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Phase I and II enzyme polymorphisms as risk factors for Barrett's esophagus and esophageal adenocarcinoma: a systematic review and meta-analysis

L. M. Bull, D. L. White, M. Bray, Z. Nurgalieva, and H. B. El-Serag

Baylor College of Medicine, Houston, Texas, USA

SUMMARY

Although several studies have examined the association between phase I/II enzyme polymorphisms and esophageal adenocarcinoma (EAC) and/or Barrett's esophagus (BE), their overall findings remain unclear. We performed a systematic review and meta-analysis to determine whether phase I/II polymorphisms are independent risk factors for either BE or EAC. We employed keyword searches in multiple databases to identify studies published before October 1, 2007. Single-nucleotide polymorphisms (SNPs) examined in 3 studies were meta-analyzed to obtain a pooled estimate of effect. Meta-analysis suggested the minor allele for *GSTP1 Val¹⁰⁵* conveys modest excess risk (odds ratio [OR]_{BE} = 1.50, 95% confidence interval [CI] 1.16–1.95; OR_{EAC} = 1.20, 95% CI 0.94–1.54). No excess risk was observed with *GSTM1 null* (OR_{BE} = 0.77, 95% CI: 0.56–1.08; OR_{EAC} = 1.08, 95% CI: 0.79–1.48), *GSTT1 null* (OR_{BE} = 1.35, 95% CI: 0.91–2.01; OR_{EAC} = 0.84, 95% CI: 0.48–1.49), or *CYP1A Val⁴⁶²* (OR_{EAC} = 0.89, 95% CI: 0.40–1.97). Insufficient data existed to meta-analyze remaining SNPs. Our review identified *GSTP1 Ile105Val* as a possible risk factor for BE and EAC in Caucasian males. No excess risk was observed for other phase I/II polymorphisms with sufficient data to meta-analyze. Additional studies are needed to determine if GSTP1 conveys excess risk in females or non-Caucasians and to evaluate other phase I/II polymorphisms.

Keywords

Barrett's esophagus; epidemiology; esophageal adenocarcinoma; genetic association; GST; single-nucleotide polymorphism (SNP)

INTRODUCTION

The incidence of esophageal adenocarcinoma (EAC) has been dramatically increasing over the last 30 years in several regions in North America, Europe, and Oceania.¹ While incidence of EAC is increasing in both genders and all ethnic groups, it is most pronounced in Caucasian males. Specifically, EAC incidence has increased 350% since the 1970s in Caucasian men in the USA, which currently results in approximately 10 000 deaths per

year.² Although increased screening practices, improved diagnostic techniques, and changes in classification likely explain some of the observed increase, the ongoing annual increases of approximately 10% suggest a real increase in the incidence of EAC.^{1,3}

It is believed that EAC arises from Barrett's esophagus (BE), an abnormal growth of columnar cells from normal esophageal interstitial tissue. BE has been reported to as much as 6–12% in patients screened for reflux disease, and in 10–25% asymptomatic patients undergoing endoscopy for other reasons.^{4,5} Patients with BE have a 50- to 100-fold increased risk of developing EAC,⁶ although the annual incidence of cancer in BE cases is thought to be between 0.4% and 0.5%. Main risk factors for BE and EAC include chronic reflux symptoms, high body mass index (BMI), and possibly tobacco smoking, alcohol use, and low dietary intake of fruit and vegetables. Together, these risk factors suggest a strong environmental component in the development of BE and EAC.

However, due to the dramatic rise in EAC in men of a single racial group even across multiple continents and cultures, there is also strong implication that there are genetic determinants involved in disease etiology and progression, and, more specifically, that the likely genetic pathways may be associated with metabolism of environmental exposures.

The primary metabolic defense against tissue damage from xenobiotic chemicals like tobacco and alcohol is mediated through phase I and II enzymes. Phase I enzymes, including cytochrome P450 (CYP) and microsomal epoxide hydrolases (mEH), are found throughout the body, but are primarily expressed in the gastrointestinal tract, liver, kidneys, and lungs. The phase II conjugating enzymes, including quinone oxidoreductase (NQO), N-acetyltransferases (NAT), and glutathione S-transferases (GST), are found ubiquitously and mediate reactions that can take place simultaneously or sequentially to the phase I reactions.

Several genetic association studies have evaluated the distribution of phase I and II metabolic genes in EAC or BE cases. However, it is difficult to obtain a collective view of these studies due to the lack of critical systematic reviews dedicated to this topic. Current reviews have summarized genetic associations with esophageal cancer irrespective of subtype (i.e., adeno vs. squamous).^{7–9} However, given the considerable differences in the distribution, temporal trends, and risk factors of these two types of esophageal cancer, it is important to focus the review specifically on EAC and its precursor, BE.

We therefore conducted a systematic review to identify studies examining phase I and phase II genetic polymorphisms as risk factors for BE or EAC. When sufficient comparable studies existed, we also performed a meta-analysis to obtain a precise estimate of overall effect and to identify potential sources of between-study heterogeneity.

METHODS

Search strategy

We followed recommended guidelines for performance and reporting of meta-analyses.¹⁰ Two investigators conducted independent searches of PubMed, Ovid, EBSCO, Web of

Science, OMIM, Dissertation Abstracts Internet, and conference abstracts for the American Society for Clinical Oncology.

