	1999	1.06	0.71	1.59
Yoshioka M et al.	1999	1.55	0.89	2.68
Zhang H et al.	1999	0.86	0.50	1.50
Butler WJ et al.	2001	0.93	0.63	1.38
Loktionov A et al.	2001	1.29	0.90	1.84
Saadat I and Saadat	2001	1.75	0.89	3.45
Laso et al.	2002	1.02	0.73	1.43
Sachse C et al.	2002	1.33	1.04	1.69
Seow A et al.	2002	1.25	0.93	1.68
Sgambato A et al.	2002	2.37	1.09	5.11
Tiemersma EW et al.	2002	1.17	0.76	1.79
Zhu Y et al.	2002	1.51	0.87	2.61
Nascimento H et al.	2003	1.19	0.76	1.87
van der Hel OL et al.	2003	0.76	0.56	1.04
Chen K et al.	2004	0.99	0.66	1.50
Kiss I et al.	2004	1.48	1.16	1.91
Ates NA et al.	2005	1.56	1.04	2.33
Landi S et al.	2005	0.99	0.68	1.43
van der Logt EM et al.	2005	1.03	0.78	1.37
Yeh C et al.	2005	0.98	0.80	1.21
Little et al.	2006	0.87	0.63	1.21
Combined OR		**1.12**	**1.02**	**1.23**

Figure 5: Meta-Analysis Odds Ratios and 95% Confidence Intervals: Colorectal Cancer and *GSTM1*



Assessment of Publication Bias

The Begg's test (p=0.20) and the Egger's test (p=0.34) did not suggest evidence of publication bias. The Begg's plot was reasonable symmetrical and the Egger's plot does not appear to deviate significantly from zero providing further evidence for a lack of publication bias.

Figure 6: Begg's Funnel Plot: Colorectal Cancer and *GSTM1*

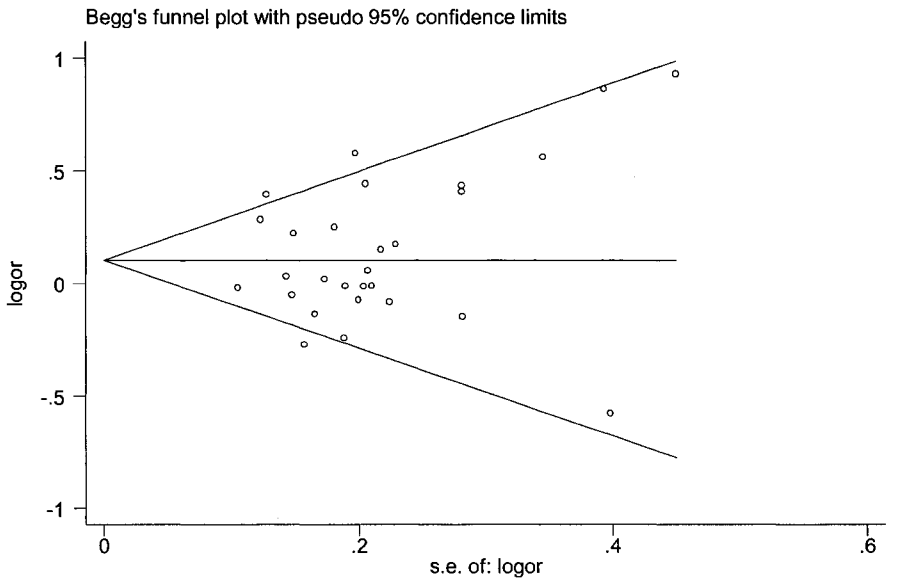
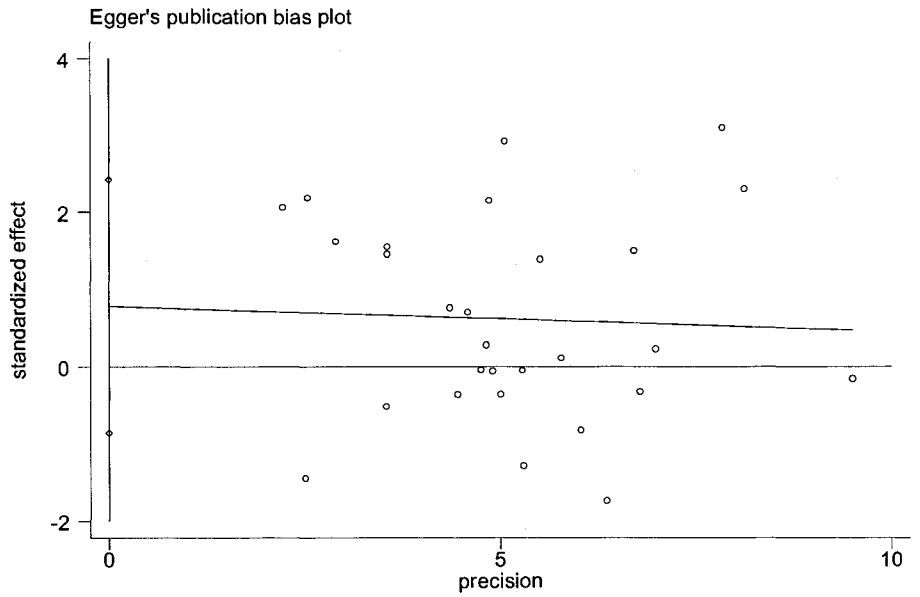


Figure 7: Egger's Publication Bias Plot: Colorectal Cancer and *GSTM1*

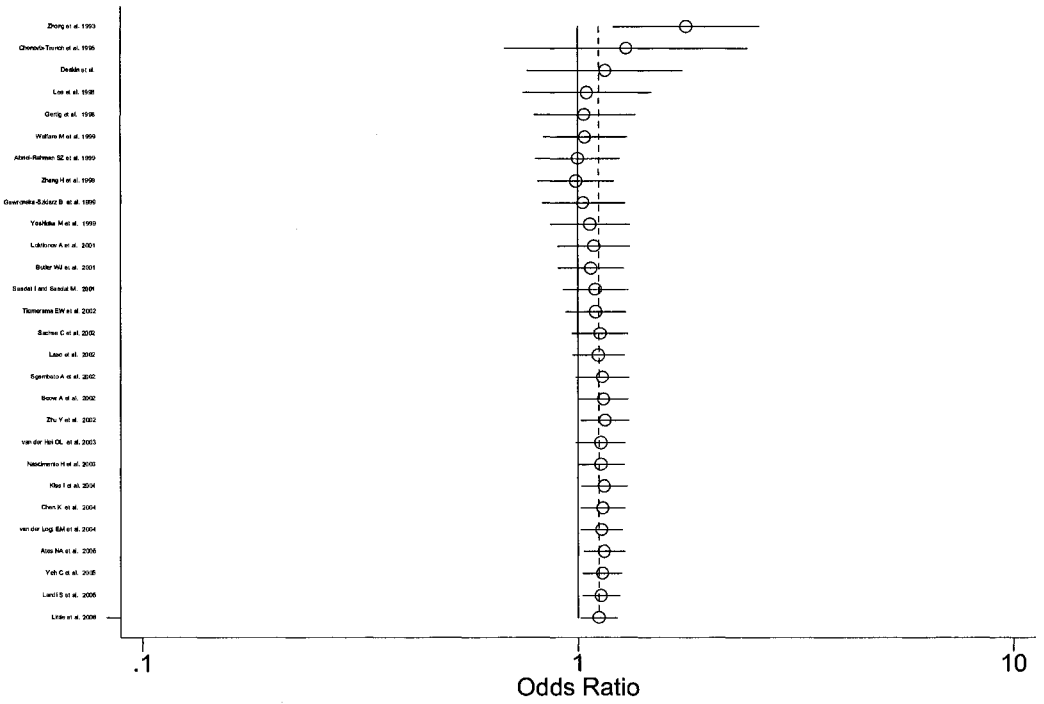


Cumulative Meta-Analysis: Colorectal Cancer and *GSTM1*

Table 13: Cumulative Meta-Analysis Results for *GSTM1* and Colorectal Cancer

Trial	Cumulative Estimate	LCI	UCI	z	Pvalue
Zhong et al. 1993	1.78	1.21	2.63	2.93	0.00
Chenevix-Trench et al. 1995	1.29	0.68	2.47	0.78	0.43
Deakin et al.	1.16	0.77	1.75	0.70	0.49
Lee et al. 1998	1.05	0.75	1.47	0.28	0.78
Gertig et al. 1998	1.04	0.79	1.36	0.27	0.79
Gawronska-Szklarz B et al. 1999	1.11	0.83	1.48	0.72	0.47
Zhang H et al. 1999	1.07	0.83	1.39	0.55	0.58
Welfare M et al. 1999	1.07	0.86	1.33	0.59	0.56
Yoshioka M et al. 1999	1.10	0.90	1.36	0.92	0.36
Abdel-Rahman SZ et al. 1999	1.07	0.86	1.32	0.60	0.55
Loktionov A et al. 2001	1.09	0.90	1.32	0.88	0.38
Butler WJ et al. 2001	1.07	0.90	1.28	0.78	0.43
Saadat I and Saadat M. 2001	1.10	0.92	1.30	1.06	0.29
Laso et al. 2002	1.09	0.93	1.27	1.06	0.29
Sgambato A et al. 2002	1.12	0.95	1.32	1.36	0.17
Tiemersma EW et al. 2002	1.12	0.96	1.30	1.47	0.14
Sachse C et al. 2002	1.14	0.99	1.31	1.81	0.07
Zhu Y et al. 2002	1.15	1.01	1.32	2.03	0.04
Seow A et al. 2002	1.16	1.02	1.31	2.28	0.02
van der Hel OL et al. 2003	1.13	0.99	1.28	1.81	0.07
Nascimento H et al. 2003	1.13	1.00	1.28	1.92	0.06
Kiss I et al. 2004	1.15	1.02	1.30	2.28	0.02
van der Logt EM et al. 2004	1.14	1.02	1.28	2.29	0.02
Chen K et al. 2004	1.14	1.02	1.27	2.27	0.02
Landi S et al. 2005	1.13	1.02	1.25	2.24	0.03
Yeh C et al. 2005	1.12	1.01	1.24	2.18	0.03
Ates NA et al. 2005	1.13	1.03	1.25	2.44	0.02
Little et al. 2006	1.12	1.02	1.23	2.26	0.02

Figure 8: Cumulative Meta-Analysis Odds Ratios and 95% Confidence Intervals: Colorectal Cancer and *GSTM1*



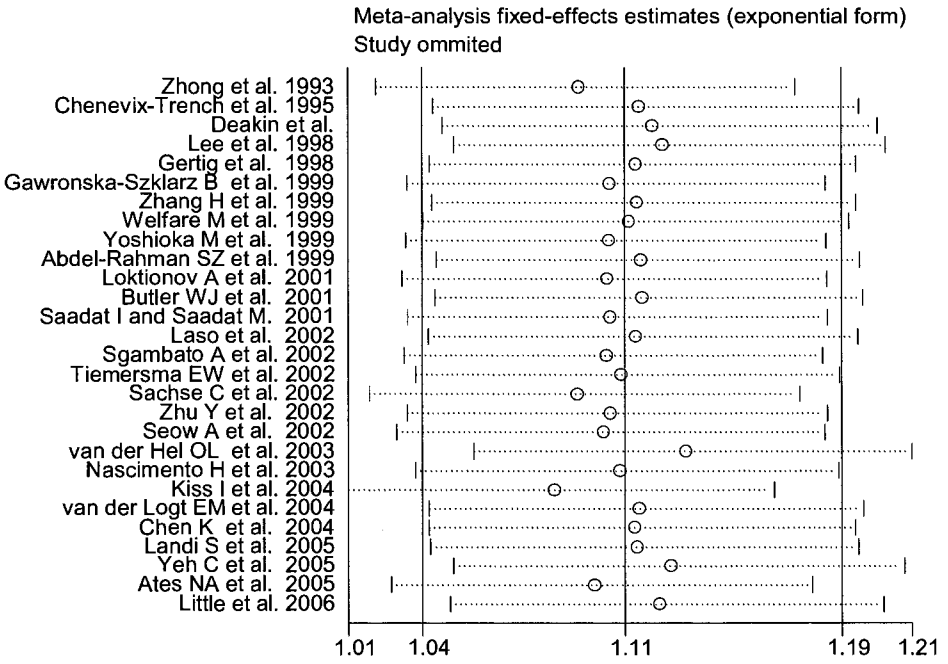
The cumulative meta-analysis demonstrates that the first few studies resulted in a stronger association than later studies. As more studies were published, the association gradually levels off. The association however remains significant.

Influence Analysis: Colorectal Cancer and *GSTM1*

Table 14: Meta-Analysis Influence Analysis Results for *GSTM1* and Colorectal Cancer

Study Omitted	e^coef.	LCI	UCI
Zhong et al. 1993	1.09	1.02	1.17
Chenevix-Trench et al. 1995	1.11	1.04	1.19
Deakin et al.	1.12	1.04	1.20
Lee et al. 1998	1.12	1.05	1.20
Gertig et al. 1998	1.11	1.04	1.19
Welfare M et al. 1999	1.11	1.04	1.19
Abdel-Rahman SZ et al. 1999	1.12	1.04	1.19
Zhang H et al. 1999	1.11	1.04	1.19
Gawronska-Szklarz B et al. 1999	1.10	1.03	1.18
Yoshioka M et al. 1999	1.10	1.03	1.18
Loktionov A et al. 2001	1.10	1.03	1.18
Butler WJ et al. 2001	1.12	1.04	1.20
Saadat I and Saadat M. 2001	1.10	1.03	1.18
Tiemersma EW et al. 2002	1.11	1.03	1.19
Sachse C et al. 2002	1.09	1.02	1.17
Laso et al. 2002	1.11	1.04	1.19
Sgambato A et al. 2002	1.10	1.03	1.18
Seow A et al. 2002	1.10	1.03	1.18
Zhu Y et al. 2002	1.10	1.03	1.18
van der Hel OL et al. 2003	1.13	1.06	1.21
Nascimento H et al. 2003	1.11	1.03	1.19
Kiss I et al. 2004	1.08	1.01	1.16
Chen K et al. 2004	1.11	1.04	1.19
van der Logt EM et al. 2004	1.11	1.04	1.20
Ates NA et al. 2005	1.10	1.03	1.18
Yeh C et al. 2005	1.13	1.05	1.21
Landi S et al. 2005	1.11	1.04	1.19
Little et al. 2006	1.12	1.05	1.20
Combined OR	**1.11**	**1.04**	**1.19**

Figure 9: Influence Analysis Plot: Colorectal Cancer and *GSTM1*



The influence analysis revealed that the overall estimate did not change as a result of the exclusion of any one particular study. The influence of two particular studies was of interest. The study conducted by van der Hel et al. (2003) included women subjects only and the study by Zhang et al. (1999) used tumor samples to genotype cases. Removal of either study did not result in a significantly different combined estimate.

Meta-Regression: Colorectal Cancer and *GSTM1*

The following variables were used in the meta-regression model: region, number of cases, language, type of control and year of publication. All variables were treated as categorical with the exception of year.

Table 15: Meta-Analysis Meta-Regression Results for *GSTM1* and Colorectal Cancer

COVARIATE 1	COVARIATE 2	p
Region		
Europe	UK	0.76
Europe	Asia	0.90
Europe	Other	0.72
Number of Cases		
>150	<150	0.39
Language		
English	Chinese	0.82
Type of Control		
Population	Healthy	0.18
Population	Hospital	0.62
Population	Other	0.31
Year of Publication		
year		0.79

The meta-regression results indicate that none of the variables included in the model contribute to heterogeneity.

