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Glutathione S-transferase P1 Ile105Val polymorphism and colorectal cancer risk: A meta-analysis and HuGE review

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ABSTRACT

Colorectal cancer is the third most common form of cancer and the fourth most frequent cause of cancer deaths worldwide. Its development is influenced by both environmental and genetic factors. The glutathione S-transferase P1 gene (*GSTP1*) is a particularly attractive candidate for colorectal cancer susceptibility because it codes the enzyme involved in the metabolism of environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs). However, epidemiologic findings have been inconsistent. To investigate a putative association of *GSTP1* Ile105Val polymorphism with the risk of colorectal cancer, we performed a meta-analysis and HuGE review of 16 published case-control studies (involving a total of 4386 colorectal cancer patients and 7127 controls). We used odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of the association. Overall, the comparison of Val versus Ile allele showed no differential susceptibility to colorectal cancer (OR = 0.98, 95% CI: 0.92–1.04). A protective effect was found in recessive, with an OR of 0.86 (95% CI: 0.76–0.98). Whereas no significant association was observed in either dominant or codominant model. In stratified subgroup analysis, no effect of Val allele was seen in subjects of Caucasian and Asian descent, and in healthy and hospital controls. In conclusion, the meta-analysis suggests that the *GSTP1* Ile105Val polymorphism is unlikely to increase considerably the risk of sporadic colorectal cancer, and it should be confirmed in further studies.

1. Background

1.1. *Gene and gene variants*

The glutathione S-transferases are a family of dimeric enzymes catalysing conjugation between glutathione and chemotherapeutic drugs, carcinogens, environmental pollutants and a broad spectrum of xenobiotics.[1] In humans, there are at least 13 GST enzymes belonging to five families, namely α (*GSTA*), μ (*GSTM*), θ (*GSTT*), π (*GSTP*) and σ (*GSTS*).[2,3] They detoxify diverse electrophiles, including carcinogens, chiefly by conjugating them with glutathione.[4] Thus, the detoxification ability of GSTs plays a role in cellular protection from environmental and oxidative stresses, yet it is also implicated in cellular resistance to drugs.[5–8] *GSTP1* possesses unique enzymatic properties, including broad substrate specificity, glutathione peroxidase activity towards lipid peroxides, low sensitivity to organic anion inhibitors, high sensitivity to active oxygen species and ligand-binding properties.[6,9–12] *GSTP1*, the only member of the GST Pi class, appears to be the most widely distributed GST isoenzyme.[13] Two *GSTP1* single nucleotide polymorphisms have been identified that are characterised by transitions at A1578G (exon 5, A313G) and C2293T (exon 6, C341T), resulting in amino acid substitutions

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Ile105Val and Ala114Val, respectively, which appear to be within the active site of the GST-pi protein.[9,14–16] The SNP of *GSTP1* exon 5 was usually recognised as rs1695, or sometimes as rs947894 (merged into rs1695 from Pubmed annotation), and the rs1695 results in a less active enzyme, which could decrease the metabolism of carcinogens.[17] Thus, the altered metabolic activity of these enzymes could influence susceptibility to cancer, including colorectal cancer.

1.2. *Population frequency*

The *GSTP1* Ile105Val polymorphisms consist of the variant genotypes *GSTP1* Val/Ile and *GSTP1* Val/Val along with the wild-type *GSTP1* Ile/Ile. The frequency of *GSTP1* Ile/Ile in healthy controls ranges from 42% to 69%. The valine-containing homozygous variant, *GSTP1* Val/Val, occurs in approximately 10% of healthy controls, whereas the heterozygous Ile-Val variant, *GSTP1* Val/Ile, occurs in approximately 35% of controls.[16,18,19]

In this meta-analysis, we combined data from 17 articles and summarised data for the Ile105Val polymorphism. The pooled frequencies are given in the results section.