To decrease the potential impact of publication bias, we did not restrict the search by language, population, or sample size. We identified all studies published prior to October 2007 using key words searches with combinations of the following terms: 'esophageal adenocarcinoma,' 'Barrett's esophagus,' 'glutathionine S-transferase,' 'GSTT1,' 'GSTP1,' 'GSTM1,' 'cytochrome P450,' 'microsomal epoxide hydrolase,' 'oxidoreductase,' and 'polymorphism.' We also reviewed bibliographies of all identified articles, as well as relevant review articles to capture studies not identified by our keyword searches.

Eligibility criteria

We included only studies in which (i) cases were clearly defined as having either BE or EAC; and (ii) there was a comparable EAC- or BE-free control group. Studies that also included cases with squamous cell carcinoma or gastric cardia were included only if the sample size and risk estimates for EAC were clearly separated from the other cancers. We excluded studies that examined somatic mutations or examined or used animal models or human cell lines.

Our searches identified 134 citations, of which 57 were reviewed in more detail. Only 11 original reports^{11–22} and two review papers^{8,9} met our eligibility criteria. Seven evaluated cases with EAC, three evaluated both BE and EAC cases, and one evaluated just BE cases in comparison with a disease-free control group. We excluded most publications because they used tumor or cell line DNA, 10 publications because they only reported combined findings for squamous cell carcinoma and EAC,^{23–32} and 1 because it included another cancer without separate reporting of EAC,³³ and 1 publication lacked sufficient detailed data.³⁴

Data abstraction

Data on study methods, including case and control identification, as well as all associated study findings, were abstracted and entered into a structured database. When insufficient data were reported, we used data from any earlier reports or contacted the corresponding author.

Analysis

We decided a priori to conduct a meta-analysis for any single-nucleotide polymorphism (SNP) evaluated in three or more comparable studies. To maintain independence among pooled studies, we included the most recent study when there was more than one publication based on the same dataset.

We evaluated between-study heterogeneity by using Higgins and Thompson's I^2 , which is a scale-free measure of variability that calculates the proportion of total between-study variation resulting from heterogeneity as opposed to random error or chance.³⁵ The decision to perform a random or fixed-effects meta-analysis was based on this heterogeneity test result and the number of studies available. If there was considerable between-study heterogeneity (defined as $I^2 = 50\%$), we employed a random effects analysis to calculate

the pooled estimator.³⁶ While if there was less than five studies available, we employed the Mantel–Haenszel method to compensate for the poor estimate of the χ^2 distributions when the study number is small. We presented odds ratios (ORs) and 95% confidence intervals (CIs) for each study in a forest plot that included the pooled estimator. We used a dominant (AA vs. Aa + aa) for the meta-analysis of CYP1A and GSTP1 genes based on the information given in the studies and the probable biological model of the gene effect in the literature.^{37–40} For the GSTM1 and GSTT1 genes, we compared cases against controls for the contrast of the null vs. the nondeleted genotype. We assessed deviations from Hardy–Weinberg equilibrium (HWE) for control groups using a χ^2 goodness-of-fit test. Publication bias was assessed by evaluation of funnel plots and Egger’s test. We also performed an analysis of influence to determine the importance of any one study on the pooled estimator. Finally, we investigated how the pooled estimator changed over time by performing a cumulative meta-analysis. All analyses were conducted with Stata, version 9.0 (Stata Corp, College Station, TX, USA).

RESULTS

All 12 original studies we identified were case-control studies, and most ($n = 7$) were conducted in mostly male, exclusively Caucasian populations from Europe or North America.^{11–14,18–22} The most common SNPs studied were in the glutathione S-transferase genes – GSTP1 in eight studies followed by GSTM1 and GSTT1 in six studies. Other SNPs examined were in the following genes: cytochrome P450 1A (CYP1A1) ($n = 3$ studies), microsomal epoxide hydrolase (EPHX1) ($n = 2$ studies), quinine oxidoreductase (NQO1) ($n = 3$ studies), and N-acetyltransferase I (NAT1) ($n = 1$ study). Characteristics of studies evaluating EAC and BE are presented in Tables 1 and 2, respectively.

Phase I enzymes

The initial metabolic defense against xenobiotic chemicals such as tobacco or other potential carcinogens is mediated through conversion of hydrophobic compounds to reactive electrophiles by phase I enzymes.

CYP 1A—CYP1A enzymes metabolize aromatic hydrocarbons found in environmental pollutants like cigarette smoke. Although expressed in most extrahepatic tissues, expression is greatest in the lung and placenta.⁴¹

Gene: The CYP1A1 gene is found on chromosome 15 in the q22–q24 region and encompasses seven exons and six introns that span 5810 bp. Although several polymorphisms have been identified in the CYP1A1 gene, the two main polymorphisms both result in increased enzymatic expression, leading individuals to be classified as extensive metabolizers. *CYP1A1 Ile462Val* involves a $A \rightarrow G$ substitution in exon 7 and leads to a twofold increase in enzymatic expression.³³ *CYP1A1 MspI* is in strict linkage disequilibrium with *CYP1A1 Ile462Val* and involves a $T \rightarrow C$ (*T6235C*) substitution in the 3′ flanking region.^{42–44} Both polymorphisms are relatively infrequent in Caucasian populations. The frequency of the rare homozygous genotype for *CYP1A1 Ile462Val* (Val^{462}/Val^{462}) is 0.6% in Caucasians and 4.9% in Asians,⁴⁵ while the frequency of the rare

homozygous genotype for *CYP1A1 MspI* (C^{6235}/C^{6235}) is 1.2% in Caucasians and 14% in Asians.⁴⁵