Stratified Analysis: Colorectal Cancer and *GSTM1*

A stratified meta-analysis was conducted to examine the study specific effects of region, number of cases, language, and type of control on the combined estimate of effect.

Table 16: Stratified Meta-Analysis Summary Crude Odds Ratios and 95% Confidence Intervals for *GSTM1* and Colorectal Cancer

	No. of studies	OR for null genotype (95%CI)	I^2 (uncertainty interval)
Overall	**28**	**1.12 (1.02,1.23)**	**45% (14%-65%)**
Region			
Europe	9	1.13 (0.92, 1.39)	59 (15%-81%)
United Kingdom	6	1.17 (0.96, 1.43)	55 (0%-82%)
Asia	4	1.11 (0.89, 1.38)	25 (0%-88%)
Other	8	1.07 (0.89, 1.28)	39 (0%-72%)
Number of cases			
‹150 cases	11	1.21 (0.98,1.49)	36 (0%-69%)
≥150 cases	17	1.09 (0.98,1.22)	51 (14%-72%)
Language			
English	26	1.12 (1.01, 1.24)	47 (17%-67%)
Chinese	2	1.17 (0.78, 1.75)	30 (NA)
Type of control			
Screening/Population based	9	1.06 (0.93, 1.21)	35 (0%-70%)
Healthy	9	1.29 (1.00, 1.65)	47 (0%-76%)
Hospital	5	0.98 (0.83, 1.15)	2 (0%-80%)
Other	5	1.21 (0.93, 1.56)	61 (0%-86%)

Grouping study results by geographical region, case sample size, language and type of control produced similar summary odds ratios.

Subgroup Analysis: Colorectal Cancer and *GSTM1*

Subgroup meta-analysis was conducted when sufficient information was reported to compute summary odds ratios by subgroup. Table 17 summarizes the results of the analysis examining the effect of sex, tumor site, and smoking on *GSTM1* and colorectal cancer risk.

Table 17: Subgroup Meta-Analysis Summary Odds Ratios and 95% Confidence Intervals for *GSTM1* and Colorectal Cancer

Sex	**Men**			**Women**		
Study	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
Abdel-Rahman al. 1999	0.83	0.29	2.38	0.30	0.09	0.99
Laso et al. 2002	0.95	0.57	1.58	1.05	0.66	1.67
Loktionov A et al. 2001	1.41	0.89	2.22	1.13	0.64	2.01
Yeh C et al. 2005	0.98	0.74	1.28	1.00	0.73	1.37
Combined OR	**1.04**	**0.85**	**1.29**	**0.97**	**0.72**	**1.31**

Tumor site	**Proximal**			**Distal**		
Study	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
Laso et al. 2002	0.83	0.51	1.36	1.13	0.77	1.66
Loktionov A et al.	1.11	0.63	1.95	1.37	0.92	2.05
van der Logt EM et al. 2001	0.93	0.59	1.48	1.03	0.75	1.42
Gertig et al. 1998	0.74	0.44	1.25	1.26	0.77	2.07
Chenevix-Trench et al. 1995	0.87	0.42	1.81	0.94	0.58	1.53
Zhong et al. 1993	3.38	1.91	6.00	1.66	1.04	2.66
Combined OR	**1.11**	**0.72**	**1.71**	**1.18**	**1.00**	**1.40**

Smoking	**Non Smokers**			**Smokers**		
Study	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
Gertig et al. 1998	1.14	0.61	2.13	1.08	0.64	1.80
Ates NA et al. 2005	1.74	1.00	3.03	1.27	0.70	2.31
Laso et al. 2002	1.11	0.72	1.70	0.89	0.51	1.55
Yoshioka M et al. 1999	1.71	0.76	3.84	1.45	0.68	3.09
van der Hel OL et al. 2003	0.94	0.65	1.37	0.50	0.29	0.88
Combined OR	**1.17**	**0.93**	**1.48**	**0.95**	**0.67**	**1.36**
Combined OR*	1.32	1.00	1.74	1.11	0.83	1.49

*Removed van der Hel OL et al – all subjects were women.

Four studies included sufficient information to examine the effect of sex on the association between *GSTM1* and colorectal cancer. In terms of colorectal cancer risk, there was no significant difference

for men and women. Six studies reported sufficient information on tumor site (proximal vs. distal) to conduct a subgroup meta-analysis. A weak but significant association between distal tumors and colorectal cancer risk was observed (OR=1.18; 95%CI: 1.00, 1.40) when compared to proximal tumors. Five studies were included in the subgroup meta-analysis examining smoking. Smoking status was categorized into either non-smokers or smokers. A significant association was not observed.

5.3.3 Pooled Analysis Results: Colorectal Cancer and *GSTM1*

The pooled analysis of *GSTM1* and colorectal cancer risk included 13 studies with 3796 cases and 5984 controls. The pooled crude (OR=1.18; 95%CI: 1.08,1.28) and adjusted (OR=1.11; 95%CI: 1.02,1.22) odds ratios were significant suggesting that a significant association between *GSTM1* and colorectal cancer exits.

Table 18: Description of Studies Included in the Pooled Analysis, Study Specific Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Colorectal Cancer and *GSTM1*

Study	Year	No. of cases	No. of controls	Type of Control	Crude OR*			Adjusted OR†		
					OR	LCI	UCI	OR	LCI	UCI
Deakin et al.	1996	231	291	Hospital	0.69	0.49	0.98	0.7	0.49	1.01
Butler WJ et al.	2001	202	200	Hospital/Healthy	0.92	0.62	1.36	0.86	0.48	1.53
Nowell/Sweeney et al.	2001	86	310	Population	1.87	1.15	3.06	1.86	1.14	3.05
Sachse C et al.	2002	490	455	Population	1.55	1.2	2.01	1.55	1.2	2.01
Seow A et al.	2002	213	1190	Population	1.25	0.93	1.68	1.22	0.9	1.64
Sgambato A et al.	2002	48	121	Hospital/Healthy	1.89	0.92	3.88	1.85	0.89	3.83
Nascimento H et al.	2003	102	300	Hospital/Healthy	1.19	0.76	1.87	1.2	0.75	1.93
van der Hel OL et al.[5]	2003	221	766	Population	0.75	0.55	1.01	0.74	0.54	1.00
Kiss I et al.	2004	500	500	Mixed**	1.48	1.16	1.91	1.49	1.16	1.91
Landi S et al.	2005	369	320	Hospital	1.22	0.9	1.64	1.22	0.9	1.65
van der Logt EM et al.	2005	370	415	Volunteers	1.03	0.78	1.37	0.81	0.55	1.20
Yeh C et al.	2005	723	733	Population	0.99	0.8	1.21	0.99	0.8	1.22
Little et al.	2006	241	383	Population	0.87	0.63	1.21	0.89	0.63	1.25
Pooled Results		**3796**	**5984**		**1.18**	**1.08**	**1.28**	**1.11**	**1.02**	**1.22**

**in and out patients and volunteers from the health status examination

* OR, odds ratio; CI, confidence interval

† adjusted by age, sex and study

[5] All subjects were female

Table 19: Model Fit Statistics: Colorectal Cancer and *GSTM1*

Predictor	Wald's χ^2	df	p	eβ (OR)	UCI	LCI	-2log likelihood
Reduced Model		1					
GSTM1	15.24	1	<0.001	1.18	1.08	1.28	13064.29
Full Model		15					
GSTM1	5.86	1	0.02	1.11	1.02	1.22	11817.38
Sex	16.97	1	<0.0001				
Age	311.35	1	<0.0001				
Study	600.44	12	<0.0001				
Likelihood Ratio Test		14	<.0001				1246.91

Removal of the Van der Hel study in which all subjects were female, resulted in an adjusted odds ratio of 1.16 (95%CI: 1.06,1.27) .

5.3.4 Comparison of Combined Overall Estimate between Meta- and Pooled-Analyses: Colorectal Cancer and *GSTM1*

When the analysis was limited to only studies that were included in the pooled analysis and the meta-analysis, the combined odds ratio from the meta-analysis was no longer significant (OR: 1.08; 95%CI: 0.95,1.23). The combined overall adjusted estimate from the pooled analysis remained statistically significant (OR: 1.10; 95%CI: 1.00,1.20).

5.4 Summary of Results: *GSTM1*

Overall 36 studies examining *GSTM1* and either colorectal cancer, colorectal adenomas, colon or rectal cancer were identified. Sufficient information was available to include five studies in the meta-analysis and two studies in the pooled analysis examining colorectal adenomas and *GSTM1*. A total of 28 studies were included in the meta-analysis and 13 studies in the pooled analysis of the association between *GSTM1* and colorectal cancer risk. A significant association between colorectal adenoma risk and *GSTM1* null was not observed.

The results of the meta-analysis (OR=1.12; 95%CI: 1.02, 1.23) and the pooled analysis (OR=1.11; 95%CI: 1.02, 1.22) suggest an association between *GSTM1* and colorectal cancer risk. The meta-regression results indicated that region, number of cases, language, type of control or year of publication did not contribute to heterogeneity. This result was backed up by the stratified meta-analysis results. Grouping study results by these same variables resulted in similar summary odds ratios. A significant but weak association was observed between distal tumors and colorectal cancer risk (OR=1.18; 95%CI: 1.00, 1.40) when compared with proximal tumors. A difference in colorectal cancer risk, *GSTM1* null and sex or smoking was not observed.

Chapter 6: *GSTT1*

6.1 Introduction

Overall, 27 studies examining the association between the *GSTT1* genotype and either colon, rectal, colorectal or adenomas were identified.

Table 20: Total number of studies examining *GSTT1* by cancer type

GSTT1	Number of Studies
Colorectal cancer	23
Colorectal adenomas	5
Colon cancer	1
Rectal cancer	0

GSTT1 and Colon Cancer

Ye and Parry (2002) were the only study to examine the association between *GSTT1* and colon cancer risk. A significant association was not observed.

6.2 Colorectal Adenomas and *GSTT1*

6.2.1 Systematic Review Results: Colorectal Adenomas and *GSTT1*

Five studies were identified that examined the association between *GSTT1* and colorectal adenomas. None of the studies reported observing a significant association. Studies included, study characteristics and study results are summarized in table 21.

Table 21: Summary of Studies of Colorectal Adenomas and *GSTT1* by Region and Date of Publication

Authors	Year	Region & Country	Study Period	Description of Cases	#of Cases	Description of Controls	#of Controls	% of controls *GSTT1* null	OR (95% CI)	Adjustments	Subgroup Analysis Reported
Inoue et al.	2000	Asia Japan	Jan95-Dec96	Men receiving pre-retirement health examination at SDF Fukuoka Hospital and SDF Kumamoto Hospital with histologically confirmed adenomas	205	Men receiving pre-retirement health examination at SDF Fukuoka Hospital and SDF Kumamoto Hospital with normal study of colonoscopy	220	49.1	Crude OR: 1.2 (0.8, 1.8) Adjusted OR: 1.1 (0.7, 1.6)	Hospital, rank in SDF, cigarette-years, alcohol use, BMI	Tumor location, size, Cigarette-years, genotype combinations: *GSTM1* and *GSTT1*
Tijhuis MJ et al.	2005	Europe Nether-lands	Jun97-Oct02	Cases recruited among patients undergoing endoscopy at the outpatient clinics of eight hospitals in the Netherlands. At least one histologically confirmed colorectal adenomatous polyp ever in their life.	746	No history of any type of polyps.	698	54.1	Not reported		Cruciferous vegetable intake

Lin et al. Original analysis: 1995	2002	North America US	Jan91-Aug93	Subjects undergoing screening sigmoidoscopy at Kaiser Permanente's Bellflower or Sunset medical centers, diagnosed for the first time with one or more histologically confirmed adenomas	459	No polyps of any type at sigmoidoscopy or history of polyps. Individually matched to cases by gender, age, date of sigmoidoscopy and center	507	24	Adjusted OR: 1.19 (0.88, 1.61)	Date of sigmoidoscopy (6-month intervals), age (5 year intervals), gender and clinic attended	Cruciferous vegetable intake, ethnic group, smoking
Moore LE et al.	2005	North America US	Sept. 1993 – Sept. 1999	Randomly selected cases drawn from the Prostate, Lung, Colorectal, and Ovarian Trial (10 screening centers throughout the US) with at least one advanced colorectal adenoma in the distal colon	772	Randomly selected controls drawn from the Prostate, Lung, Colorectal, and Ovarian Trial (10 screening centers throughout the US) with a negative sigmoidoscopy screening. Frequency matched to cases by gender and ethnicity	777	16.8	Not reported		Smoking, broccoli intake
Gunter MJ et al.	2005	UK	NR	Nested case-control study, UK Flexible Sigmoidoscopy Screening Trial, (randomized controlled trial of 368,583 participants from 14 geographic areas). Histologically confirmed adenoma	768	Age and sex matched individuals with a negative flexible sigmoidoscopy result	814	17.4	Adjusted OR: 0.9 (0.7, 1.2)	Age, sex, center	Gender, Interaction between diet, smoking & genotype

6.2.2 Meta-Analysis Results: Colorectal Adenomas and *GSTT1*

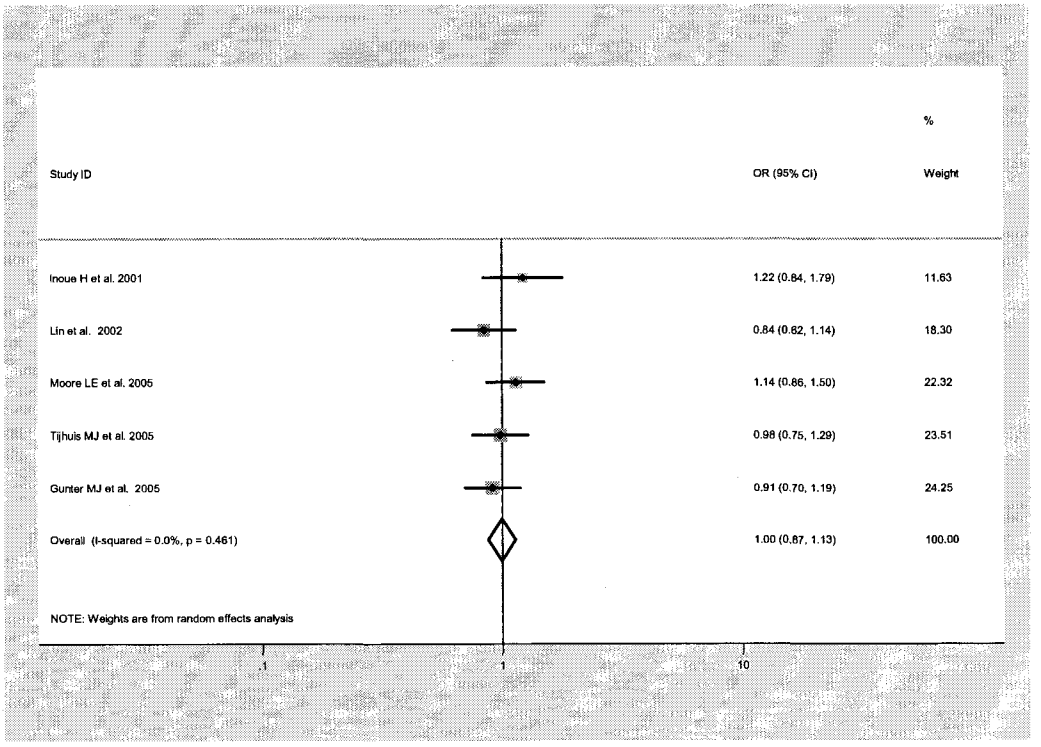
Five studies were identified that examined the association between *GSTT1* and colorectal adenomas. All of the studies used population based controls. The study by Inoue et al. (2000) included men only in their study. The studies were conducted in various regions, Japan, Europe, North America and the United Kingdom. All of the studies were published in the English language. In terms of sample size all five of the studies included a minimum of 200 cases and controls.