1.3. *Disease*

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer deaths globally.[20] Among different regions, there were large variation in rates, with the lowest rates in Africa and Asia and the highest in Europe, Northern America and Australasia.[21] In Europe, the incidence of colorectal cancer is increasing, particularly in southern Europe and eastern Europe, where rates were originally lower than in western Europe.[22] In 2006, there were an estimated 307,432 new cases of CRC in the European Union (EU). The rates varied by a factor of two for women and three for men. The lowest rates were in Greece and the highest were in Hungary and the Czech Republic.[23]

From a comprehensive overview of most recent European trends,[24] the incidence of CRC among males increased modestly in most countries and markedly in Austria, Croatia, Slovenia, Spain and the Czech Republic. While among females, the incidence rates were stable with some decreases in Scotland, Northern Ireland and Poland, contrasting a clear increase in Spain.

On account of its typically slow development, it is important to research the aetiology and preventive measures to reduce the burden of the disease. Colorectal cancer (CRC) is usually considered to be a multifactorial disease, in which multiple exposures to endogenous factors and dietary carcinogens interact with individual genetic background in a complex manner resulting in modulation of the risk.[25]

Epidemiological studies have revealed the importance of some environmental factors in the carcinogenesis of sporadic colorectal cancer.[26] Cigarette smoking, alcohol use and the consumption of diets high in red meat are probably important aetiological factors increasing the risk of developing colorectal cancer.[27,28] Polycyclic aromatic hydrocarbons (PAHs) from cigarette smoke and certain dietary components can form DNA adducts and lead to DNA mutations.[29] Thus, it has been postulated that genes involved in PAH metabolism may modify colorectal cancer risk.[30] As a member of phase II metabolic enzymes, *GSTP1* is an important candidate for involvement in susceptibility to carcinogen-associated colorectal cancer.

Previous studies, including the sixteen studies we have recruited have been conducted to assess the risk of colorectal cancer with the variant Val *GSTP1* allele; however, the results are quite equivocal.[42–58] Chen and colleagues[59] performed a meta-analysis in 2005 and demonstrated no significant association with *GSTP1* Ile105Val polymorphism and colorectal cancer risk. Since then, the previous studies did not find confirmed outcomes based on small sample sizes or no investigating *GSTP1*, respectively, or probably potential publication bias, aiming to have a more comprehensive picture of the potentially functional polymorphisms with *GSTP1*, we carried out a meta-analysis to evaluate the influence of Ile105Val *GSTP1* polymorphism on susceptibility to CRC with 17 published studies published from August 1998 to January 2009.

2. Materials and methods

2.1. *Search strategy and data extraction*

A literature search of Pubmed, Embase and HuGENet database was conducted using the combined keywords: '*GSTP1*', 'glutathione s-transferase P1', 'polymorphism', 'genetics', 'colon cancer', 'rectal cancer' and 'colorectal cancer'. The latest search was done in January 2009, without any language restriction. Additional articles were identified through the references cited in the first series of articles selected. Articles included in the meta-analysis were in any language, with human subjects, published in the primary literature and had no obvious overlap of subjects with other studies. Among overlapping reports, only the studies with more information on origin of cases/controls were retained. The inclusion criteria were as follows: (1) independent case-control or cohort studies that quantitatively assessed the relationship of *GSTP1* polymorphism and risk of sporadic colorectal cancer. (2) studies that contained colorectal cancer cases and colorectal cancer-free controls. (3) cases with colorectal cancer regardless of tumour stage or histological type. And for the exclusion criteria, we provided as follows: (1) studies without raw data we need. (2) studies that focused on HNPCC or FAP. Family-based studies of pedigrees with several affected cases per family were also excluded, because their analysis is based on linkage considerations.

Two investigators independently extracted the data and reached consensus on all items. The following information was sought from each study: authors; year of publication; country of origin; selection and characteristics of colorectal cancer cases and controls; number of cases and controls for each *GSTP1*; source of controls (categorised as hospital, healthy and mixed); match (categorised as age, gender, area, etc.); and racial descent of the study population (categorised as Caucasian, African and Asian).