Studies: We identified three studies that examined *CYP1A1 Ile462Val* and two that examined *CYP1A1 MspI* as risk factors for EAC^{11,12,20,22} (see Fig. 1). One study also examined these polymorphisms as risk factors for BE²⁰ (see Fig. 2). Findings were highly variable for both polymorphisms and across both conditions, and only one study reported a significant association.²⁰

Meta-analysis: Sufficient comparable studies existed to perform a meta-analysis examining *CYP1A1 Ile462Val* in 103 EAC cases and 503 controls pooled from three studies.^{11,20,22} The individual study ORs ranged from 0.65 to 1.60. We independently confirmed that controls in HWE in all studies. There was no detectable heterogeneity ($I^2 = 0\%$), so we adopted a fixed-effects model. We assumed a dominant model of inheritance in the meta-analysis because there were insufficient reported cases of minor allele homozygote (Val^{462}/Val^{462}) in the three studies. The minor allele for *CYP1A1* was not a significant risk factor for EAC (OR = 0.89, 95% CI 0.40–1.97) (Fig. 3). Our analysis of influence showed that removal of any of the studies affected the pooled estimator, as was expected by the small number of studies available for the analysis. Finally, our cumulative meta-analysis by year of publication showed no trend in risk over time (data not shown).

mEH—mEH) encoded by the *EPHX1* gene, hydrolyzes polycyclic aromatic hydrocarbons found in cigarette smoke, but may also subsequently produce highly toxic and mutagenic transdihydrodiols by-products. The *EPHX1* gene expression is greatest in the kidney, liver, lung, and epithelial cells.^{46–48}

Gene: The *EPHX1* gene is found on chromosome 1 at position q42.1 and encompasses nine exons and eight introns that span 35.48 kb. Two SNPs known to affect enzymatic activity are *EPHX1 Tyr113His* (*EPHX1 exon3*) and *EPHX1 His139Arg* (*EPHX1 exon4*). While *EPHX1 exon3* conveys a 40–50% reduction in enzymatic activity, *EPHX1 exon4* conveys a 25% increase in enzymatic activity.⁴⁹ Both SNPs have been associated with oropharyngeal carcinoma and esophageal squamous cell carcinoma.^{50,51} *EPHX1* polymorphisms have also been associated with lung,⁵² colorectal,⁵³ and liver cancer.⁵⁴ The reported minor allele frequency (MAF) for *EPHX1 exon3* is 34% in Caucasians and 51% in Asians. The reported MAFs for *EPHX1 exon4* are lower – 19% in Caucasians and 14% in Asians.⁵⁵

Studies: We identified two studies examining these SNPs^{12,13} (see Fig. 1). Casson (2006)¹³ included cases with BE as well as EAC, while Casson (2003)¹² only included cases with EAC. While carriage of the minor allele for *EPHX1 exon3* was associated with moderate increased risk of EAC, carriage of the minor allele for *EPHX1 exon4* was associated with moderate decreased risk. However, neither association was significant.

Phase II enzymes

Phase II enzymes increase the hydrophilicity of potentially toxic compounds so they can be excreted in urine or bile. Phase II enzymes mediate reactions that can take place simultaneously or sequentially to the phase I reactions.

NAD(P)H: quinone oxidoreductase 1 (NQO1)—NQO1 detoxifies quinones derived from the oxidation of benzene. It also protects cells from free radicals and reactive oxygen species. It is expressed in all human tissues⁵⁶ in response to oxidative stress and has been shown to be overexpressed in breast,⁵⁷ pancreatic,⁵⁸ colorectal,⁵⁹ and esophageal squamous cell cancers.⁶⁰

Gene: The NQO1 gene is located on chromosome 16 in the q22.1 region, spanning 35 kb and consisting of six exons and five introns. A *-C609T* substitution leads to a proline to a serine amino acid substitution. Rare homozygotes for *NQO1*_{C609T} (*Ser*⁻⁶⁰⁹/*Ser*⁻⁶⁰⁹) have only 2–4% of the enzymatic activity observed in the wild type.⁶¹ The frequency for the minor allele among Caucasians ranges from 12 to 23 %, while the frequency among Asians is more than twice as great and ranges from 35 to 61%.^{62,63}

Studies: We identified three case-control studies that examined *NQO1 Ser*⁻⁶⁰⁹ in EAC^{14,19,21} (Fig. 1), and one that also examined it in BE¹⁴ (Fig. 3). Although two of the EAC studies appear to be performed with the same case population,^{19,21} the von Rahden *et al.* study expanded upon the original study of Sarbia *et al.*, including doubling in size the original case group. Given the correlation in these findings due to the partially shared case group, we had an insufficient number of comparable studies to perform a meta-analysis. The di Martino study was the only study to evaluate *NQO1 Ser*⁻⁶⁰⁹ as a risk factor for BE. Similar to their results for EAC, a strong decreased risk of BE was observed with *NQO1 Ser*⁻⁶⁰⁹ minor allele (OR = 0.22, 95% CI 0.07–0.76, *P* = 0.01).