Due to the limited number of studies, sensitivity analysis was not conducted. The estimated odds ratios ranged from 0.84 to 1.14. The combined OR was not significant for either the crude odds ratios (OR=1.00; 95%CI: 0.87, 1.13; I^2=0%; 0%-77%) or the adjusted (when reported) odds ratios (OR=1.03; 95%CI: 0.89, 1.17; I^2=0%; 0%-66%).

Table 22: Meta-Analysis Study Specific Crude Odds Ratios, Summary Odds Ratio and 95% Confidence Intervals for Colorectal Adenomas and *GSTT1*

Study	Year	OR	LCI	UCI
Inoue H et al.	2001	1.23	0.84	1.79
Lin et al.	2002	0.84	0.62	1.14
Moore LE et al.	2005	1.14	0.86	1.50
Tijhuis MJ et al.	2005	0.98	0.75	1.29
Gunter MJ et al.	2005	0.91	0.70	1.19
Combined OR		**1.00**	**0.87**	**1.13**

Figure 10: Meta-Analysis Odds Ratios and 95% Confidence Intervals: Colorectal Adenomas and *GSTT1*



Assessment of Publication Bias

The Begg's test (p=0.33) and the Egger's test (p=0.48) do not provide evidence of publication bias. The Begg's funnel plot is reasonably symmetrical and the intercept in the Egger's plot does not deviate significantly from zero. Results should be interpreted cautiously due to the limited number of studies.

Figure 11: Begg's Funnel Plot: Colorectal Adenomas and *GSTT1*

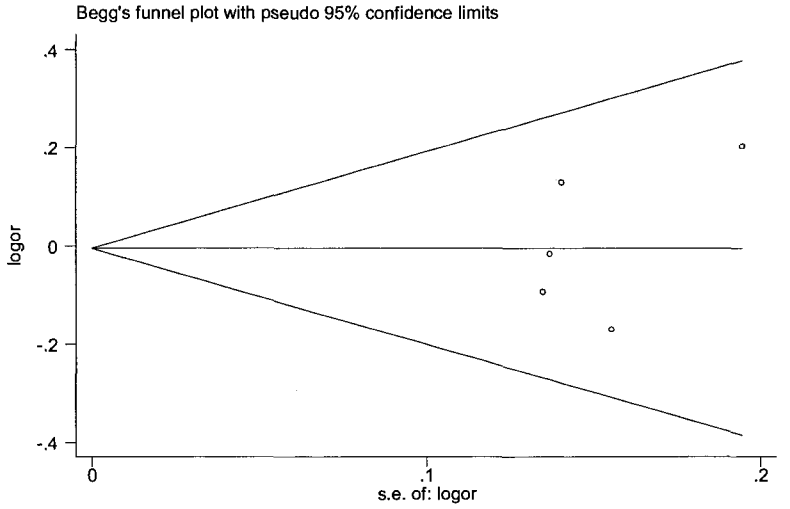
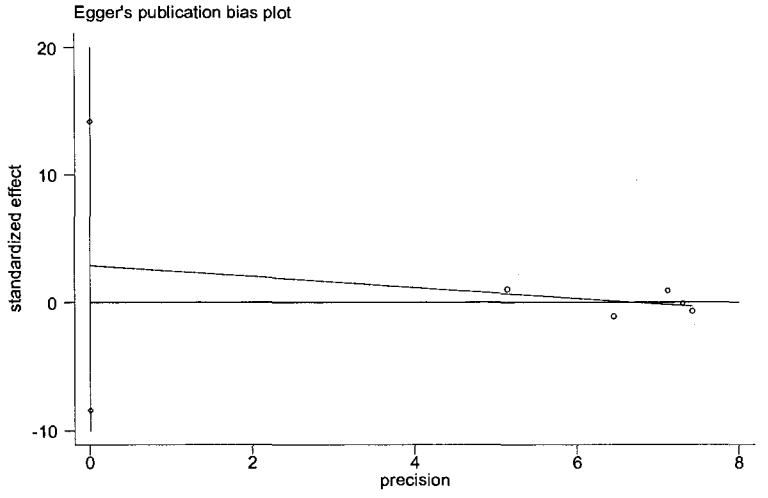


Figure 12: Egger's Publication Bias Plot: Colorectal Adenomas and *GSTT1*



6.2.3 Pooled Analysis Results: Colorectal Adenomas and *GSTT1*

The pooled analysis of *GSTT1* and colorectal adenoma risk included two studies with a total of 1136 controls and 1123 cases. Both studies used population based controls. Table 23 presents the combined crude and adjusted odds ratios which were not significant, suggesting that there is no association between colorectal adenoma risk and *GSTT1*.

Table 23: Pooled Analysis Study Specific Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Colorectal Adenomas and *GSTT1*

Study	Year	No. of cases	No. of controls	Crude OR*			Adjusted OR†		
				OR	LCI	UCI	OR	LCI	UCI
Moore et al.	2005	689	702	1.15	0.87	1.52	1.18	0.90	1.56
Tiemersma et al.	2005	434	434	0.87	0.60	1.26	0.89	0.60	1.33
Combined		**1123**	**1136**	**1.04**	**0.83**	**1.30**	**1.08**	**0.86**	**1.36**

Table 24: Model Fit Statistics: Colorectal Adenomas and *GSTT1*

Predictor	Number of Predictors	Wald's χ^2	df	p	eβ (OR)	UCI	LCI	-2log likelihood
Null Model	Intercept							3131.56
Reduced Model			1					3131.44
GSTT1	1	0.12	1	0.73	1.04	0.83	1.30	
Full Model	4		4					3005.67
GSTT1		0.46	1	0.4971	1.08	0.86	1.36	
Sex		10.91	1	0.001				
Age		100.21	1	<.0001				
Study		21.36	1	<.0001				
Likelihood Ratio Test			3					125.77

6.2.4 Comparison of Combined Overall Estimate between Meta- and Pooled-Analyses: Colorectal Adenomas and *GSTT1*

The pooled analysis resulted in an adjusted odds ratio of 1.08 (95%CI: 0.86,1.36). A similar result was obtained for the meta-analysis where only these two studies were considered (OR: 1.05; 95%CI: 0.87,1.28).

6.3 Colorectal Cancer and *GSTT1*

6.3.1 Systematic Review Results: Colorectal Cancer and *GSTT1*

Twenty-three studies examining the association between *GSTT1* and colorectal cancer were identified. A summary of the studies, the study characteristics and study results are outlined in table 25. Six studies reported the observation of a significant association between *GSTT1* and colorectal cancer risk. Zhang H et al. (1999) reported an increased frequency of the *GSTT1* null genotype in cases (53%) in comparison with controls (20%) ($p<0.0001$). Butler WJ et al. (2001) reported a significant association between *GSTT1* and colorectal cancer risk (OR=2.18, 95%CI: 1.38,3.43). However once an adjustment was made for age, this association just failed to reach significance (OR=1.91, 95%CI: 0.99,3.70). Laso et al. (2002) observed a borderline significant association between the *GSTT1* genotype and colorectal cancer (OR=1.68; 95%CI:1.0,2.82). Rajagopal et al. (2005) observed a significant association between the *GSTT1* null genotype and colorectal cancer (OR=1.65; 95%CI:1.22,2.24). Ates NA et al. (2005) reported a significant association between the *GSTT1* null genotype and an increased risk of developing colorectal cancer (OR=1.64; 95%CI:1.10,2.59). Yeh C et al. (2005) reported a moderate association between *GSTT1* and risk of developing colorectal cancer ($p<0.06$).

Table 25: Summary of Studies of Colorectal Cancer and *GSTT1* by Region and Date of Publication

Authors	Date	Region & Country	Study Period	Description of Cases	# of Cases	Description of Controls	# of Controls	% of controls *GSTT1* null	OR (95% CI)	Adjustments	Subgroup Analysis Reported
Abdel-Rahman SZ et al.	1999	Africa Egypt	NR	Newly diagnosed patients from 3 cancer hospitals	59	Healthy controls friends of other cancer patients from the same centers, matched on age	51	41.2	Crude OR: 0.85 (0.37,1.97)		Age, rural/ urban; site; gender
Lee et al.	1995	Asia Singapore	NR	CRC patients from surgical department	300	Patients with no history of neoplasms from the clinical chemistry department of a local hospital	183	49	Not Reported		Tumor site and histology
Yoshioka M et al.	1999	Asia Japan	Sept91-Jun95	Consecutive histologically confirmed from University of Occupational & Environmental Health Hospital, Mitsubishi Chemical Hospital, & Kitakyushu Medical Center	106	Individuals who had visited local medical clinics in Kitakyushu City (Sept93-Apr95) for regular medical check-ups with no current of previous diagnosis of cancer	100	41	Crude OR: 1.33 (0.77, 2.32)		Genotype combinations: *GSTM1*, *GSTT1*, *GSTP1*, *NAT1* and *NAT2*), smoking
Zhu et al.	2002	Asia China		Sporadic colorectal adenocarcinoma patients	104	Healthy controls	101	47.5	Not significant		Smoking, age, Dukes Stage, differentiation, tumor location
Chen K et al.	2004	Asia China			125		339	20.4	Not reported	Age, sex	Colon/rectal, smoking, *GSTM1* and *GSTT1* combinations

Yeh C et al.	2005	Asia China	Jan95-Jan99	Histologically confirmed cases newly diagnosed at the Chang Gung Memorial Hospital	723	Controls were recruited from the Physical Check-Up Department for comprehensive health examinations (incl. colonoscopies) matched by age and sex	733	49.1	Not significant	Sex, age, physical activity, coffee, cigarettes, alcohol, meat, vegetable/ fruit, and fish/shrimp	Sex, tumor site, age at diagnosis, vegetable/fruit consumption, smoking
Chenevix-Trench et al.	1995	Australia	NR	Patients with colorectal adenocarcinoma	132	Unselected subjects (source not stated n=94) and geriatric patients without cancer or family history of cancer (n=54)	94 54	19 9	0.7 (0.3, 1.4) 1.5 (0.6, 4.3)	None	Age, position of tumor
Butler et al.	2001	Australia	NR	Queen Elizabeth Hospital, white adults	203	Queen Elizabeth Hospital, white adults attending a voluntary blood donation blood bank	200	20.0	Crude OR: 2.18 (1.38, 3.43) Adjusted OR: 1.91 (0.99, 3.70)	Age	Age, gender, tumor site, combinations of genotypes
Zhang H et al.	1999	Europe Sweden	1989-1997	Samples from tumors obtained from patients with primary CRC diagnosed at the Dept. of Pathology, Linkoping University Hospital & Norrkiping Central Hospital	99	Subjects were randomly selected from a healthy control group of residents in South-Eastern Sweden	109	20%	Significant (53% for cases, 20% in controls), OR not reported		
Laso et al.	2002	Europe Spain	1996-1997	Consecutive patients undergoing surgery from the University of Barcelona Hospital Clinic, Spain	247	Consecutively recruited patients presenting to the Trauma Service of the Hospital Clinic during the same time period as case accrual	296	11.1	Crude OR: 1.68 (1, 2.82) Adjusted OR: 1.75 (1.02, 2.99)	Age, sex	Smoking status, gender, stage, differentiation, tumor location
van der Hel OL et al.	2003	Europe Nether-lands	1987-1996	Netherlands Population-based screening program for early detection of breast cancer	234	Random selection of women from the source population	765	29.3	Not significant	Smoking	

Kiss I et al.	2004	Europe Hungary	NR	Histologically confirmed from the Central Hospital of the Ministry of Internal Affairs and from the area of Baranya and Vas County, Hungary	500	Cancer-free controls from the same regions including non cancer patients from in- or out-patient wards and volunteers from the health status examination study. Matched according to sex and age	500	21.6	Crude OR: 1.29 (0.95, 1.74)		Genotype combinations, high risk vs. low risk allele combinations
van der Logt EM et al.	2004	Europe Nether-lands	NR	Patients recruited from the Depts of Gastroenterology and General Surgery, University Medical Centre St. Radboud, Nijmegen	371	Recruited from local newspaper	415	16.6	Crude OR: 1.2 (0.84, 1.7) Adjusted OR: 1.0 (0.64, 1.7)	Age, gender	Proximal vs. Distal, Dukes staging
Luchtenborg M et al.	2005	Europe Nether-lands	1989-1994	Incident cases participating in the Prospective Netherlands Cohort Study on Diet and Cancer, (204 municipals) Nested case-only design		Prospective Netherlands Cohort Study on Diet and Cancer participants			Not reported		
Saadat I and Saadat M.	2001	Middle East Iran	NR	Pathologically confirmed	46	Healthy blood donor matched to cases on age and gender	131	11.5	Crude OR: 1.41 (0.70, 2.88)		Sex, genotype combinations: *GSTM1 & GSTT1*
Ates NA et al.	2005	Middle East Turkey	Sept01 - Apr04	Consecutive histologically confirmed patients from surgery depts of Mersin & Kocaeli University hospital (in and out patients)	181	Unrelated healthy controls recruited from two hospitals in Turkey	204	26.0	Adjusted OR: 1.64 (1.10, 2.59)	Age, sex	Age, smoking status, tumor site genotype combinations (*GSTM1*, *GSTT1*, *GSTP1*)

Nascimento H et al.	2003	South America Brazil	Aug99-Aug01	Consecutive histologically confirmed cases treated at the University Hospital of the State University of Campinas	102	Blood donors from the same hospital	300	17.3	Crude OR: 0.95 (0.50, 1.80) Adjusted OR: 1.08 (0.99, 1.18)	Age, gender	Age, gender, ethnic origin, smoking, tumor location, stage, differentiation genotype combinations *GSTM1 & GSTT1*
Seow A et al.	2002	Southeast Asia Singapore	Apr93-Dec98	Participants of Singapore Chinese Health Study (population based, prospective investigation of diet and cancer risk). Incident cases identified through population based Singapore Cancer Registry	213	Participants of the Singapore Chinese Health Study (population based, prospective investigation of diet and cancer risk), matched according to sex, dialect group, year of recruitment, and date of birth	1194	40.2	Adjusted OR: 0.88 (0.64, 1.21)	Education, BMI, smoking, physical activity, alcohol, saturated fat	Colon vs. Rectal cancer, GST genotype combinations (M1,T1 and P1), ITC intake (low vs. high), smoking (never vs. ever)
Welfare M et al.	1999	UK	Dec94-Sept95	Histologically confirmed cases from Newcastle & North Tyneside health district were invited to participate	201	Age and sex matched community controls were recruited for each case, identified from GP records	187	16.9	Adjusted OR: 1.21 (0.63, 2.0)	Matched pair design: age, sex, and area of residence	Sex, age group, stage, site of tumor, genotype combinations: *GSTM1*, *GSTT1*, *GSTP1*, & *NAT2*
Loktionov A et al.	2001	UK	1997-1999	Histologically confirmed cases treated at the Department of Surgery, Norwich and Norfolk Health Care NHS Trust	206	Participants of the on-going UK Flexible Sigmoidoscopy Screening Trial with a normal result	355	15.2	Crude OR: 1.43 (0.89, 2.28)		Colon proximal vs. Colorectal distal