2.2. *Statistical analysis*

The association between *GSTP1* polymorphism and colorectal cancer was first determined using the per-allele approach. Allele frequencies were calculated for studies reporting only

genotype data. The per-allele analysis compared CRC patients against controls for the contrast of Val versus Ile alleles. We also estimated the association with colorectal cancer risk under certain genotypic models, namely codominant (Val/Val versus Ile/Ile; Val/Ile versus Ile/Ile), recessive (Val/Val versus Val/Ile + Ile/Ile) and dominant (Val/Ile + Val/Val versus Ile/Ile). The effect of association was indicated as odds ratio (OR) with the corresponding 95% confidence interval (CI). Based on the individual ORs, the pooled OR was estimated.

For each genetic contrast, we estimated the between-study heterogeneity across all eligible comparisons using the chi-square based Q statistic, which was the weighted sum of the squared difference between the overall effect size and the effect size from each study. (Considered significant for $P < 0.10$).[31] The fixed effect model was used to estimate overall effect size if the effect sizes were homogeneous across studies; otherwise, a random effect model was used. Random effects incorporate an estimate of the between-study variance and provide wider 95% confidence intervals (95% CI), when the results of the constituent studies differ among themselves.

To further explore the source of heterogeneity, subgroup analysis and sensitivity analysis were performed. In subgroup analysis, the controls were performed by group studies that showed similar characteristics, such as ethnicity and control source. The ethnic subgroups were categorised into three ethnic groups (Caucasian, Asian and mixed), while the control source subgroups were considered as 2 groups hospital controls [patients recruited within a hospital setting] and healthy controls [healthy blood donors or individuals selected through population-based sampling methods]. In sensitivity analysis, each study was excluded one at a time to determine the magnitude of influence on the overall summary estimate.[32]

For publication bias assessing, inverted funnel plot, Begg's test and Egger's test were employed. To obtain the evidence of population stratification, the distribution of genotypes in control group was tested by χ^2 method for Hardy–Weinberg equilibrium (HWE; $P \geqslant 0.05$).[33] Studies with controls that violated or deviated from HWE were subjected to a sensitivity analysis. Articles where HWE could not be assessed were treated as studies that deviate from HWE in the sensitivity analysis. All analyses were performed using Stata version 9.0 (Stata Corporation, USA). All the *P* values were two-sided.

3. Results

3.1. Study characteristics

A total of 183 abstracts were retrieved through searching PubMed, Embase and HuGENet database. We identified 25 relevant studies that described the association between the *GSTP1* Ile105Val polymorphism and colorectal cancer, but after reading the full articles, we excluded three case-only studies[34–36] and three studies without raw data.[37–39] Four studies were overlapped[40–43], and only two were retained[42,43] as the criteria mentioned above. Finally, 17 studies met the inclusion criteria and were included.[42–58] Fig. 1 shows the process of study selection and exclusion, with specification of reasons.

Among the eligible studies, 11 studies were about Caucasian,[44,45,48–57] four studies were about Asians,[42,43,46,47] and only one study[58] contained subjects of different racial descents, including Japanese American, African American, Latino, White, Native Hawaiian. All included studies were case-control designed. With the exception of two studies[51,58] that did not clarify the exact diagnostic criteria, most of the studies selected colorectal cancer patients based on a histological diagnosis from biopsy. While three studies defined cases by International Classification of Diseases (ICD)[49], medical history[39] and sigmoidoscopy screening,[55] respectively. Five studies mentioned about the tumour status of CRC,[42,46,48,51,55] while other studies did not comment on clinical stages. Three reports[42,53,58] mentioned positive family history of CRC in 4%, 11.7% and 10% of patients, respectively, whereas the remaining did not comment on family history.

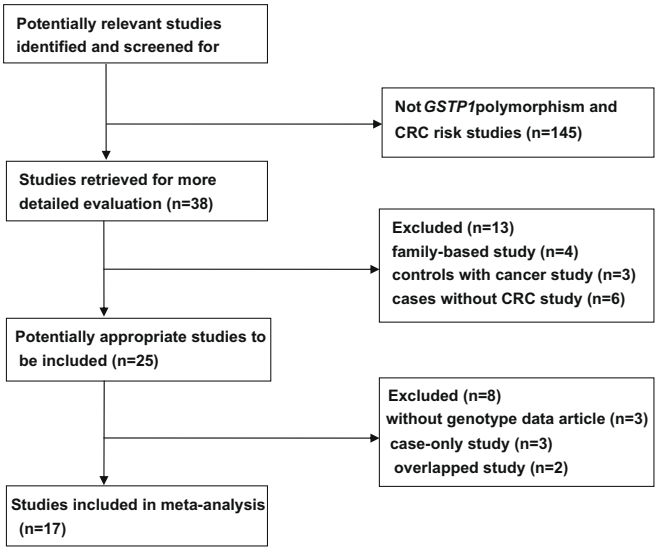

Fig. 1 – The process of study selection and exclusion, with specifications of reasons.