NAT1—Arylamine NATs catalyze the detoxification of xenobiotics by N-acetylation. Slow acetylation has been associated with bladder cancer, while fast acetylation has been associated with colon cancer.⁶⁴ NAT1 variants have also been associated with lung and breast cancer.⁶⁵

Gene: The NAT1 locus is found on chromosome 8p21.3–23⁶⁶ and spans a 870 bp open reading frame.⁶⁷ Combinations of nucleotide insertions/deletions and substitutions result in more than 24 different allelic variants in humans. *NAT1*3* (*C1095A*), *NAT1*4* (wild-type referent), *NAT1*10* (*T1088A*, *C1095A*), and *NAT1*11* (*Val149Ile*, *Ser214Ala*) are the most commonly examined polymorphisms in the literature. Because of the variant polyadenylation signal, *NAT1*10* is associated with increased *N*-acetyltransferase activity in the colon, liver, and bladder.^{68,69} The frequency of *NAT1* wild-type referent (*NAT1*4*) is reported to range from 44 to 62% in Caucasians and 38% in Asians, while the frequency for the *NAT1*10* is approximately 16–20% in Caucasians^{68,70} and 30–53% in Asians.^{71,72}

Studies: We identified only one study that examined *NAT1* variants, which only examined cases with EAC (Fig. 1).²² There was no significant association between the *NAT1**10 alleles and EAC.

GST—The most frequently studied SNPs in EAC research involve genes of the GST family. GSTs are expressed ubiquitously but are especially prevalent in the liver, lung, and upper gastrointestinal tract. There are six gene families identified for GST, among them theta (*GSTT1*), mu (*GSTM1* and *GSTM3*), and pi (*GSTP1*).^{73,74} GSTs have broad and overlapping substrates and have been associated with bladder, head and neck, and lung cancer.^{75,76}

Gene: The *GSTP1* gene is found on chromosome 11 at the p13 region. There are two variants of the *GSTP1* gene, *Ile105Val* and *Ala114Val*, and both produce decreased levels of enzymatic activity.^{37,77,78} Rare homozygotes for *GSTP1*_{*Ile105Val*} (*Ile*¹⁰⁵/*Ile*¹⁰⁵) increased IgE and resulted in an immune-mediated inflammatory response, a potentially important process in EAC pathogenesis.⁷⁹

The *GSTT1* gene is found on chromosome 22 in the q11.2 region, while the *GSTM1* and *GSTM3* genes are found on chromosome 1 in the p13.3 region. *GSTT1* and *GSTM1* both have homozygous deletion polymorphisms (*GSTT1 null* and *GSTM1 null*) that result in loss of enzymatic activity. *GSTM3*, which is in tight disequilibrium with *GSTM1*, has a 3 bp deletion mutation on intron 6 that similarly results in a loss of function. *GSTM3**A is recognized as the wild type, and *GSTM3**B is the less common deletion polymorphism.

The minor allele frequency (MAF) for *GSTP1*_{*Ile105Val*} is 31% in Caucasians and 17% in Asians. Frequency of the *GSTM1 null* genotype ranges from 42% to 57% in Caucasians and from 42% to 54% in Asians,^{45,80} while the frequency of the *GSTT1 null* genotype ranges from 13 to 26% in Caucasians and from 35 to 53% in Asians. *GSTM3**B, the minor allele for *GSTM3*, is rare and occurs in only 16% of Caucasians.^{50,81}

Studies: Our review identified seven studies that evaluated the association between EAC and GST polymorphisms, including seven that evaluated *GSTP1*_{*Ile105Val*},^{11–13,15,18,20,22} six that also evaluated *GSTM1* and *GSTT1 null*,^{11–13,15,20,22} and one that also examined *GSTM3* (Table 1).¹⁶ Controls were tested and were within HWE in all of the studies with the exception of Abbas *et al.*¹¹ and Casson *et al.* (2003),¹² which reported HWE among the controls in the manuscript text.

Sufficient comparable studies ($n = 3$) existed to perform meta-analyses examining the association between *GSTP1*, *GSTM1*, and *GSTT1* in BE and EAC, the results of which are described in the meta-analysis section below. The single study¹⁶ that evaluated *GSTM3* found a strong and significant excess risk of EAC with the minor allele (*GSTM3**B) (OR = 2.2; 95% CI 1.4–3.3) (Fig. 1).

Meta-analysis

GSTP1

EAC: There were a total of 432 genotyped EAC cases and 1086 controls pooled from seven studies.^{11–13,15,18,20,22} The individual study ORs ranged from 0.89 to 4.62 (Fig. 4). We used a dominant model in the meta-analysis combining *Ile*¹⁰⁵/*Val*¹⁰⁵ and *Val*¹⁰⁵/*Val*¹⁰⁵ genotypes. The pooled estimator suggested that carriage of the minor or ‘high-risk’ allele for *GSTP1* results in modest excess risk of EAC ($OR_{EAC} = 1.20$, 95% CI 0.94–1.54). While Egger’s test indicated a potential small study or publication bias ($P = 0.06$), our evaluation of the funnel plot (Appendix I) suggested that much of this apparent effect may be attributable to a single study.²⁰ Further, our analysis of influence indicated that the pooled estimator was robust and not substantially affected by removal of the smallest (Casson 2003)¹² or largest (Murphy 2007)¹⁸ studies (data not shown). Finally, our cumulative meta-analysis by year of publication suggested a potential diminution in risk over time. However, this trend was not monotonic (data not shown).