Sachse C et al.	2002	UK	Aug97-Feb01	Incident cases recruited at either Ninewells Hospital, Dundee, Perth Royal Infirmary, Leeds General Infirmary, St. Jame's Hospital, Leeds or York District Hospital	490	Healthy population based controls with no history of previous cancer (GP controls), recruited by age, sex, and general practitioner matching of incident cases	593	63.7	Unmatched analysis: 0.87 (0.67, 1.13)		Genotype combinations: *GSTM1* null/*NAT2* slow, *GSTM1* null/*GSTT1* null, and *GSTM1* null/*CYP1a1*2B*
Rajagopal R et al.	2005	UK	1990-2000	Caucasian cases with operative and histological confirmation from the University Hospital of North Staffordshire undergoing potentially curative surgery	361	Unrelated controls with non-malignant, non-inflammatory conditions recruited from the same hospital	881	17.9	Crude OR: 1.65 (1.22, 2.24)		Sex, tumor site, differentiation, Dukes' stage, Host lymphocyte reaction
Little et al.	2006	UK	Sept98-Feb00	Population-based case-control study in North-East Scotland. Histologically confirmed cases identified from the database of the pathology laboratory	264 189 colon/ 75 rectal	Selected from the Grampian Community Health Index (list of everyone registered with a GP) matched by age and sex	408	17	Adjusted OR: 1.25 (0.81, 1.93) Adjusted OR: 1.23 (0.74, 2.02)	Age, sex Age, sex, family history of CRC, aspirin use, use of other NSAIDs, and physical activity	Green leafy vegetable intake (high, low) Interaction: *GSTT1* and *CYP1A1*
Gertig et al.	1998	U.S.	NR	White male cases with CRC from the prospective Physician's Health Study.	212	White male participants not diagnosed with CRC within the prospective Physician's Health Study.	221	23	0.8 (0.5, 1.2)	Age, smoking, BMI, physical activity, alcohol intake	Site, smoking, *GSTM1* and *GSTT1*, age

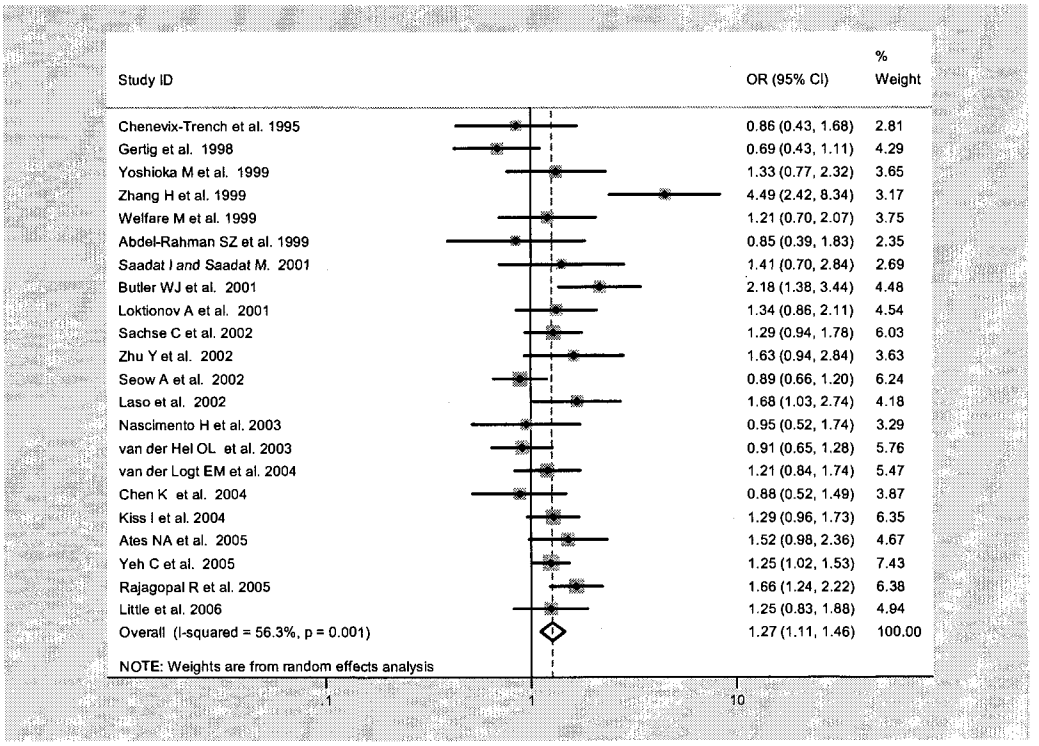
6.3.2 Meta-Analysis Results: Colorectal Cancer and *GSTT1*

A total of 22 studies were deemed eligible for inclusion for the investigation of the potential association between *GSTT1* and colorectal cancer risk. The estimated odds ratios of the individual studies ranged from 0.69 to 4.49. The combined crude OR for all studies was 1.27 (95%CI: 1.11, 1.46) for the random effects model. The formal test for heterogeneity suggests moderate heterogeneity exists across the studies (I^2=56%; 30%-73%). Using the adjusted odds ratio when reported resulted in a similar combined estimate (OR=1.12; 95%CI: 1.00, 1.24; I^2=28%; 0%-57%).

Table 26: Meta-Analysis Study Specific Crude Odds Ratios, Summary Odds Ratio and 95% Confidence Intervals for Colorectal Cancer and *GSTT1*

Author(s)	Year	OR	LCI	UCI
Chenevix-Trench et al.	1995	0.86	0.43	1.69
Gertig et al.	1998	0.69	0.43	1.11
Yoshioka M et al.	1999	1.33	0.77	2.32
Zhang H et al.	1999	4.49	2.42	8.34
Welfare M et al.	1999	1.21	0.70	2.07
Abdel-Rahman SZ et al.	1999	0.85	0.39	1.83
Saadat I and Saadat	2001	1.41	0.70	2.84
Butler WJ et al.	2001	2.18	1.38	3.44
Loktionov A et al.	2001	1.34	0.86	2.11
Sachse C et al.	2002	1.29	0.94	1.78
Zhu Y et al.	2002	1.63	0.94	2.84
Seow A et al.	2002	0.89	0.66	1.20
Laso et al.	2002	1.68	1.03	2.74
Nascimento H et al.	2003	0.95	0.52	1.74
van der Hel OL et al.	2003	0.91	0.65	1.28
van der Logt EM et al.	2005	1.21	0.84	1.74
Chen K et al.	2004	0.88	0.52	1.49
Kiss I et al.	2004	1.29	0.96	1.73
Ates NA et al.	2005	1.52	0.98	2.36
Yeh C et al.	2005	1.25	1.02	1.53
Rajagopal R et al.	2005	1.66	1.24	2.22
Little et al.	2006	1.25	0.83	1.89
Combined OR		**1.27**	**1.11**	**1.46**

Figure 13: Meta-Analysis Odds Ratios and 95% Confidence Intervals: Colorectal Cancer and *GSTT1*



Publication Bias Assessment

Both the Begg's test (p=0.74) and the Egger's test (p=0.73) suggest that publication bias is not present.

Figure 14: Begg's Funnel Plot: Colorectal Cancer and *GSTT1*

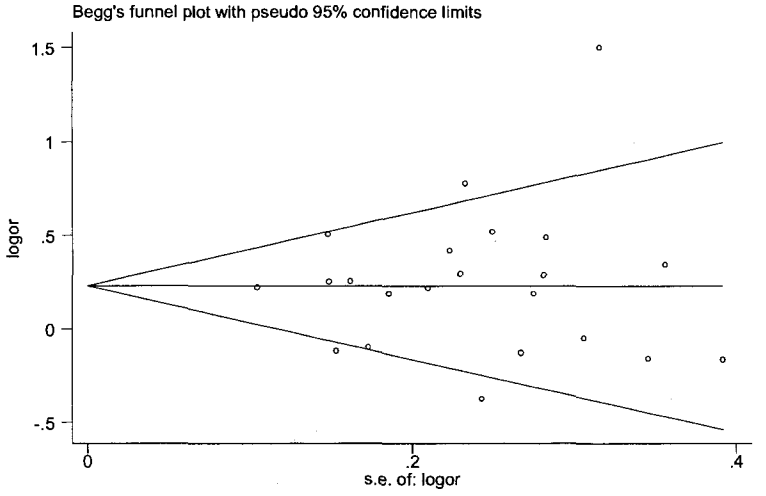
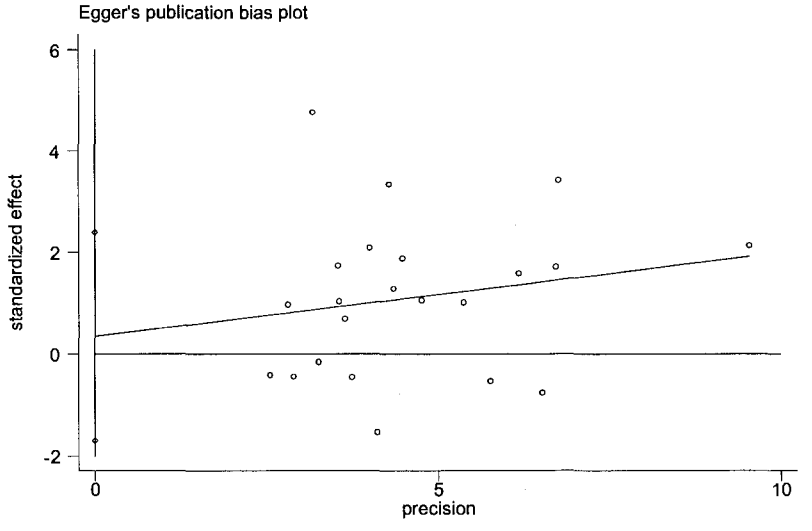


Figure 15: Egger's Plot: Colorectal Cancer and *GSTT1*



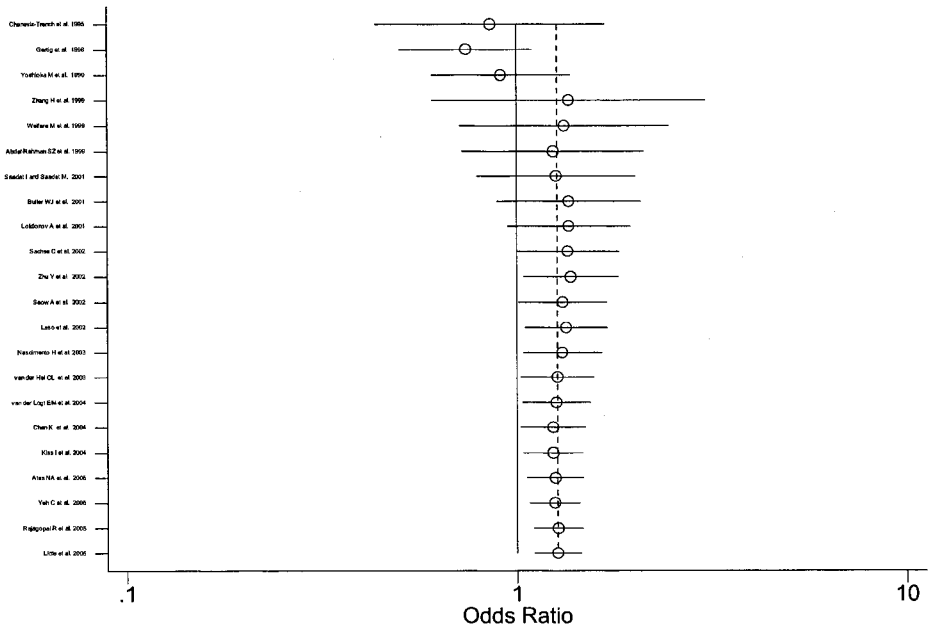
Cumulative Meta-Analysis: Colorectal Cancer and *GSTT1*

Table 27: Cumulative Meta-Analysis Results for *GSTT1* and Colorectal Cancer

Trial	Cumulative Estimate	LCI	UCI	z	P value
Chenevix-Trench et al. 1995	0.86	0.43	1.69	-0.45	0.65
Gertig et al. 1998	0.74	0.50	1.09	-1.51	0.13
Yoshioka M et al. 1999	0.91	0.61	1.37	-0.44	0.66
Zhang H et al. 1999	1.36	0.61	3.05	0.75	0.45
Welfare M et al. 1999	1.33	0.72	2.45	0.90	0.37
Abdel-Rahman SZ et al. 1999	1.24	0.73	2.12	0.79	0.43
Saadat I and Saadat M. 2001	1.26	0.79	2.01	0.98	0.33
Butler WJ et al. 2001	I.36	0.89	2.08	1.43	0.15
Loktionov A et al. 2001	1.36	0.95	1.95	1.66	0.10
Sachse C et al. 2002	1.35	1.00	1.83	1.96	0.05
Zhu Y et al. 2002	1.38	1.04	1.81	2.26	0.02
Seow A et al. 2002	1.31	1.01	1.70	2.04	0.04
Laso et al. 2002	1.34	1.05	1.71	2.34	0.02
Nascimento H et al. 2003	1.31	1.04	1.65	2.28	0.02
van der Hel OL et al. 2003	1.27	1.02	1.58	2.15	0.03
van der Logt EM et al. 2004	1.26	1.03	1.54	2.29	0.02
Chen K et al. 2004	1.24	1.02	1.50	2.18	0.03
Kiss I et al. 2004	1.24	1.04	1.47	2.40	0.02
Ates NA et al. 2005	1.25	1.06	1.48	2.65	0.01
Yeh C et al. 2005	1.25	1.08	1.45	2.93	0.00
Rajagopal R et al. 2005	1.27	1.10	1.47	3.29	0.00
Little et al. 2006	1.27	1.11	1.46	3.43	0.00

The strength of the association between *GSTT1* and colorectal cancer gradually increases with time and then stabilizes at 1.27 (95%CI: 1.11,1.46).

Figure 16: Cumulative Analysis Plot: Colorectal Cancer and *GSTT1*



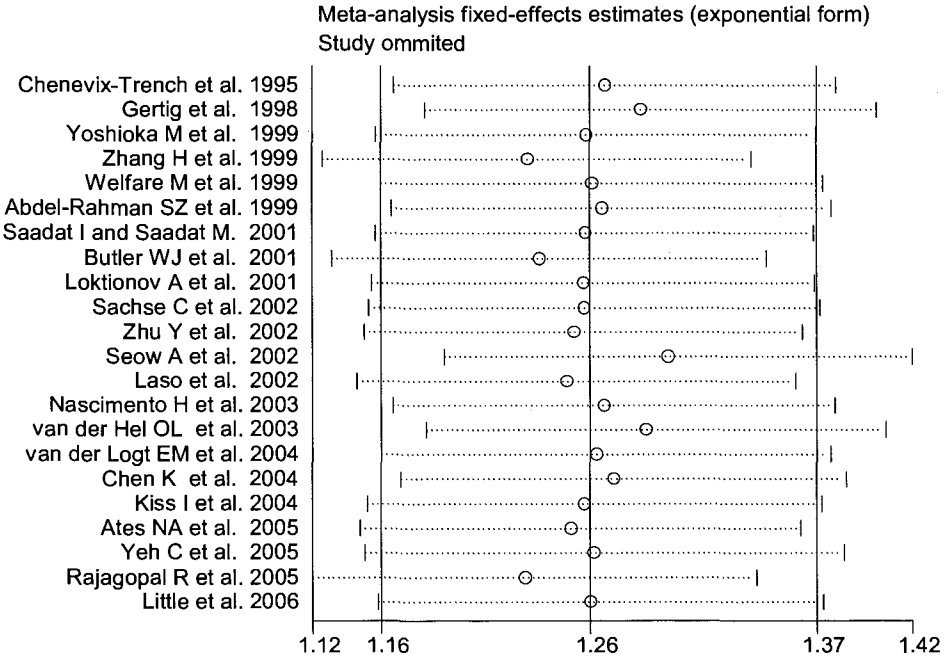
Influence Analysis: Colorectal Cancer and *GSTT1*

Table 28: Meta-Analysis Influence Analysis Results for *GSTT1* and Colorectal Cancer

Study Omitted	e^coef.	LCI	UCI
Chenevix-Trench et al. 1995	1.27	1.16	1.38
Gertig et al. 1998	1.28	1.18	1.40
Welfare M et al. 1999	1.26	1.16	1.37
Abdel-Rahman SZ et al. 1999	1.26	1.16	1.38
Yoshioka M et al. 1999	1.26	1.15	1.37
Zhang H et al. 1999	1.23	1.13	1.34
Loktionov A et al. 2001	1.26	1.15	1.37
Saadat I and Saadat M. 2001	1.26	1.15	1.37
Butler WJ et al. 2001	1.23	1.13	1.35
Sachse C et al. 2002	1.26	1.15	1.37
Seow A et al. 2002	1.30	1.19	1.42
Zhu Y et al. 2002	1.25	1.15	1.36
Laso et al. 2002	1.25	1.14	1.36
van der Hel OL et al. 2003	1.29	1.18	1.40
Nascimento H et al. 2003	1.27	1.16	1.38
Chen K et al. 2004	1.27	1.17	1.38
Kiss I et al. 2004	1.26	1.15	1.37
van der Logt EM et al. 2004	1.26	1.16	1.38
Yeh C et al. 2005	1.26	1.15	1.38
Ates NA et al. 2005	1.25	1.15	1.36
Rajagopal R et al. 2005	1.23	1.12	1.34
Little et al. 2006	1.26	1.15	1.37
Combined	**1.26**	**1.16**	**1.37**

The overall combined estimate of *GSTT1* does not change dramatically as a result of the exclusion of any particular study. In addition, removal of the study by Zhang et al. (1999) in which tumor samples were used to genotype cases did not result in a significantly different combined estimate.