Table 1 – Summary of the studies of colorectal cancer risk and GSTP1 Ile105Val polymorphism.

Ref.	Investigator, year	Place of study	Selection/characteristics of cases and controls (age range [mean ± SD])		Race	Eligible subjects		Source of controls	Method
			Case	Controls		Case	Control		
44	Harris et al. (1998)	Australia	Histologically confirmed diagnosis	Randomly selected normal individuals. 58% male, 42% female, (18–69 [39])	Caucasian	88	199	Healthy	PCR
45	Welfare et al. (1999)	United Kingdom	Histologically confirmed diagnosis. 58% male, 42% female, [69]	Age- and sex-matched community individuals identified from the records of the general practitioner. 58% male, 42% female, [69]	Caucasian	178	178	Healthy	PCR
46	Katoh et al. (1999)	Japan	Histologically confirmed diagnosis. 65% male, 35% female, [62.2 ± 12.1]	Individuals recruited from health check-ups, without current or previous diagnosis of cancer. 59% male, 41% female, [62.4 ± 16.5]	Asian	103	122	Healthy	PCR-RFLP
47	Yoshioka et al. (1999)	Japan	Histologically confirmed diagnosis. 65.1% male, 34.9% female, [62.3 ± 12], smoking 50%	Individuals for heath check-up, without a history of any type of tumour. 58% male, 42% female, [61.7 ± 16], smoking 55%	Asian	106	100	Healthy	PCR
48	Loktionov et al. (2001)	United Kingdom	Histologically confirmed diagnosis, excluded cases with family history of early onset CRC or multiple cancer. 59.7% male, 40.3% female, [68.7 ± 9.7], smoking 18.8%	Individuals with a normal flexible sigmoidoscope. 67.5% male, 32.5% female, (55–66 [60.6 ± 2.8]), smoking 15.0%	Caucasian	206	345	Healthy	PCR
49	Sachse et al. (2002)	United Kingdom	Incident patients identified through ICD, without FAP, inflammatory bowel disease, ulcerative colitis, diverticular disease or previous malignancy; 61% male, 39% female, (45–80 [67.7 ± 8.5])	Individuals without history of previous cancer, recruited by age, sex and general practitioner matching of incident cases. 54% male, 46% female, [68.6 ± 8.9]	Caucasian	490	593	Healthy	TaqMan
50	Kiss et al. (2004)	Hungary	Histologically confirmed diagnosis, excluding FAP, HNPCC and ulcerative colitis	Cancer-free patients from in- or outpatient wards and volunteers for health status examination, matched to case by age, gender, smoking habit and red meat consumption	Caucasian	500	500	Hospital	PCR-RFLP
51	van der Logt et al. (2004)	Netherlands	Not clarified. 57.1% male, 42.9% female, [64 ± 11]	Individuals recruited by advertisement in a local paper. 40.5% male, 59.5% female, [42 ± 12]	Caucasian	371	415	Healthy	PCR-RFLP
52	Ates et al. (2005)	Turkey	Histologically confirmed diagnosis. 58.6% male, 41.4%female, [59.8 ± 13.3], smoking 50.3%	Selected among healthy people with no history of CVD, cancer, chronic degenerative neurological disease, COPD, hepatitis, diabetes, hypertension, atopy, autoimmune diseases, or allergies. 56.4% male, 43.6% female, [62.1 ± 7.1], smoking 41.2%	Caucasian	181	204	Healthy	Real-Time PCR