BE: A total of 434 cases and 738 controls were available to pool from four studies.^{13,17,18,20} ORs of the individual studies varied from 0.84 to 3.86, the majority of which indicated excessive risk for EAC. The pooled estimator suggested carriage of the minor allele for *GSTP1* (i.e. *Ile*¹⁰⁵/*Val*¹⁰⁵ and *Val*¹⁰⁵/*Val*¹⁰⁵) results in moderate excess risk of BE ($OR_{BE} = 1.50$, 95% CI 1.16–1.95) (Fig. 5). We did not detect any evidence of publication bias ($P = 0.54$) (Appendix II), and our cumulative meta-analysis by year of publication did not suggest a trend in the effect reporting over time. However, our analysis of influence indicated that the pooled estimator was vulnerable to removal of the largest study (Murphy 2007),¹⁸ which is not unexpected with only four studies included in the analysis (data not shown).

GSTM1 null

EAC: There were a total of 225 genotyped EAC cases and 863 controls pooled from six studies.^{11–13,15,20,22} The individual study ORs ranged from 0.90 to 2.10. Given between-study heterogeneity <30%, we employed a fixed-effects model. The pooled OR suggested neither strong nor significant risk of EAC with *GSTM1 null* genotype ($OR = 1.08$, 95% CI 0.79–1.48) (Fig. 6). While Egger’s test ($P = 0.04$) was significant, our evaluation of the funnel plot (Appendix III) indicated little evidence of small study or publication bias, suggesting the one outlier study¹⁵ may be influencing the result. Additionally, our analysis of influence again indicated that the pooled estimator was robust to removal of the smallest (Casson 2003)¹² or largest (Widerff 2007)²² study. Finally, our cumulative meta-analysis by year of publication did not show any trend in the data over time (data not shown).

BE: Three studies also examined the *GSTM1 null* variants in BE cases, which included 245 BE cases compared with 515 controls.^{13,17,20} All three studies showed that *GSTM1 null* variant was protective for BE, although not significantly so, and individual ORs ranged between 0.51 and 0.97. The pooled OR indicated the effect was also moderately protective ($OR = 0.77$ 95% CI 0.56–1.08) (Fig. 7). We did not detect any evidence of publication bias ($P = 0.6$) (Appendix IV), and our cumulative meta-analysis by year of publication did not suggest a trend in the effect reporting over time. However, because there were only three

studies included in the analysis of influence, we found that the pooled estimator was vulnerable to removal of the largest study (van Lieshout 1999)²⁰ (data not shown).

GSTT1 null

EAC: There was a total of 225 genotyped EAC cases and 863 controls from six studies.^{11–13,15,20,22} The individual study ORs ranged from 0.59 to 1.78. Given between-study heterogeneity ($I^2 = 38\%$), we employed a fixed-effects model. The pooled estimator suggested that the *GSTT1* null genotype conveys neither strong nor significant risk of EAC (OR = 0.84, 95% CI 0.48–1.49) (Fig. 8). Both Egger's test ($P = 0.26$) and our evaluation of the funnel plot (Appendix V) indicated that there was no substantial small study or publication bias. Our analysis of influence again suggested that the pooled estimator was robust to removal of the smallest¹³ or largest²² study, and our cumulative meta-analysis by year of publication did not show any trend in the data over time.

BE: All three studies with BE cases in the *GSTM1* null meta-analysis were included in the *GSTT1* null meta-analysis, so a total of 245 cases and 515 controls were pooled.^{13,17,20} The individual study ORs ranged from 1.31 to 1.50, and they all trended toward excessive risk for BE (Fig. 9). The *GSTT1* null genotype conveys some excess risk of BE (OR = 1.35, 95% CI 0.91–2.01). Although there were only three studies included in the analysis, we found no evidence of publication bias either from the funnel plot or by Egger's test statistic ($P = 0.2$) (Appendix VI), and our cumulative meta-analysis showed no trend in the reported study affect over time. Our analysis of influence again suggested that the pooled estimator was significantly affected by the removal of any of the studies.

DISCUSSION

This is the first meta-analysis and systematic review to examine phase I and II metabolic enzymes in EAC and its precursor condition BE, evaluating a total of 786 cases with EAC and 509 BE cases in 12 studies. Four phase I and six phase II genes were examined. Eight SNPs were examined in BE cases and ten in EAC cases. While other review studies have examined genetic variants in esophageal squamous cell carcinoma or in unspecified esophageal cancer, little work has focused in EAC or BE.

The most frequently examined SNPs were phase II GST genes. We found that carriage of *GSTP1* Val¹⁰⁵ allele was associated with a modest increase in the risk of having BE (OR_{BE} = 1.50, 95% CI 1.16–1.95), and was also a suggestive factor in the risk of EAC (OR_{EAC} = 1.20, 95% CI 0.94–1.54). The difference in magnitude between the effect sizes found in the BE and EAC suggests GST-pi may play a greater role in the initial etiology of the precursor condition than in the transition of cells to cancer. However, increased levels of GST-pi expression have been found in malignant esophagus and gastric biopsies,⁸² indicating strong biological plausibility for its association with the development of EAC, and, therefore, survival bias in EAC studies is a possible explanation of the nonsignificant findings. GST-pi is the main GST enzyme expressed in the esophagus, and the *GSTP1* Val¹⁰⁵ has been associated with increase risk of reflux esophagitis⁸³ and esophageal squamous cell carcinoma.^{84,85} While the mechanism by which an amino acid change from isoleucine to valine in the *GSTP1* gene affects EAC or BE susceptibility is unknown, the resulting