Figure 17: Influence Analysis: Colorectal Cancer and *GSTT1*



Meta-Regression Results: Colorectal Cancer and *GSTT1*

The following variables were used in the meta-regression model: region, number of cases, language, type of control and year of publication. All variables were treated as categorical with the exception of year.

Table 29: Meta-Regression Results for *GSTT1* and Colorectal Cancer

COVARIATE 1	COVARIATE 2	p
Region		
Europe	UK	0.76
Europe	Asia	0.49
Europe	Other	0.17
Number of Cases		
≥150	<150	0.76
Language		
English	Chinese	0.78
Type of Control		
Population	Healthy	0.46
Population	Hospital	0.10
Population	Other	0.15
Year of Publication		
year		0.70

The meta-regression results indicate that none of the variables included in the model contribute to heterogeneity.

Stratified Analysis: Colorectal Cancer and *GSTT1*

A stratified meta-analysis was conducted to examine the study specific effects of region, number of cases, language, and type of control on the combined estimate of effect. The results of the meta-regression are presented in table 30.

Table 30: Stratified Meta-Analysis Summary Odds Ratios and 95% Confidence Intervals for *GSTT1* and Colorectal Cancer

	No. of studies	OR for null genotype (95%CI)	I^2 (uncertainty interval)
Overall	**22**	**1.27 (1.11, 1.46)**	**56% (30%,73%)**
Region			
Europe	5	1.52 (1.01, 2.27)	81% (55%,92%)
United Kingdom	5	1.39 (1.18, 1.65)	0% (0%,61%)
Asia	4	1.24 (1.05, 1.48)	0% (0%,83%)
Other	8	1.10 (0.82, 1.48)	61% (16%,82%)
Number of cases			
‹150 cases	8	1.31 (0.90, 1.93)	68% (33%,85%)
≥150 cases	14	1.25 (1.09, 1.44)	50% (8%,73%)
Language			
English	20	1.28 (1.11, 1.48)	58% (31%,75%)
Chinese	2	1.19 (0.65, 2.17)	60% (0%,91%)
Type of control			
Screening/Population based	8	1.13 (1.00, 1.27)	4% (0%,69%)
Healthy	7	1.26 (0.90, 1.75)	60% (9%,83%)
Hospital	3	1.60 (1.28, 2.01)	0% (0%,59%)
Other	4	1.53 (0.90, 2.60)	82% (53%,93%)

Grouping study results by geographical region, case sample size, language and type of control did not produce considerable variation in terms of the summary odds ratios computed. Some variation was observed in terms of the type of control used in the study. Specifically, the studies which used hospital controls resulted in greater risk of colorectal cancer than those using population based, healthy or other types of controls.

Subgroup Analysis: Colorectal Cancer and *GSTT1*

Subgroup meta-analysis was conducted when sufficient information was reported to compute summary odds ratios by subgroup. Table 31 summarizes the results of the analysis examining the effect of sex, tumor site, and smoking on *GSTT1* and colorectal cancer risk.

Table 31: Subgroup Meta-Analysis Summary Odds Ratios and 95% Confidence Intervals for *GSTT1* and Colorectal Cancer

Sex	**Men**			**Women**		
Study	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
Abdel-Rahman et al. 1999	0.71	0.28	1.81	0.35	0.09	1.37
Laso et al. 2002	1.78	0.86	3.67	1.52	0.77	3.00
Loktionov A et al. 2001	1.31	0.74	2.31	1.42	0.67	3.00
Yeh C et al. 2005	1.30	0.99	1.71	1.20	0.88	1.64
Pooled OR	**1.29**	**1.03**	**1.62**	**1.20**	**0.85**	**1.69**
Tumor site	**Proximal**			**Distal**		
Study	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
Laso et al. 2002	1.93	1.00	3.72	1.56	0.90	2.70
Loktionov A et al. 2001	1.42	0.71	2.86	1.31	0.79	2.17
van der Logt EM et al. 2004	0.86	0.45	1.63	1.29	0.86	1.94
Gertig et al. 1998	0.89	0.48	1.66	0.57	0.29	1.10
Chenevix-Trench et al. 1995	0.44	0.10	1.99	1.22	0.60	2.47
Pooled OR	**1.12**	**0.76**	**1.66**	**1.18**	**0.87**	**1.59**
Smoking	**Non Smokers**			**Smokers**		
Study	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
Gertig et al. 1998	0.83	0.39	1.76	0.78	0.40	1.50
Ates NA et al. 2005	1.47	0.83	2.63	1.72	0.87	3.39
Laso et al. 2002	1.29	0.69	2.42	2.50	1.11	5.63
Yoshioka M et al. 1999	1.88	0.83	4.25	0.99	0.47	2.12
Yeh C et al. 2005	1.53	0.94	2.48	1.30	0.92	1.83
van der Hel OL et al. 2003	0.88	0.57	1.36	0.92	0.53	1.61
Pooled OR	**1.22**	**0.95**	**1.56**	**1.22**	**0.91**	**1.63**
Pooled OR*	1.32	0.94	1.85	1.32	0.79	2.20

*Excluded van der Hel (all subjects were women) and Yeh (all subjects were men)

Four studies included sufficient information to examine the effect of sex on the association between *GSTT1* and colorectal cancer. A weak but significant association between *GSTT1* and colorectal cancer risk was observed in men (OR=1.29; 95%CI: 1.03,1.62), but not in women (OR=1.20;

95%CI: 0.85,1.69). Five studies reported sufficient information on tumor site (proximal vs. distal) to conduct a subgroup meta-analysis. A significant association between tumor site and colorectal cancer risk was not observed. Six studies were included in the subgroup meta-analysis examining smoking. Smoking status was categorized into either non-smokers or smokers. A significant association was not observed.

6.3.3 Pooled Analysis Results: Colorectal Cancer and *GSTT1*

The pooled analysis of *GSTT1* and colorectal cancer risk included 13 studies with a total of 5988 controls and 3827 cases. A significant association between *GSTT1* and colorectal cancer was observed for both the crude (OR=1.10; 95%CI:1.00,1.20) and adjusted (OR=1.22; 95%CI:1.10,1.35) odds ratios.

Table 32: Description of Studies Included in the Pooled Analysis, Study Specific Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Colorectal Cancer and *GSTT1*

Author(s)	Year	No. of cases	No. of controls	Type of Control	Crude OR*			Adjusted OR†		
					OR	LCI	UCI	OR	LCI	UCI
Butler WJ et al.	2001	189	200	Hospital/Healthy	1.21	0.84	1.74	1.92	0.99	3.71
Nowell /Sweeney et al.	2001	86	307	Population	1.15	0.63	2.12	1.16	0.63	2.12
Sachse C et al.	2002	500	471	Population	1.31	0.93	1.84	1.31	0.93	1.84
Seow A et al.	2002	213	119	Population	0.89	0.66	1.2	0.88	0.65	1.21
Sgambato et al.	2002	48	121	Hospital/Healthy	0.92	0.36	2.35	1.12	0.43	2.97
Nascimento H et al.	2003	102	299	Hospital/Healthy	0.95	0.52	1.73	1.19	0.63	2.23
van der Hel OL et al.	2003	221	766	Population	0.92	0.66	1.28	1.00	0.72	1.41
Kiss I et al.	2004	500	500	Mixed**	1.29	0.96	1.73	1.29	0.96	1.73
Deakin/Rajagopal R et al.	2005	268	283	Hospital	1.25	1.02	1.54	1.93	1.26	2.94
Landi et al.	2005	365	320	Hospital	0.96	0.65	1.43	0.99	0.67	1.47
van der Logt EM et al.	2005	371	415	Volunteers	1.21	0.84	1.74	1.04	0.64	1.71
Yeh C et al.	2005	723	733	Population	1.25	1.02	1.54	1.26	1.02	1.54
Little et al.	2006	241	383	Population	1.25	0.83	1.89	1.25	0.81	1.93
Combined		**3827**	**4917**		**1.10**	**1.00**	**1.20**	**1.22**	**1.10**	**1.35**

**in and out patients and volunteers from the health status examination
* OR, odds ratio; CI, confidence interval
† adjusted by age and sex
ς All subjects were female

Table 33: Model Fit Statistics: Colorectal Cancer and *GSTT1*

Predictor	Wald's χ^2	df	p	eβ (OR)	UCI	LCI	-2log likelihood
Reduced Model		1					13126.76
GSTT1	4.02	1	0.045	1.10	1.20	1.00	
Full Model		15					11858.50
GSTT1	14.76	1	0.00	1.22	1.35	1.10	
Sex	18.09	1	<.0001				
Age	320.94	1	<.0001				
Study	605.84	12	<.0001				
Likelihood Ratio Test		14					1268.26

6.3.4 Comparison of Combined Overall Estimate between Meta- and Pooled-Analyses: Colorectal Cancer and *GSTT1*

When the analysis was limited to only studies that were included in the pooled analysis and the meta-analysis, the combined odds ratio from the meta-analysis was no longer significant (OR: 1.25; 95%CI: 0.83,1.89). The combined overall adjusted estimate from the pooled analysis remained statistically significant (OR: 1.24; 95%CI: 1.12,1.38).

6.4 Summary of Results: *GSTT1*

Overall 27 studies examining *GSTT1* and either colorectal cancer, colorectal adenomas, colon or rectal cancer were identified. Sufficient information was available to include five studies in the meta-analysis and two studies in the pooled analysis examining colorectal adenomas and *GSTT1*. A total of 22 studies were included in the meta-analysis and 13 studies in the pooled analysis of the association between *GSTT1* and colorectal cancer risk.

A significant association between colorectal adenoma risk and *GSTT1* null was not observed.

The results of the meta-analysis (OR=1.27; 95%CI: 1.11, 1.46) and the pooled analysis (OR=1.22; 95%CI: 1.10, 1.35) suggest an association between *GSTT1* and colorectal cancer risk. The meta-regression results indicated that region, number of cases, language, type of control or year of publication did not contribute to heterogeneity. This result was backed up by the stratified meta-

analysis results. Grouping study results by these same variables resulted in similar summary odds ratios. An exception was observed in terms of type of control used. Studies using hospital controls resulted in a greater risk of colorectal cancer than those using population based, healthy or other controls. A significant but weak association was observed in men and colorectal cancer risk (OR=1.29; 95%CI: 1.03, 1.62) but not in women (OR=1.20; 95%CI: 0.85, 1.69). A difference in colorectal cancer risk, *GSTT1* null and tumor site or smoking was not observed.

Chapter 7: *GSTP1*

7.1 Overview

Overall, 14 studies examining the association between the *GSTP1* genotype and either colon, rectal, colorectal or adenomas were identified.

Table 34: Total number of studies examining *GSTP1* by type of cancer.

GSTP1	Number of Studies
Colorectal cancer	11
Colorectal adenomas	3
Colon cancer	0
Rectal cancer	0

Two genetic polymorphisms for *GSTP1* have been identified, both of which result in an amino acid change in the protein. The first polymorphism has been identified at codon 105 where a substitution of isoleucine (Ile) to valine (Val) in the protein amino acid sequence results (I105V). The other polymorphic site for *GSTP1* is at codon 114 where an adenosine (A) to guanine (G) transition results in an alanine to valine substitution (A114V). The Val allele encoded enzyme is associated with lower activity and is therefore thought to result in less effective capability of detoxification. It is therefore hypothesized that individuals carrying the Val allele are at a greater risk for developing cancer than among those carrying the Ile genotype.

7.2 *GSTP1* and Colorectal Adenomas

7.2.1 Systematic Review Results: Colorectal Adenomas and *GSTP1*

Three studies investigated the association between *GSTP1* and colorectal adenomas (Lin et al. 2001, Moore et al. 2005 and Tijhuis et al. 2005). The analysis for the association between *GSTP1* and colorectal adenomas was therefore limited to the systematic review. Only one study reported a main effect OR for the association between *GSTP1* and colorectal adenomas (Moore et al. 2005) and the association was not significant. The other two studies focused on the interaction between diet, vegetable intake, *GSTP1* and colorectal adenomas.

7.3 *GSTP1* and Colorectal Cancer

7.3.1 Systematic Review Results: Colorectal Cancer and *GSTP1*

Eleven studies were identified that investigated the association between *GSTP1* and colorectal cancer. None of the studies identified support a relationship between *GSTP1* and colorectal cancer. Table 35 summarizes the studies identified.