53	Landi et al. (2005)	Spain	Histologically confirmed diagnosis. 59.7% male, 40.3% female, smoking 46.8%, family history 11.7%	Randomly selected patients, excluded cancer or other chronic diseases, frequency matching to cases by sex and age. 53.1% male, 46.9% female, smoking 44.1%, family history 3.8%.	Caucasian	377	326	Hospital	Oligonucleotide micro-array and APEX
54	Martinez et al. (2006)	Spain	Histologically confirmed diagnosis. 53.5% male, 46.5% female, male [67.0 ± 9.6], female [65.4 ± 12.9]	Unrelated healthy individuals recruited among medical and nursery staff and students. History-medical examination and laboratory tests excluding pre-existing disorders. 60.2% male, 39.8% female, male [42.1 ± 11.5], female [44.6 ± 9.6]	Caucasian	144	329	Healthy	PCR-RFLP
42	Probst-Hensch et al. (2006)	Singapore	Histologically confirmed diagnosis. 57% male, 43% female, [61.2 ± 7.5], family history 4%,smoking 41.0%	Individuals without a history of CRC. 43% male, 57% female, [56.5 ± 8.1], family history 2%, smoking 27.0%	Asian	300	1169	Healthy	TaqMan
43	Yeh et al. (2007)	Taiwan, China	Histologically confirmed diagnosis, excluded FAP, HNPCC, inflammatory bowel disease and other malignancies	Individuals recruited from health check-ups, excluded other colorectal diseases, a history of other cancers or the existence of a family history of colorectal cancer; matched to case by age and sex	Asian	727	736	Healthy	PCR-RFLP
55	Skjelbred et al. (2007)	Norway	Individuals identified in the NORCCAP screening group based on flexible sigmoidoscopy examination and patients operated on hospitals. 54.7% male, 45.3% female, [67.3 ± 11.2], smoking 61.5%	Individuals drawn from the NORCCAP study, with normal findings at flexible sigmoidoscopy screening. 39.3% male, 60.7% female, [54.2 ± 3.3], smoking 53.3%	Caucasian	234	400	Healthy	Multiplex PCR
56	Vlaykova et al. (2007)	Bulgaria	Operated patients identified by the accepted protocols in Bulgaria for surgical interventions and obtaining of human biopsy materials. 62.5% male, 37.5% female, (40–82 [64]).	Unrelated normal volunteers attended the regular annual prophylactic examinations and had no indications for CRC. 57% male, 43% female, (20–83 [51])	Caucasian	80	126	Healthy	PCR-RFLP
57	Kury et al. (2008)	France	Patients recruited in regional hospitals and clinics, with a personal history of colorectal cancer, excluded patients suspected of having a familial form of colorectal cancer. 61.8% male, 38.2% female, [65.7 ± 10.1]	Individuals recruited from health check-ups, matched cases according to sex, age and geographic origins, without a familial history of CRC or polyps. 54.3% male, 45.7% female, [61.9 ± 10]	Caucasian	1023	1121	Healthy	TaqMan
58	Epplein et al. (2009)	America	Identified through tumour registries of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute. 61% male, 49% female, [69.5 ± 8.3], family history 10%, smoking 60.0%	Individuals remained alive and free of colorectal cancer at the age of the case's diagnosis. 60% male, 40% female, [69.2 ± 8.2], family history 8%, smoking 53%	Mix[a]	173	313	Healthy	TaqMan

Abbreviations: CRC, colorectal cancer; ICD, International Classification of Diseases; FAP, familial adenomatous polyposis coli; HNPCC, hereditary non-polyposis colorectal cancer; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; NORCCAP, Norwegian Colorectal Cancer Prevention study; APEX, arrayed primer extension technique.

a 41.6% Japanese American, 17.9%African American, 19.1% Latino, 15.0% White, 6.4% Native Hawaiian.

Of the eligible studies, most matched controls to cases by age and/or gender, and 12 studies further matched controls to cases by age and at least one other risk factor.[42,43,45,47,52,54,58] Two reports used hospital patients as controls and 11 studies used healthy controls.

All controls did not have a clinical diagnosis of CRC, but the amount of additional screening to exclude colorectal cancer differed substantially across studies. A list of details abstracted from the studies included in the meta-analysis is provided in Table 1.