conformational change may affect the enzyme-binding site, thus changing substrate stability.⁸⁶ Pandya *et al.* showed that the amino acid switch in the hetero and homozygotes reduce the enzymes catalytic activity more than 85% toward some alkylating agents.⁸⁷ This decreased catalytic activity could result in increased risk of cancer. The second most frequently examined SNPs were the deletion polymorphisms *GSTM1 null* and *GSTT1 null*. We found that neither variant showed a strong or significant effect for EAC ($OR_{GSTM1\ null} = 1.08, 95\% CI\ 0.79-1.48$; $OR_{GSTT1\ null} = 0.84, 95\% CI\ 0.48-1.49$) or BE ($OR_{GSTM1\ null} = 0.77, 95\% CI\ 0.56-1.08$; $OR_{GSTT1\ null} = 1.35, 95\% CI\ 0.91-2.01$). The *GSTM3*B* SNP was also evaluated as a risk allele in EAC cases. *GSTM3* gene is in tight disequilibrium with the *GSTM1*, and the *GSTM3*B* variant has been suggested to modify the *GSTM1* enzyme expression, thereby effecting susceptibility to some cancers like basal cell carcinoma.⁸⁸ Jain *et al.* found strong significant excess risk with the *GSTM3*B* allele in one study that evaluated EAC cases in Northern India ($OR = 2.2; 95\% CI\ 1.4-3.3$). However, further evaluation examining the *GSTM1* *GSTM3* haplotypes is needed to determine importance of this variant.

Sufficient studies also existed to meta-analyze one of the gene variants in the *CYP1A* in EAC cases. We found that the *CYP1A1 Val⁴⁶²* allele effect was neither strong nor significant for EAC ($OR = 0.89, 95\% CI\ 0.40-1.97$) or BE. However, several systematic reviews found the *CYP1A1 Ile⁴⁶²/Val⁴⁶²* to be a significant risk factor for the development of cancer. Hiyama *et al.* suggested a possible relationship between *CYP1A1 Ile⁴⁶²/Val⁴⁶²* and esophageal squamous cell carcinoma, citing that seven studies showed an $OR > 1.0$.⁸ Similarly, Yang *et al.* also found that patients with *CYP1A1 Ile⁴⁶²/Val⁴⁶²* substitution were at increased risk for unspecified esophageal cancer ($OR = 2.52$ for *Ile⁴⁶²/Val⁴⁶²* heterozygotes).⁹ This is again important because it emphasizes the need to differentiate between the cancer cell types.

Other phase I and phase II enzymes investigated in the literature, *CYP1A1 MspI*, *EPHX1 His113Tyr (EH3)* or *EPHX1 Arg139His (EH4)*, and *NAT1*10*, *NAT1*11* alleles, were not available in sufficient numbers to meta-analyze, being only identified in one or two studies. Results were generally nonsignificant and variable both within and across conditions, thereby making interpretation of the results more difficult. For example, we found the studies that examined the *NQO1 C609T* SNPs had highly disparate results. Where one study found the minor *Ser⁻⁶⁰⁹* allele to increase risk for EAC,¹⁹ another later study found the allele had no effect,²¹ and a third study found that the effect of the minor allele was protective.¹⁴ These differing results may be a result of the study limitations or a result of the Proteus phenomenon, coined by Ioannidis,⁸⁹ where early studies produce extreme contradictory results. Further validation research is needed to parse out whether the discrepancy is due to chance, analytical manipulation, or other study restrictions.

Meta-analyses and reviews are affected by the quality, availability, and comparability of studies. The studies included in our review were highly comparable because they were all case-control study designs and largely included the same target population. Therefore, population admixture was not an important factor in these studies.⁹⁰ The quality of the studies can in part be measured by calculating whether the alleles are within HWE. We independently confirmed HWE for each SNP, with the exception of controls in the Abbas¹¹

and Casson (2003)¹² studies, which did not provide enough data to confirm equilibrium compliance; however, HWE was reported in the manuscript. The low number and small sample size of available studies investigating either EAC or BE has affected the precision of the risk estimates even when meta-analysis was possible. Sample sizes within our review varied, with 400 enrolled in the largest study, and 90 enrolled in the smallest study. However, the sample size needed to adequately detect an allelic OR of less than 1.5 in a common variant is over a thousand.^{91,92} Future validation research might select cases with early onset of disease or cases with family history to select those with a strong genetic predisposition.

Our meta-analysis identified *GSTP1 Val¹⁰⁵* as a possible determinant of increased BE risk and may also effect the risk of EAC. The decreased level of enzymatic activity resulting from the *Val¹⁰⁵* may predispose individuals to BE; however, it is still unclear if the decreased activity also affects disease progression to EAC. Larger genetic association studies for BE and for EAC separate from esophageal squamous cell carcinoma are necessary to further examine possible genetic determinants. Future research could also involve more diverse populations, such as women and different racial group, and include more or newly identified variants in the phase I and phase II metabolic pathways.