Table 35: Summary of Studies of Colorectal Cancer and *GSTP1* by Region and Date of Publication

Author	Year	Region & Country	Cancer	# Cases	# Controls	Codon	Alleles	OR (CI)	Adjusted OR (CI)	Variable used in Adjustment
Harris et al.	1998	Australia	CRC	131	199	I105V		Not reported		
						A114V		Not reported		
Yoshioka M et al.	1999	Asia	CRC	106	100	I105V	AA	1.0		
		Japan					With G	1.5 (0.81-2.76)		
Yeh C et al.	2005	Asia	CRC	727	747	I105V	AA	1.0		
		China					With G	MEN: 1.36 (0.98-1.89)		
						I105V	AA	1.0		
							With G	WOMEN: 1.00 (0.70-1.43)		
Seow A et al.	2002	Southeast Asia Singapore	CRC	213	1194	I105V	AA		1.0	Education, BMI, smoking, physical activity, alcohol, saturated fat
							AG		0.94 (0.67-1.33)	
Welfare M et al.	1999	UK	CRC	201	187	I105V	AA	1.0		
		England					AG	1.01 (0.65-1.6)		
							GG	0.57 (0.25-1.2)		
						A114V	CC	1.0		
							CT	1.0 (0.5-1.9)		
							TT	0.18 (0.01-1.58)		
Loktionov A et al.	2001	UK	CRC	206	355	I105V	AA	1.0		
							With G	0.84 (0.58-1.21)		

Sachse C et al.	2002	UK	CRC	490	593	I105V	AA	1.0		
							AG	1.26 (0.97-1.63)		
							GG	0.99 (0.67-1.47)		
						A114V	CC	1.0		
							CT	0.98 (0.72-1.35)		
							TT	0.54 (0.20-1.51)		
							GG		0.54 (0.20-1.41)	
Kiss I et al.	2004	Europe Hungary	CRC	500	500	I105V	AA	1.0		
							With G	1.11 (0.85-1.43)		
van der Logt EM et al.	2004	Europe Netherlands	CRC	371	415	I105V	AA	1.0	1.0	Age, gender
							With G	0.94 (0.77-1.2)	0.87 (0.65-1.2)	
Ates NA et al	2005	Middle East Turkey	CRC	181	204	I105V	AA	1.0		
							AG	1.44 (0.91-2.27)		
							GG	0.82 (0.45-1.51)		
Landi S et al.	2005	Europe Spain	CRC	322	303	I105V	AA	1.0		
							AG	1.04 (0.75-1.46)		
							GG	0.73 (0.42-1.27)		
							With G	0.98 (0.71-1.34)		
						A114V	CC	1.0		
							CT	0.94 (0.57-1.57)		
							TT	Rare		
							With T	0.89 (0.54-1.47)		

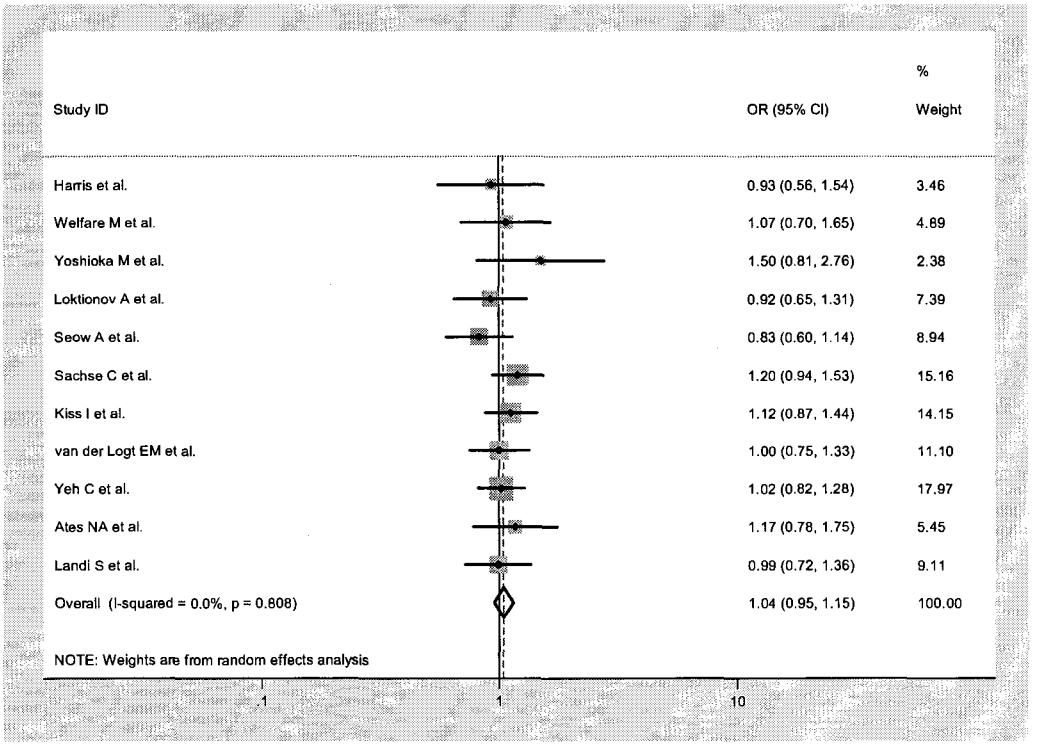
7.3.2 Meta-Analysis Results: Colorectal Cancer and *GSTP1*

A total of 11 studies were deemed eligible for inclusion for the investigation of the potential association between *GSTP1* and colorectal cancer risk. The estimated odds ratios for the individual studies ranged from 0.83 to 1.20. The combined OR for all studies was not significant (OR=1.04; 95% CI: 0.95, 1.15; I^2=0%; 0%-35%) for the random effects model. The combined OR for all studies using the adjusted odds ratio when reported was 1.03 (95%CI: 0.92, 1.13; I^2=0%; 0%-20%).

Table 36: Meta-Analysis Study Specific Crude Odds Ratios, Summary Odds Ratio and 95% Confidence Intervals for Colorectal Cancer and *GSTP1* (ILE/ ILE compared with ILE /VAL and VAL / VAL)

Author(s)	Year	OR	LCI	UCI
Harris et al.	1998	0.93	0.56	1.54
Welfare M et al.	1999	1.07	0.70	1.65
Yoshioka M et al.	1999	1.50	0.81	2.76
Loktionov A et al.	2001	0.92	0.65	1.31
Sachse C et al.	2002	1.20	0.94	1.53
Seow A et al.	2002	0.83	0.60	1.14
Kiss I et al.	2004	1.12	0.87	1.44
van der Logt EM et al.	2005	1.00	0.75	1.33
Yeh C et al.	2005	1.02	0.82	1.28
Ates NA et al.	2005	1.17	0.78	1.75
Landi S et al.	2005	0.99	0.72	1.36
Pooled OR		**1.04**	**0.95**	**1.15**

Figure 18: Meta-Analysis Odds Ratios and 95% Confidence Intervals: Colorectal Cancer and *GSTP1*



Tests for Publication Bias

The tests for publication bias did not support evidence of publication bias. Both the Begg's test (p=0.64) and the Egger's test (p=0.90) were not significant.

Figure 19: Begg's Plot: Colorectal Cancer and *GSTP1*

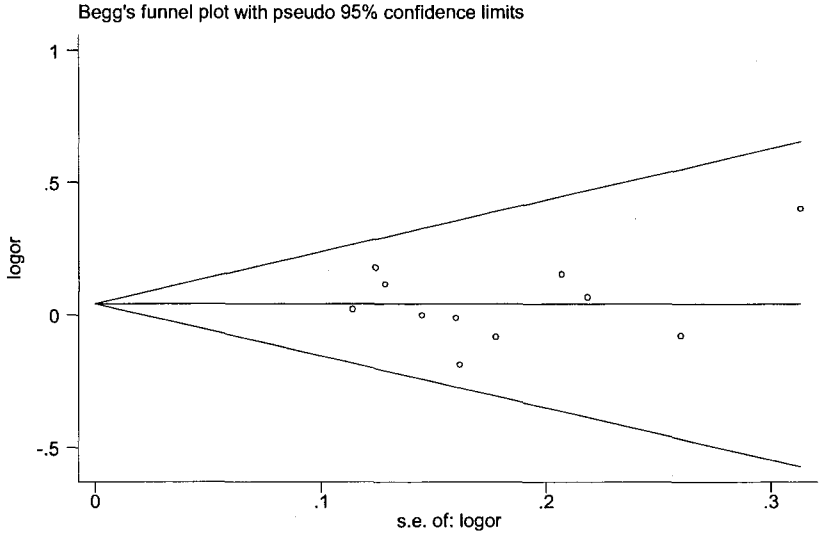
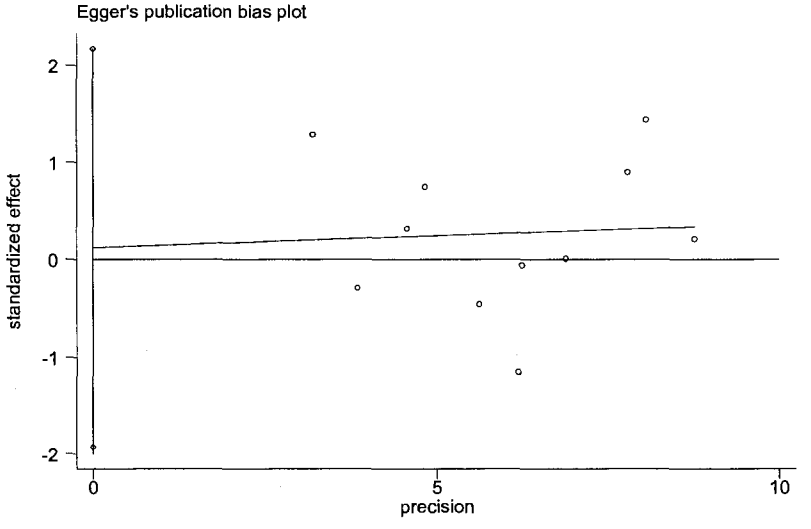


Figure 20: Egger's Plot: Colorectal Cancer and *GSTP1*

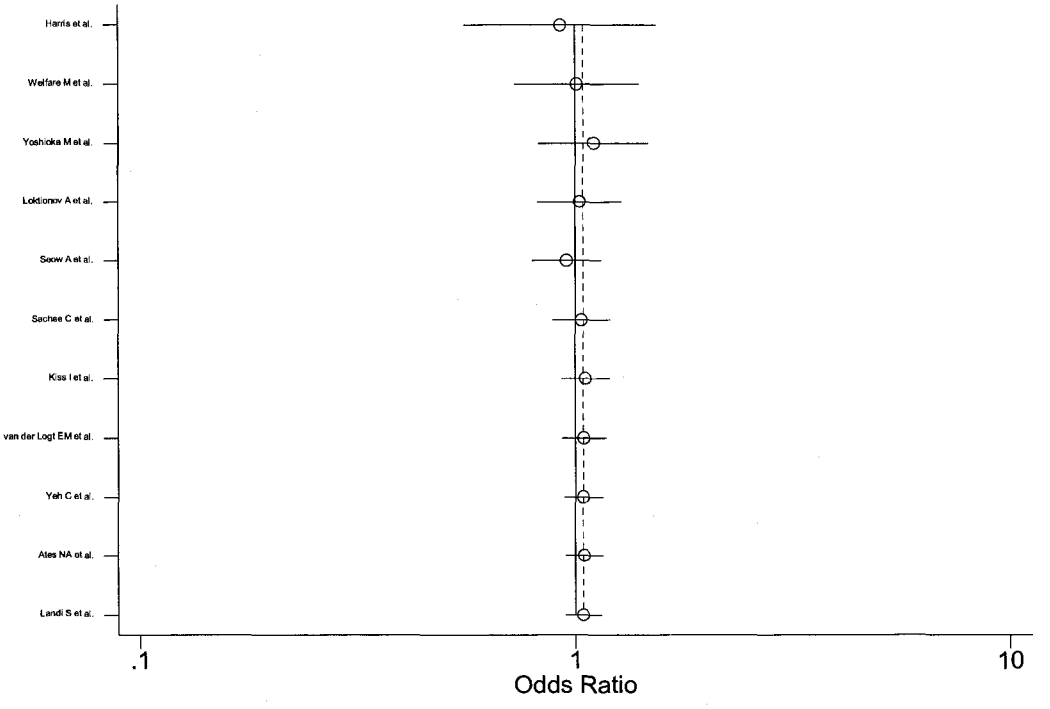


Cumulative Analysis

Table 37: Cumulative Meta-Analysis Results for *GSTP1 (I105V)* and Colorectal Cancer

Trial	Cumulative Estimate	LCI	UCI	z	Pvalue
Harris et al.	0.93	0.56	1.54	-0.29	0.77
Welfare M et al.	1.01	0.73	1.40	0.05	0.96
Yoshioka M et al.	1.10	0.83	1.47	0.65	0.51
Loktionov A et al.	1.03	0.82	1.28	0.21	0.83
Seow A et al.	0.96	0.80	1.15	-0.49	0.63
Sachse C et al.	1.03	0.89	1.20	0.44	0.66
Kiss I et al.	1.06	0.93	1.20	0.86	0.39
van der Logt EM et al.	1.05	0.93	1.18	0.79	0.43
Yeh C et al.	1.04	0.94	1.16	0.80	0.43
Ates NA et al.	1.05	0.95	1.16	0.96	0.34
Landi S et al.	1.04	0.95	1.15	0.89	0.37

Figure 21: Cumulative Analysis Plot: Colorectal Cancer and *GSTP1*



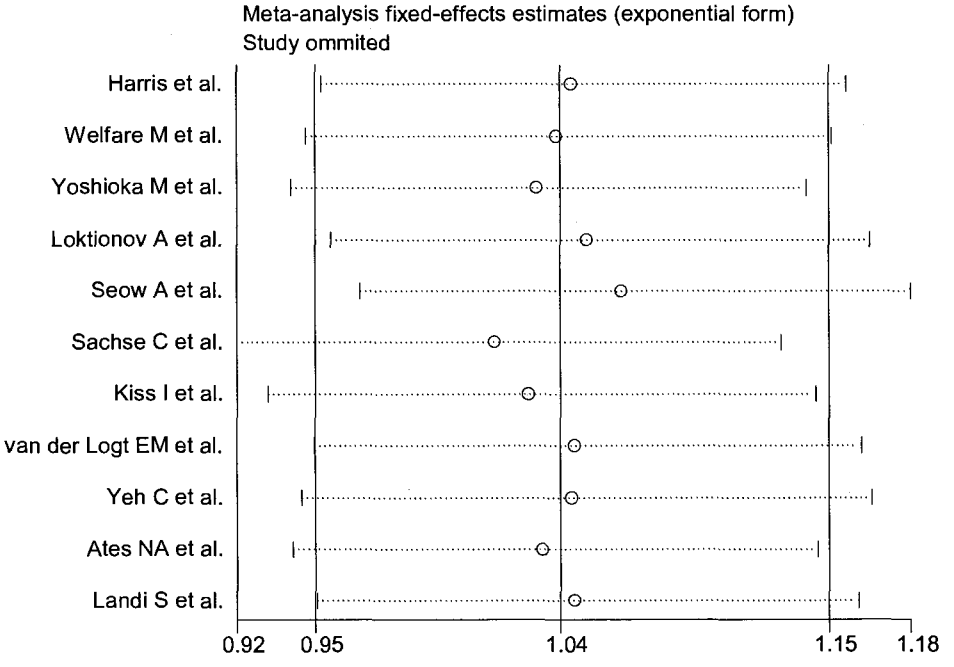
Influence Analysis

Table 38: Meta-Analysis Influence Analysis Results for *GSTP1 (I105V)* and Colorectal Cancer

Study ommited	e^coef.	LCI	UCI
Harris et al.	1.05	0.95	1.15
Welfare M et al.	1.04	0.95	1.15
Yoshioka M et al.	1.04	0.94	1.14
Loktionov A et al.	1.05	0.96	1.16
Seow A et al.	1.07	0.97	1.18
Sachse C et al.	1.02	0.92	1.13
Kiss I et al.	1.03	0.93	1.14
van der Logt EM et el.	1.05	0.95	1.16
Yeh C et al.	1.05	0.94	1.16
Ates NA et al.	1.04	0.94	1.14
Landi S et al.	1.05	0.95	1.16
Combined	**1.04**	**0.95**	**1.15**

The overall estimate does not change dramatically as a result of the exclusion of any particular study.

Figure 22: Influence Analysis Plot: Colorectal Cancer and *GSTP1*



Meta-Regression

Due to the limited number of studies that examined the association between *GSTP1* and colorectal cancer, a meta-regression was not conducted.

Stratified Analysis

Table 39: Stratified Meta-Analysis Summary Odds Ratios and 95% Confidence Intervals for *GSTP1 (I105V)* and Colorectal Cancer

	No. of studies	OR for null genotype (95%CI)	I^2 % (uncertainty interval)
Overall	**11**	**1.04 (0.95, 1.15)**	**0% (0%,42%)**
Region			
Europe	3	1.05 (0.89, 1.23)	0% (0%,59%)
United Kingdom	3	1.09 (0.91, 1.31)	0% (0%,86%)
Asia	3	1.00 (0.79, 1.27)	34% (0%,78%)
Other	2	1.07 (0.78, 1.47)	NA
Number of cases			
‹200 cases	4	1.23 (0.89, 1.42)	0% (0%,69%)
≥200 cases	7	1.03 (0.93, 1.14)	0% (0%,58%)
Type of control			
Screening/Population based	5	1.02 (0.90, 1.16)	0% (0%,77%)
Healthy	2	1.07 (0.78, 1.47)	NA
Hospital	2	1.11 (0.77, 1.61)	NA
Other	2	1.07 (0.88,1.29)	NA

Grouping the study results by geographic region, number of cases or type of control used did not result in notably different summary odds ratios.