3.2. Meta-analysis database

Overall, the eligible studies included 5281 cases and 7176 controls, and a total of 5121 cases and 7035 controls were genotyped. The Val allele was more highly represented among controls of Caucasian descent [33.6%, 95% CI: 32.6–34.6] than in controls of Asian descent [17.9%, 95% CI: 16.7–19.0]. The prevalence of Val/Val homozygosity was 12.1% and 4.0% in control subjects of Caucasian and Asian descent, respectively. The respective prevalence rates of Val/Ile heterozygosity were 42.9% and 27.6%. The majority of studies that reported genotype frequencies in controls were consistent with HWE ($p > 0.05$). Deviations from Hardy–Weinberg proportions in controls were observed in three studies.[46,52,58] The gene distribution and all the *P*-values for HWE testing are shown in Table 2.

3.3. Overall effects for alleles

There was no strong evidence that the Val allele conferred increased susceptibility to colorectal cancer. The pooled OR was 0.98 by fixed effects (95% CI: 0.92–1.04), without between-study heterogeneity ($P = 0.31$). Fig. 2 shows the odds ratio for the risk of colorectal cancer associated with the Val allele.

In race specific analysis, no association was observed that the Val allele conferred increased susceptibility to CRC risk in any subgroups. The summary OR was 1.00 (95%CI: 0.93–1.06) for Caucasians and 0.95 (95%CI: 0.83–1.09) for Asians. When restricting the analysis to the source of controls, the overall OR for healthy and hospital are 0.97 (95%CI: 0.91–1.03) and 1.05 (95%CI: 0.91–1.22), respectively.

In sensitivity analysis, the estimated ORs were insensitive to the removal of individual studies. Three studies[46,52,58] that deviated from HWE were among the studies that if removed the overall significance did not change.

For publication bias assessing, the inverted funnel plot was potentially asymmetric and the results of Begg's test ($P = 0.23$) and Eggar's test ($P = 0.05$) implied some evidence of publication bias.

All the results for the association between *GSTP1* Ile105Val polymorphism and the risk of colorectal cancer are shown in Table 3.

3.4. Other contrasts

No evidence for an association with colorectal cancer was discerned when dominant and codominant models were examined for the effect of Val. Under dominant model, the fixed effects OR was 1.02 (95% CI: 0.94–1.10), without between-study heterogeneity ($P = 0.29$). Compared with Ile/Ile wildtype, the fixed effects OR was 0.88 (95% CI: 0.77–1.01) for Val/Val genotype, and 1.06 (95% CI: 0.98–1.14) for Val/Ile genotype.

Whereas little evidence was found when the recessive model was employed. The fixed effects OR was 0.86 (95% CI: 0.76–0.98) for the contrast of Val/Val versus Val/Ile + Ile/Ile. No between-study heterogeneity was seen for the recessive genetic model.

In sensitivity analysis, pooled estimates for all genetic models were insensitive to the removal of individual studies. And the removal of the three studies that deviated from HWE had a negligible effect. All the summary ORs for different genetic models are shown in Table 3 and Figs. 3–6.

Table 2 – Frequency of GSTP1 Ile105Val polymorphism in different populations included in a meta-analysis.