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APPENDIX I

Begg's funnel plot of log odds ratios and pseudo 95% confidence intervals for studies evaluating associations between *GSTP1Ile105Val* single-nucleotide polymorphism and esophageal adenocarcinoma. Circle size is proportional to the study weight. Dominant model used, combining heterozygote genotype with homozygote minor allele genotype.

APPENDIX II

Begg's funnel plot of unadjusted odds ratios and corresponding 95% confidence intervals for studies evaluating associations between *GSTP1Ile105Val* SNP and Barrett's esophagus. Circle size is proportional to the study weight. Dominant model used, combining heterozygote genotype with homozygote minor allele genotype.

APPENDIX III

Begg's funnel plot of log odds ratios and pseudo 95% confidence intervals for studies evaluating associations between *GSTM1* null variant and esophageal adenocarcinoma. Circle size is proportional to the study weight.

APPENDIX IV

Begg's funnel plot of log odds ratios and pseudo 95% confidence intervals for studies evaluating associations between *GSTM1* null variant and Barrett's esophagus. Circle size is proportional to the study weight.

APPENDIX V

Begg's funnel plot of log odds ratios and pseudo 95% confidence intervals for studies evaluating associations between *GST1null* variant and esophageal adenocarcinoma. Circle size is proportional to the study weight.

APPENDIX VI

Begg's funnel plot of log odds ratios and pseudo 95% confidence intervals for studies evaluating associations between *GST1null* variant and Barrett's esophagus. Circle size is proportional to the study weight.

Fig. 1.

Odds ratios (ORs) and 95% confidence intervals reported by studies evaluating *GSTM3*, *CYP1*, *EPHX1*, *NQ01*, and *NAT1* single-nucleotide polymorphisms as risk factors for esophageal adenocarcinoma. Jain adjusted for age, sex, smoking status, and alcohol use. Sarbia adjusted for age. di Martino adjusted for age, sex, reflux, body mass index, and smoking status. Wideroff adjusted for age, sex, and study center.

Fig. 2. Odds ratios (ORs) and 95% confidence intervals reported by studies evaluating *CYP1*, *mEH*, *NQO1*, *NAT1* and *GST* single-nucleotide polymorphisms as risk factors for Barrett's esophagus. di Martino adjusted for age, sex, reflux, body mass index, and smoking status.

Fig. 3.

Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *CYP1A1* *Ile462Val* single-nucleotide polymorphisms and esophageal adenocarcinoma. Box size is proportional to the meta-analysis study weight. Dominant model used, combining heterozygote genotype with homozygote minor allele genotype.

Fig. 4.

Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *GSTP1*Ile105Val single-nucleotide polymorphism and esophageal adenocarcinoma. Box size is proportional to the meta-analysis study weight. Dominant model used, combining heterozygote genotype with homozygote minor allele genotype.

Fig. 5. Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *GSTP1Ile105Val* SNP and Barrett's esophagus. Box size is proportional to the meta-analysis study weight. Dominant model used, combining heterozygote genotype with homozygote minor allele genotype.

Fig. 6. Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *GSTM1* null variant and esophageal adenocarcinoma. Box size is proportional to the meta-analysis study weight.

Fig. 7. Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *GSTM1null* variant and Barrett's esophagus. Box size is proportional to the meta-analysis study weight.

Fig. 8. Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *GSTT1* null variant and esophageal adenocarcinoma. Box size is proportional to the meta-analysis study weight.

Fig. 9.

Forest plot of fixed-effect pooled odds ratio and corresponding 95% confidence intervals (CIs) from studies evaluating associations between *GSTT1null* variant and Barrett's esophagus. Box size is proportional to the meta-analysis study weight.

Table 1

Characteristics of eligible studies evaluating the effect of phase I and phase II metabolic enzyme polymorphisms on risk of esophageal adenocarcinoma

Study (first author [year published], country, study time period, reference no.)	Case ascertainment	Sample size (case/control)	Controls ascertainment	SNPs	Cases			Controls		
					Age	Race (%)	Men (%)	Age	Race (%)	Men (%)
van Lieshout (1999), Netherlands, unknown time, 4-year span ²⁰	Hospital based	21/247	Healthy, unmatched, source – unknown	<i>CYP1A1</i> (<i>MspI</i>), <i>CYP1A1</i> (<i>Ile462Val</i>), <i>GSTM1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (<i>null</i>)	Mean = 64, SE 2	White (100)	Unknown (86)	Mean = 52, SE 2	100% white	Unknown (40)
Casson (2003), Canada, 1992–1997 ¹²	Hospital based	45/45	Healthy, population based, age and sex, matched	<i>CYP1A1</i> (<i>MspI</i>), <i>GSTM1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (<i>null</i>), <i>EPHX1</i> (<i>exon 3</i>), <i>EPHX1</i> (<i>exon 4</i>)	Unknown	Unknown	38 (84)	Unknown	Unknown	38 (84)
Casson (2006), Canada, 2001–2003 ¹³	Hospital based	56/95	Healthy, clinic based, frequency matched but unknown on what	<i>GSTM1</i> (<i>null</i>), <i>GSTT1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>EPHX1</i> (<i>exon 3</i>), <i>EPHX1</i> (<i>exon 4</i>)	<50 years = 4%, 50 years = 96%	White (100)	50 (89)	<50 years = 38%, 50 years = 62%	White (100)	64 (67)
Jain (2006), India, 2003–2006 ¹⁵	Hospital based	9/137	Cancer free, clinic based, age and sex, matched	<i>GSTM1</i> (<i>null</i>), <i>GSTT1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>)	Mean = 56.5, SD 13.1	North Indian (100)	Unknown (76)	Mean = 55, SD 9.4	North Indian (100)	Unknown (71)
Jain (2007), India, 2003–2006 ¹⁶	Hospital based	9/200	Cancer free, clinic based, age and sex, matched	<i>GSTM3</i> (*B)	Mean = 58.5, SD 12.4	North Indian (Asian)	Unknown (76)	Mean = 55, SD 9.4	North Indian (Asian)	Unknown (71)
Abbas (2004), France, unknown ¹¹	Clinic based	27/130	Cancer free, clinic based, age and sex, matched	<i>CYP1A1</i> (<i>Ile462Val</i>), <i>GSTM1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (<i>null</i>)	Mean = 66, range (51–85)	Unknown	25 (93)	Mean = 56, range = 19–87	Unknown	87 (67)
Sarbia (2003), Germany, 1983–2001 ¹⁹	Hospital based	61/252	Healthy, population based, unmatched	<i>NQO1</i> (<i>Pro609Ser</i>)	Mean = 62, range (33–81)	White (100)	53 (86.9)	Mean = 39, range = 24–70	White (100)	193, (76.6)
von Rahden (2005), Germany,	Hospital based	140/260	Cancer free, hospital based,	<i>NQO1</i> (<i>Pro609Ser</i>)	Mean = 62.6,	White (100)	128 (91.4)	Mean = 57.5, SD 18	White (100)	172, (66.2)