Subgroup Analysis

Sufficient data was not reported to conduct a subgroup analysis for *GSTP1*.

Hardy-Weinberg Equilibrium

Departure from Hardy-Weinberg equilibrium was assessed for *GSTP1*. It has been hypothesized that departure from Hardy-Weinberg equilibrium in the control population may be responsible for spurious results, in addition to suggesting the presence of genotyping errors or other biases in the study (79) . A significant deviation from Hardy-Weinberg equilibrium was detected in the controls for two studies (Ates et al and Welfare et al.). As a result, a sensitivity analysis was conducted to assess the impact of these two studies on the combined OR. The combined OR resulting from the removal of these two studies was not substantially different from the combined OR that included these two studies (OR=1.04; 95%CI: 0.94,1.15).

7.3.3 Pooled Analysis Results: Colorectal Cancer and *GSTP1*

The pooled analysis of *GSTP1* I105V and colorectal cancer risk included eight studies with a total of 3962 controls and 2867 cases. A significant result was obtained for the crude odds ratio (OR=1.19; 95%CI: 1.08,1.13) but not the adjusted odds ratio (OR=1.06; 95%CI: 0.96,1.18).

Table 40: Description of Studies Included in the Pooled Analysis, Study Specific Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Colorectal Cancer and *GSTP1* I105V

Study	Year	No. of cases	No. of controls	Type of Control	Crude OR*			Adjusted OR†		
					OR[x]	LCI	UCI	OR[x]	LCI	UCI
Deakin et al.	1996	191	110	Hospital	1.56	0.97	2.50	1.45	0.85	2.48
Nowell /Sweeney et al.	2001	64	261	Population	1.13	0.64	2.03	1.11	0.62	2.00
Sachse C et al.	2002	490	455	Population	1.20	0.92	1.55	1.20	0.92	1.55
Seow A et al.	2002	212	1185	Population	0.83	0.60	1.14	0.89	0.64	1.23
Kiss I et al.	2004	500	500	Mixed**	1.12	0.87	1.44	1.12	0.87	1.45
Landi S et al.	2005	319	303	Hospital	1.01	0.74	1.38	1.01	0.73	1.38
van der Logt EM et a	2005	369	414	Volunteers	1.00	0.75	1.33	0.93	0.62	1.38
Yeh C et al.	2005	722	734	Population	1.02	0.82	1.28	1.02	0.82	1.28
Total		**2867**	**3962**		**1.19**	**1.08**	**1.31**	**1.06**	**0.96**	**1.18**

* OR, odds ratio; CI, confidence interval
† adjusted by age and sex
**in and out patients and volunteers from the health status examination
[ξ]ILE/ILE vs. ILE/VAL and VAL/VAL

The pooled analysis of *GSTP1* A114V and colorectal cancer risk included two studies with a total of 758 controls and 809 cases. A significant association was not observed.

Table 41: Model Fit Statistics: Colorectal Cancer and *GSTP1* I105V

Predictor	Number of Predictors	Wald's χ^2	df	p	eβ (OR)	UCI	LCI	-2log likelihood
Null Model	Intercept							9290.67
Reduced Model	1							9278.05
GSTP1 (I114V)		12.61	1	0.0004	1.19	1.08	1.31	
Full Model	4							8361.13
GSTP1 (I114V)		1.22	1	0.27	1.06	0.96	1.18	
Sex		16.03	1	<.0001				
Age		176.68	1	<.0001				
Study		515.37	7	<.0001				
Likelihood Ratio Test			3					916.92

Table 42: Description of Studies Included in the Pooled Analysis, Study Specific Crude and Adjusted Odds Ratios and 95% Confidence Intervals for Colorectal Cancer and *GSTP1* A114V

Author(s)	Year	No. of cases	No. of controls	Type of Control	Crude OR*			Adjusted†		
					OR[ξ]	LCI	UCI	OR[ξ]	LCI	UCI
Landi et al.	2005	319	303	Hospital	1.02	0.60	1.72	1.01	0.59	1.71
Sasche et al.	2002	490	455	Population	0.89	0.64	1.23	0.89	0.64	1.23
					0.926	**0.704**	**1.218**	**0.912**	**0.692**	**1.203**

* OR, odds ratio; CI, confidence interval
† adjusted by age and sex
[ξ]ILE/ILE vs. ILE/VAL and VAL/VAL

Table 43: Model Fit Statistics: Colorectal Cancer and *GSTP1* A114V

Predictor	Number of Predictors	Wald's χ^2	df	p	eβ (OR)	UCI	LCI	-2log likelihood
Null Model	Intercept							2170.66
Reduced Model	1							2170.36
GSTP1 (I114V)		0.31	1	0.58	0.93	0.70	1.22	
Full Model	4							2166.80
GSTP1 (I114V)		0.42	1	0.52	0.92	0.69	1.20	
Sex		2.21	1	0.14				
Age		1.38	1	0.24				
Study		0.03	1	0.86				
Likelihood Ratio Test			3					3.56

7.4 Comparison of Combined Overall Estimate between Meta- and Pooled-Analyses: Colorectal Cancer and *GSTP1*

When the analysis was limited to only studies that were included in the pooled analysis and the meta-analysis, the combined odds ratio from the meta-analysis (OR: 1.04; 95%CI: 0.93,1.16) and the pooled analysis (OR: 1.04; 95%CI: 0.93,1.16) were not significant.

7.5 Results Summary

Overall 13 studies examining *GSTP1* and either colorectal cancer, colorectal adenomas, colon or rectal cancer were identified. The investigation of the association between colorectal adenomas and *GSTP1* was restricted to the systematic review due to the limited number of studies available. In terms of colorectal cancer, the meta-analysis was limited to *GSTP1* I105V where a total of 10 studies were included. The pooled analysis included eight studies examining *GSTP1* I105V and two studies examining *GSTP1* A114V and colorectal cancer risk.

The results of the systematic review did not support an association between colorectal adenoma risk and *GSTP1* I105V. Only one study examined the effect of *GSTP1* IA114V and the association was not significant.

In terms of colorectal cancer risk, the meta-analysis did not result in a significant association between *GSTP1* I105V (OR=1.04; 95%CI: 0.95, 1.15). The pooled analysis resulted in inconsistent results. A significant result was obtained for the crude odds ratio (OR=1.19; 95%CI: 1.08,1.13) but not the adjusted odds ratio (OR=1.06; 95%CI: 0.96,1.18). The pooled analysis for *GSTP1* A114V did not support an association between *GSTP1* A114V and colorectal cancer risk. Due to the limited number of studies available a meta-regression to examine potential sources of heterogeneity was not possible. The stratified analysis however did not result in significantly different odds ratios for region, number of cases or type of control used. Language was not considered since all the studies were published in English.

Chapter 8: Joint Effects of *GSTM1*, *GSTT1*, *GSTP1* and Colorectal Cancer

8.1 Introduction

Interactions aim to describe how genetic and/or environmental factors jointly influence the risk of disease susceptibility (80). With the exception of a handful of cancers, cancer is thought to be the result of the combined effects of both environmental and genetic susceptibilities. Environmental exposure is thought to play a predominant role in determining cancer risk and the majority of variation in risk across individuals can now be explained by environmental risk factors for most major cancers (81). Established environmental risk factors for cancer include smoking, alcohol, obesity, hormone replacement therapy and physical inactivity. If the joint effects of environmental factors play an important role in disease susceptibility then analyses examining only the main effects of genes will result in biased results for both the main effects of gene and environmental factors resulting in very weak associations. It has been further suggested that even if the relevant environmental factors are assessed and modeled with the genetic factor of interest, these effects are likely to be diluted by the existence of other unmeasured environmental and genetic factors (82). This supports the importance of examining gene-gene and gene-environment joint effects which may be crucial to understanding cancer susceptibility. Gene-environment interaction may therefore be an explanation for the variability in results observed by studies examining colorectal cancer susceptibility (83).

Depending on the number of genes and exposures measured, individual studies often have the opportunity to examine a vast number of potential joint effects. The comparison of results across studies is difficult due to the lack of consistency in the genotypes and exposures considered. In addition, the lack of detailed reporting made it difficult to determine how many interactions were actually investigated by the various individual studies. Often studies reported only significant findings.

The focus of this chapter is on the combined effects of *GSTM1*, *GSTT1* and *GSTP1*. Due to the limited number of studies that reported adequate information to compute odds ratios and the many potential combinations that were explored across studies, the interactive effects of diet, smoking, *CYP1A1*, *NAT1* and *NAT2* genotypes were limited to the systematic review (see Chapter 3 for more detailed information).

8.2 Systematic Review Results

Combined Effect of *GSTM1*, *GSTT1* and *GSTP1*

Several studies examined various genotype combinations and their association with colorectal cancer risk. Many compared the effect of "high risk" allele combinations compared with individuals with "no risk" alleles. The examination of such combinations allows for the possibility that colorectal cancer risk may not be associated with one particular gene but a combination of genes. Five studies identified a significant association between colorectal cancer risk and genotype combinations. The potential number of combinations of the various genotypes is vast depending on the number and type of genotype(s) considered. Lack of reporting on this issue made it difficult to compare results across the various studies. Table 44 presents the significant genotype combinations that were observed for colorectal cancer risk.

Table 44: Summary of Individual Study Results Examining Significant Genotype Combinations

Study	*GSTM1*	*GSTT1*	*GSTP1*	*NAT1*	*NAT2*	OR (95% CI)
Yoshoioka et al. (1999)	present		Ile/Ile			2.15 (1.21,3.79)
Welfare et al. (1999)		null		slow		2.33 (1.10,5.0)
Welfare et al. (1999)		null			slow	2.24 (1.00,4.99)
Zhu Y et al. (2002)	null	null				4.33 (1.56,12.04)
Kiss et al. (2004)	null				fast	2.39 (1.75, 3.26)
Kiss et al. (2004)	null			fast	fast	3.28 (2.06, 5.23)
Ates NA et al. (2005)	null	present	Val			2.34 (1.15,4.77)
Ates NA et al. (2005)	null	null	Val			2.69 (1.02,7.11)

Various statistically significant joint effects were observed across studies. Due to the inconsistency in reporting it is difficult to determine whether or not the results are inconsistent across studies or the various studies did not consider the same joint effects. Further studies are required to determine whether or not the joint effects observed by these various studies are meaningful.

8.3 Meta-Analysis Results

Several studies examined the combined effects of various combinations of GST genotypes. Sufficient information was reported to conduct a subgroup meta-analysis examining the effect of *GSTM1* and *GSTT1* status and colorectal cancer risk. In total, eight studies were included in the subgroup analysis. Genotype status was categorized into three categories: *GSTM1* and *GSTT1* functional (reference group), *GSTM1* or *GSTT1* null and *GSTM1* null and *GSTT1* null.

Table 45: Meta-Analysis Results: Combined Effects of *GSTM1*, *GSTT1* and Colorectal Cancer

Author(s)	Interaction	OR	LCI	UCI
Deakin et al.	M1 or T1 Null	1.30	0.91	1.86
	Both Null	1.54	0.88	2.72
Gertig et al.	M1 or T1 Null	0.75	0.50	1.13
	Both Null	0.94	0.49	1.81
Abdel-Rahman SZ et al.	M1 or T1 Null	0.92	0.35	2.38
	Both Null	0.49	0.17	1.41
Yoshioka M et al.	M1 or T1 Null	1.20	0.64	2.27
	Both Null	1.96	0.93	4.14
Saadat I and Saadat	M1 or T1 Null	1.61	0.74	3.51
	Both Null	2.73	0.96	7.79
Seow A et al.	M1 or T1 Null	1.63	1.18	2.24
	Both Null	1.85	1.11	3.10
Nascimento H et al.	M1 or T1 Null	1.11	0.70	1.78
	Both Null	1.18	0.51	2.72
Ates NA et al.	M1 or T1 Null	1.85	1.18	2.91
	Both Null	2.53	1.27	5.05
Combined OR	**M1 or T1 Null**	**1.27**	**1.01**	**1.60**
	Both Null	**1.53**	**1.11**	**2.11**

A significantly increased risk for colorectal cancer was observed for individuals with either the *GSTM1* or *GSTT1* null genotype (OR=1.27; 95%CI: 1.01,1.60) and those with both null genotypes (OR=1.53; 95%CI: 1.11,2.11). The result suggests that individuals with both null genotypes are at a greater risk than those with only one of the null genotypes.

8.4 Pooled Analysis

Interaction among *GSTM1*, *GSTT1* and *GSTP1* was investigated for colorectal cancer and colorectal adenomas using the individual level data provided by GSEC. Multiplicative joint effects were not observed for any of the gene-gene combinations, indicating no evidence of interaction among the GST genetic polymorphisms included in the analysis.

Potential interaction between *GSTM1*, *GSTT1* or *GSTP1* and smoking was also investigated. Smoking status was categorized as 'never' or 'ever' for the analysis. No interaction between smoking status and any of the GST polymorphisms was observed for either colorectal cancer or colorectal adenomas. The information available for smoking may have contributed to the null result. In order to combine variables across all studies, detailed information collected by individual studies was lost. For instance not all studies categorized smoking status similarly. Some studies collected information on pack-years smoked, number of cigarettes smoked etc. whereas others did not. It is acknowledged that the categorization of never and ever is not optimal.

The systematic review identified several studies which investigated the joint effects of *GSTM1* and *GSTT1*. Therefore, a pooled analysis examining the combined effect of *GSTM1* and *GSTT1* null genotypes was also conducted. Sufficient data was available for 13 studies. This was done by categorizing genotype status as either: both *GSTM1* and *GSTT1* null, either *GSTM1* null or *GSTT1* null, or both *GSTM1* and *GSTT1* functional. A significant association between joint *GSTM1* and *GSTT1* status was observed. The results are presented in table 46. Individuals with either *GSTM1* or *GSTT1* null were at a significantly greater risk of colorectal cancer (OR=1.12; 95%CI: 1.03,1.23), as were individuals with both *GSTM1* and *GSTT1* null (OR=1.32; 95%CI: 1.16,1.50), than those with both functional genotypes. The adjusted odds ratio by sex, age and center resulted in very similar results.

Table 46: Pooled Analysis Results: Combined Effects of *GSTM1*, *GSTT1* and Colorectal Cancer

	Crude OR*			Adjusted OR†		
Gene Combination	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
GSTM1/GSTT1						
GSTM1 and *GSTT1* not null	Ref.			Ref.		
GSTM1 or *GSTT1* null	1.12	1.03	1.23	1.12	1.02	1.24
GSTM1 null and *GSTT1* null	1.32	1.16	1.50	1.37	1.19	1.58

* OR, odds ratio; CI, confidence interval

†OR adjusted for study, sex, and age

8.5 Results Summary

The analysis conducted did not provide evidence for a multiplicative interaction between any of the GST genotypes investigated. Evidence of a gene-environment multiplicative interaction between *GSTM1*, *GSTT1* or *GSTP1* and smoking was not supported. Non-significant results were obtained for both colorectal cancer and adenomas.

The analysis examining combined genotypes suggest that individuals who have either both the *GSTM1* or *GSTT1* null genotype or one of the two null genotypes are at greater risk of colorectal cancer than those who have both functional genes. Insufficient information was available to examine *GSTP1*.