Study (first author [year published], country, study time period, reference no.)	Case ascertainment	Sample size (case/control)	Controls ascertainment	SNPs	Cases			Controls		
					Age	Race (%)	Men (%)	Age	Race (%)	Men (%)
1991–2003 ²¹			unmatched		SD 10.8					
Wideroff (2007), US A, 1993–1995 ²²	Registry based	67/209	Healthy, population based, age and sex, matched	<i>CYP1A1</i> <i>Ile462Val</i> , <i>GSTM1</i> (null), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (null), <i>NAT1</i> (*10, *11)	Median = 63	White (100)	Unknown	Median = 66	White (100)	Unknown
Murphy (2007), Ireland, 2002–2004 ¹⁸	Hospital based	207/223	Healthy, clinic bases, age and sex, matched	<i>GSTP1</i> (<i>Ile105Val</i>)	Mean = 64.4, range (35– 85)	White (100)	172 (83)	Mean = 64.4, range (35– 86)	White (100)	184 (83)
di Martino (2007), UK, 2000–2003 ¹⁴	Hospital based	144/94	Symptomatic but disease free, hospital based, unmatched	<i>NQO1</i> (<i>Pro609Ser</i>)	Mean = 72, range (41– 94)	Unknown	117 (81)	Mean = 56 (29–88)	Unknown	42 (45)

SE, standard error; SD, standard deviation; SNPs, single-nucleotide polymorphisms.

Table 2
Characteristics of eligible studies evaluating the effect of phase I and phase II metabolic enzyme polymorphisms on risk of Barrett's esophagus

Study (first author [year published], country, study time period, reference no.)	Case ascertainment	Sample size (case/control)	Controls ascertainment	SNPs	Cases			Controls		
					Age	Race (%)	Men (%)	Age	Race (%)	Men (%)
van Lieshout (1999), Netherlands, 4-year span ²⁰	Hospital based	98/247	Healthy, source-unknown, unmatched	<i>CYP1A1</i> (<i>MspI</i>) <i>CYP1A1</i> (<i>Ile462Val</i>), <i>GSTM1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (<i>null</i>)	Mean = 64, SD 2	White (100)	(86)	Mean = 52, SD 2	White (100)	Unknown (40)
Casson (2006), Canada, 2001–2003 ¹³	Hospital based	125/95	Healthy, clinic based, freq. match but unknown on what	<i>GSTM1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (<i>null</i>), <i>EPHX1</i> (<i>exon 3</i>), <i>EPHX1</i> (<i>exon 4</i>)	<50years = 4%, 50years = 96%	White (100)	50 (89)	<50 years = 38%, 50years = 62%	White (100)	64 (67)
Kala (2007), Czech Republic, Unknown ¹⁷	Hospital based	22/173	Healthy, clinic based, age, sex and smoking, matched	<i>GSTM1</i> (<i>null</i>), <i>GSTP1</i> (<i>Ile105Val</i>), <i>GSTT1</i> (<i>null</i>)	Mean = 46.4, SD 12.7	White (100)	18 (82)	Mean = 44.7, SD 10.7	White (100)	107 (62)
Murphy (2007), Ireland ¹⁸	Hospital based	189/223	Healthy, population based,	<i>GSTP1</i> (<i>Ile105Val</i>)	Mean = 62.2 (24– 85)	White (100)	156 (83)	Mean = 64.4 (35–86)	White (100)	184 (83)
di Martino (2007), UK ¹⁴	Hospital based	200/94	Symptomatic but disease free, hospital based, unmatched	<i>NQO1</i> (<i>Pro609Ser</i>)	Mean = 65 (25– 97)	Unknown	148 (74)	Mean = 56 (29–88)	Unknown	42 (45)

SD, standard deviation; SNPs, single-nucleotide polymorphisms.