Chapter 9: Discussion

Meta-Analysis

The results of the meta-analysis suggest an association between *GSTM1* and *GSTT1* and colorectal cancer risk. A significant association between *GSTM1* null (OR=1.12; 95%CI: 1.02 1.23) and *GSTT1* null (OR=1.27; 95%CI: 1.11,1.46) and colorectal cancer risk was observed. A significant association between colorectal cancer risk and *GSTP1 I105V* was not observed. There was insufficient data to perform a meta-analysis examining the association between *GSTP1* A114V and colorectal cancer. Significant associations between *GSTM1*, *GSTT1*, *GSTP1* and colorectal adenomas were not observed.

There is debate over whether or not the unadjusted or adjusted odds ratio should be used in the meta-analysis. The basis for using adjusted odds ratios is the notion that the adjusted odds ratio provides the most optimal risk estimate. The challenge of using adjusted odds ratios is the fact that the adjustments made to each odds ratio is not comparable across studies. The choice of variables to be collected is not only dependent on the primary objective of the study but also the subjective opinion of the study authors. As a result, the variables used to adjust the odds ratios computed and control for confounding was not standard across all studies. In addition how the variables were defined and categorized varied from study to study. Whether or not these measures should therefore be combined is questionable. In the current analysis using the computed crude odds ratio or the adjusted odds ratio when reported did not dramatically impact the summary risk estimate with the exception of *GSTM1*. Using the computed odds ratios for *GSTM1* resulted in a significant association between *GSTM1* and colorectal cancer risk (OR=1.12; 95%CI: 1.01,1.23), whereas using the adjusted odds ratio when reported resulted in a non significant overall risk estimate (OR=1.05; 95%CI: 0.95,1.14).

In order to account for variation both between and within studies, the random effects model was used for all meta-analysis results produced. The meta-regression results suggested that none of the covariates considered contributed to the heterogeneity observed. Region of study conducted, sample size, language, and sample size were considered. Summary odds ratios were not significantly different for any of these subgroups. Limitations of the meta-regression technique include the fact that group level variables are subject to ecological bias (84). The sample size of the meta-regression is also limited to the number of studies included in each category. The limited number of studies may have contributed to the null result. In addition, within study variation across a covariate may not

be captured due to the fact that summary statistics are less likely to vary than the individual values upon which they are based (85). Finally, studies did not report the same information across studies. As a result, not all potential confounders could be considered such as smoking, diet and other lifestyle factors. The stratified analysis revealed similar results thereby supporting the results of the meta-analysis.

Stratified meta-analysis was conducted for *GSTM1*, *GSTT1* and *GSTP1* I105V by geographic region, number of cases, type of control, and language (except *GSTP1* I105V*)*. In addition sufficient data was reported to conduct subgroup meta-analysis for *GSTM1* and *GSTT1* and sex, tumor site and smoking status. Tumor site was significantly associated with *GSTM1*. Individuals who were *GSTM1* null and were diagnosed with a distal tumor were at a moderately increased risk of colorectal cancer (OR=1.18; 95%CI: 1.00, 1.40) than those with a proximal tumor (OR=1.11; 95%CI: 0.72,1.71). A significant association was observed for sex and *GSTT1*. Men who were *GSTT1* null (OR=1.29; 95%CI: 1.03,1.62) were observed to be at a greater risk for colorectal cancer than women (OR=1.20; 95%CI: 0.85,1.69).

Finally the combined effect of *GSTM1* and *GSTT1* and colorectal cancer was investigated. A significantly increased risk for colorectal cancer was observed for individuals with either the *GSTM1* or *GSTT1* null genotype (OR=1.27; 95%CI: 1.01,1.60) and those with both null genotypes (OR=1.53; 95%CI: 1.11,2.11) when compared with individuals with both functional genotypes. Due to the inconsistency of findings across studies that examined the joint effect of *GSTM1* and *GSTT1* more studies are required to replicate this result before a conclusive relationship can be established. This finding highlights the importance of studying genes that may act together, particularly within a biological pathway, in cancer susceptibility.

A disadvantage of meta-analyses is that they are dependent on the information reported by the investigators. It is more than likely that ethnicity, sex, tumor location, dietary and lifestyle factors play a role in colorectal cancer susceptibility. Yet, in many studies these factors were not reported and therefore the analysis could not account for these factors. Power is also a consideration. Often studies are already underpowered in order to detect modest gene-disease associations. As a result stratifying by other variables is often not possible.

Pooled Analysis Results

The results of the pooled analysis were similar to those of the meta-analysis. A significant association between *GSTM1*, *GSTT1* and *GSTP1* polymorphisms and colorectal adenomas was not observed. In terms of colorectal cancer, a significant association between *GSTM1* null (OR=1.11; 95%CI: 1.02,1.23) and *GSTT1* null (OR=1.22; 95%CI: 1.10,1.35) was observed. A significant association between *GSTP1* I105V or *GSTP1* A114V and colorectal cancer was not observed. Table 47 summarizes the results of the meta- and pooled analysis results for *GSTM1*, *GSTT1*, *GSTP1* and colorectal cancer.

Table 47: Comparison between Meta-Analysis and Pooled Analysis Results, *GSTM1*, *GSTT1*, *GSTP1* and Colorectal Cancer

	Meta-Analysis						Pooled Analysis					
	Crude OR*			Adjusted OR†			Crude OR*			Adjusted OR†		
Genotype	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**	**OR**	**LCI**	**UCI**
GSTM1	1.12	1.01	1.23	1.05	0.95	1.14	1.18	1.08	1.28	1.11	1.02	1.23
GSTT1	1.27	1.11	1.46	1.17	1.03	1.31	1.10	1.00	1.20	1.22	1.10	1.35
GSTP1												
I105V	1.05	0.95	1.16	1.03	0.93	1.14	1.19	1.08	1.31	1.06	0.96	1.18
A114V							0.93	0.70	1.22	0.91	0.69	1.20

* OR, odds ratio; CI, confidence interval
†OR adjusted for study, sex, and age

Significant joint effects of *GSTM1* and *GSTT1* were also observed. Individuals with either the *GSTM1* null genotype or the *GSTT1* null genotype were at greater risk for colorectal cancer than those with both functional genotypes (OR=1.12; 95%CI: 1.03,1.23). The risk of colorectal cancer increased for individuals with both null genotypes (OR=1.32; 95%CI: 1.16,1.50). Similar results were obtained in the meta-analysis.

Pooled analysis of individual data is advantageous over meta-analysis for a variety of reasons. The availability of individual data allowed for the same adjustment of confounding factors over all studies included. In addition by pooling the individual data a much larger sample size is achieved. The larger sample size provides an opportunity to examine interactions that individual studies with

limited statistical power are unable to examine. In addition, pooled analysis offers the opportunity to conduct subgroup analysis. A disadvantage was that not all studies were included in the GSEC database and therefore inclusion bias is possible. In addition, publication bias is also of concern in a meta-analysis and pooled analysis. An attempt to address this issue was undertaken by examining the grey literature. In addition the statistical tests performed indicated that substantial publication bias was not present.

Gene-gene and gene-environment interactions were investigated when sufficient information was available. In terms of environmental factors, only smoking was investigated. Limited information in terms of smoking meant that less optimal categorization had to be employed. No significant multiplicative gene-gene interactions (among the various GSTs) or gene-environment interactions were detected.

To date, numerous studies have been published using pooled data. Results however remain inconclusive. The weak association between the metabolic genes polymorphisms under study and cancer risk, the small sample size of the individual studies and the ethnic heterogeneity of populations under study have been proposed as some of the reasons for this inconclusiveness (52).

Quality Assessment

As the number of studies published on genetic associations increases, the inadequacy of reporting in this area is of great concern. Methodological issues such as insufficient power, flawed designs, suboptimal conduct, biased analysis, selective reporting of significant results, lack of standardization among studies and poor reporting even among well conducted studies have all been raised as issues which need to be addressed (86). In addition gene-disease association studies are confronting new challenges which are the result of novel attributes not encountered by traditional epidemiological studies such as the unprecedented amount of data they generate and the small marginal effects observed in the study of complex diseases.

The process of assessing study quality was extremely challenging due to the inadequate reporting of several studies. Many studies did not report sufficient information in terms of recruitment and characteristics of the study participants, inclusion and exclusion criteria, participation rates, blinding, genotyping methods and quality control. Due to the inadequacy of reporting it was very difficult to

assess whether or not cases and controls were recruited from the same source population, the internal validity and generalizability of the study and potential biases that the study may have encountered. Many of these issues may contribute to the inconsistent findings encountered across studies.

Previous Evidence

Previous meta- and pooled analyses have been conducted examining the association between *GSTM1*, *GSTT1*, *GSTP1* and colorectal cancer susceptibility. Houlston and Tomlinson (2001) conducted a systematic review and meta-analysis of 13 genes which included *GSTM1* (10 studies), *GSTT1* (8 studies) and *GSTP1* (3 studies) (44). They concluded that there was no evidence to suggest an association between *GSTM1* or *GSTT1* and colorectal cancer, whereas sufficient evidence was not available to make a reliable conclusion for *GSTP1*. This study also identified three studies which reported evaluating a possible interaction between *GSTM1* and *GSTT1* (Gertig et al. 1998, Abdel-Rahman et al. 1999 and Deakin et al. 1996). No statistical interaction was observed in any of the three studies. A meta-analysis was conducted by de Jong et al. (2002) for 30 polymorphisms in 20 different genes that had been reported in more than one colorectal adenoma or cancer study (42). In terms of the GSTs studied, an increased risk of colorectal cancer risk was observed for *GSTT1* (11 studies) (OR=1.37; 95%CI: 1.17,1.60) but not for *GSTM1* (20 studies) or *GSTP1* (5 studies). Significant associations were not observed for colorectal adenomas. Smits et al. (2003) evaluated the associations and interaction between *GSTM1* null, smoking behaviour and the development of colorectal cancer by conducting a meta- and pooled analysis (45). The pooled analysis included six studies from GSEC whereas the meta-analysis identified an additional three studies. Overall no significant association between *GSTM1* null and colorectal cancer was observed (OR=0.92; 95%CI: 0.73,1.14). In addition the findings did not find any modification of smoking and colorectal cancer by *GSTM1* genotype. The results of the Smits et al. (2003) study are not directly comparable to the current study. The focus of the study was on smoking and therefore only studies that had sufficient information on smoking were retained for the analysis. In addition they were able to obtain unpublished information that was not included in the current study. Ye and Parry (2003) conducted a meta-analysis of 18 case-control studies examining the association between *GSTM1* and colorectal cancer risk (40). A significant association was not obtained. The authors highlighted the potential importance of gene-gene interactions and identified eight studies which examined the association between multiple putative high risk genotypes and colorectal cancer risk; five of which demonstrated a significant increased risk (Abdel-Rahman et al. 1999, Welfare et al. 1999, Kiss et al. 2000, Saadat

and Saadat 2001 and Loktionov et al. 2001). The current meta-analysis included all of the studies identified in the Ye and Parry study with the exception of a few studies. The Strange et al. (1991) study was excluded due to the fact that phenotype was used instead of genotype. The Guo et al. (1996) study was excluded due to the fact that it was impossible to separate out the adenoma cases from the colorectal cancer cases. Finally the study by Moisio et al. (1998) was excluded due to the fact that their cases were individuals with HNPCC. As a result, the study population of Ye and Parry is more heterogeneous than the current study and may account for the difference in results obtained. Cotton et al. conducted a HuGE review of *GSTM1* and *GSTT1* and colorectal cancer which was published in 2000. They reported that no consistent associations between *GSTM1* and *GSTT1* genotypes and colorectal cancer have been observed. Cotton et al. also highlighted the fact that many of the studies suffered from methodological limitations which may account for the discrepancy in results across studies. This current study builds on this previously published review by extending the study to include studies published after 1998, including the genotype *GSTP1* and the inclusion of a meta- and pooled analysis. It is therefore the most current and inclusive study to date.

In terms of joint effects of genes, this is the first known study to conduct a meta- and pooled analysis examining the joint effects of *GSTM1* and *GSTT1*. A significantly increased risk for colorectal cancer was observed for individuals with either the *GSTM1* or *GSTT1* null genotype and those with both null genotypes for both the meta- and pooled analysis. The result suggests that individuals with both null genotypes are at a greater risk than those with only one of the null genotypes. This result highlights the potential of considering multigenic models for cancer susceptibility.

Overall Summary

The current study offers an up-to-date and comprehensive examination of the evidence available concerning the association between *GSTM1*, *GSTT1*, *GSTP1* and colorectal cancer. Overall, both *GSTM1* and *GSTT1* appear to be modestly associated with colorectal cancer risk. In addition there may be a joint effect of *GSTM1* and *GSTT1* and colorectal cancer risk. There appears to be no association between the GSTs investigated and colorectal adenomas. The issue of disease development and genetic susceptibility is challenging. It is unlikely that environmental or genetic factors act alone in disease causation and susceptibility. It is therefore important that these risk factors be studied not only as independent risk factors but as modifiers of each other. To date, many of the studies conducted have had limited statistical power to detect interactions. In addition the methods used and genes considered in the studies conducted have differed making it difficult to combine the evidence across studies. As a result, greater efforts to study gene-environment and gene-gene interactions should be made. Efforts are continually being made by GSEC to expand the database. Increase in subject numbers, addition of new genes being investigated and the inclusion and collection of additional environmental exposures will potentially provide new opportunities to study the complex interaction between various genes and environmental factors and genes.

List of Acronyms

CYP: Cytochrome P

FAP: Familial adenomatous polyposis

HNPCC: Hereditary non-polyposis colorectal cancer

HWE: Hardy-Weinberg equilibrium

GSEC: International collaborative study on genetic susceptibility to environmental carcinogens

GST: Glutathione S- transferase

NOPHG: National office of public health genomics

HuGE: Human Genome Epidemiology

PAH: Polycyclic aromatic hydrocarbons

Appendix A: Search Strategy

1 exp colonic neoplasms/
2 exp colorectal neoplasms/
3 exp rectal neoplasms/
4 exp intestinal neoplasms/
5 exp adenomatous polyps/
6 exp colonic polyps/
7 exp intestinal polyps/
8 1 or 2 or 3 or 4 or 5 or 6 or 7
9 cancer$.tw.
10 neoplas$.tw.
11 malignan$.tw.
12 tumour$.tw.
13 tumor$.tw.
14 carcinoma$.tw.
15 adenocarcinom$.tw.
16 9 or 10 or 11 or 12 or 13 or 14 or 15
17 colon$.tw.
18 colorect$.tw.
19 colo-rect$.tw.
20 rectum$.tw.
21 rectal$.tw.
22 bowel$.tw.
23 caecum$.tw.
24 cecum$.tw.
25 rectosigmoid$.tw.
26 recto-sigmoid$.tw.
27 splenic flexure$.tw.
28 hepatic flexure$.tw.
29 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28
30 or (16 and 29)
31 polymorphism, restriction fragment length/
32 polymorphism, single-stranded conformational/
33 "polymorphism (genetics)"/
34 mutation/
35 point mutation/
36 exp genotype/
37 31 or 32 or 33 or 34 or 35 or 36
38 polymorph$.tw.
39 mutation$.tw.
40 genotyp$.tw.
41 38 or 39 or 40
42 glutathione transferase/
43 gstm$.tw.
44 gstp$.tw.
45 gstt$.tw.
46 glutathione transferase$.tw.
47 43 or 44 or 45 or 46
48 37 and 42
49 37 and 47
50 41 and 42
51 41 and 47
52 48 or 49 or 50 or 51
53 30 and 52

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