Ref.	Investigator (year)	Race	Cases (%)			Controls (%)			*P* value for HWE
			Val/Val	Val/Ile	Ile/Ile	Val/Val	Val/Ile	Ile/Ile	
44	Harris et al. (1998)	Caucasian	12.5	45.5	42	9	50.8	40.2	0.08
45	Welfare et al. (1999)	Caucasian	7.6	45.4	47	12.3	41.6	46.1	0.41
48	Loktionov et al. (2001)	Caucasian	11.7	46.1	42.2	11	48.7	40.3	0.21
49	Sachse et al. (2002)	Caucasian	11.6	49	39.4	13	43.2	43.8	0.28
50	Kiss et al. (2004)	Caucasian	17.6	42.4	40	14.8	42.4	42.8	0.07
51	van der Logt et al. (2004)	Caucasian	10.6	47.4	42	13.3	44.7	42	0.6
52	Ates et al. (2005)	Caucasian	14.9	44.8	40.3	19.6	36.3	44.1	0.001
53	Landi et al. (2005)	Caucasian	8.4	42.9	48.7	11.2	40.3	48.5	0.26
54	Martinez et al. (2006)	Caucasian	3.6	45.7	50.7	10.4	41	48.6	0.53
55	Skjelbred et al. (2007)	Caucasian	6.5	46.3	47.2	13.4	46.8	39.8	0.91
56	Vlaykova et al. (2007)	Caucasian	8.8	22.5	68.7	7.1	38.9	54	0.97
57	Kury et al. (2008)	Caucasian	10.9	43.7	45.4	10.5	41.2	48.3	0.18
47	Katoh et al. (1999)	Asian	0	32	68	4.1	19.7	76.2	0.05
46	Yoshioka et al. (1999)	Asian	0	32	68	3	21	76	0.31
42	Probst-Hensch et al. (2006)	Asian	3	26.7	70.3	4.5	29.6	65.9	0.08
43	Yeh et al. (2007)	Asian	2.8	27.8	69.4	3.4	26.7	69.9	0.25
58	Epplein et al. (2009)	Mix[a]	8	31.6	60.4	12.1	32.4	55.5	0.000

a 41.6% Japanese American, 17.9% African American, 19.1% Latino, 15.0% White, 6.4% Native Hawaiian.

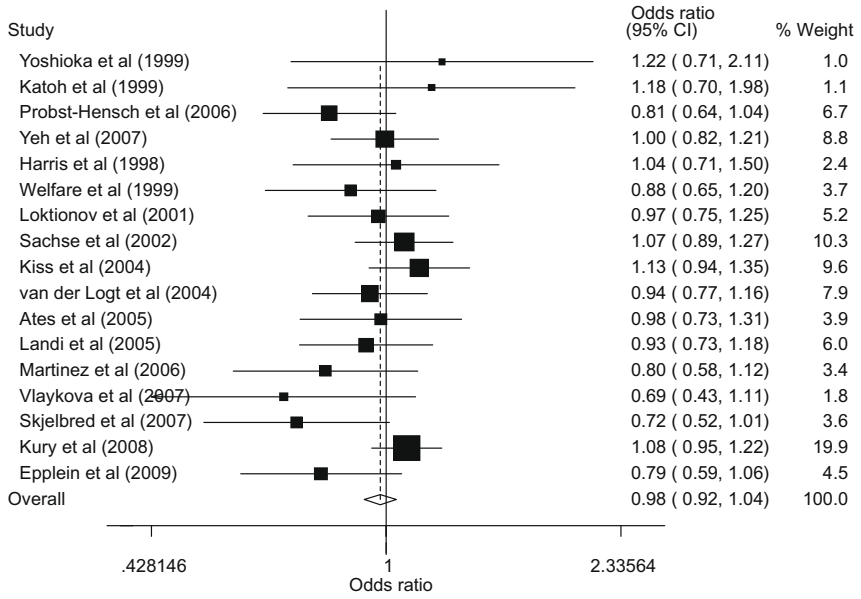

Fig. 2 – Meta-analysis with a fixed-effect model for the association between colorectal cancer risk and the *GSTP1* Ile105Val polymorphism (Val versus Ile allele). Each comparison is presented by the name of the first author and the year of publication. The point estimate of the OR and the accompanying 95% CI for each comparison is shown. Also shown is the summary fixed effects estimate for the comparison along with the respective 95% CI. Values of OR > 1, denote an increased risk for colorectal cancer with Val.

Table 3 – Odds ratios and 95% confidence intervals for the association of GSTP1 Ile105Val polymorphism and colorectal cancer – meta-analyses.

Model	Comparisons	Genotype cases	Genotype controls	FE[a] OR	95% CI	*P* for heterogeneity
Val versus Ile alleles	17	10242	10470	0.98	0.92, 1.04	0.31
Caucasians	12	7406	9142	1	0.93, 1.06	0.33
Asians	4	2462	4250	0.95	0.83, 1.09	0.35
Mixed races	1	374	678	0.79	0.59,1.06	–
Healthy controls	15	8598	8864	0.97	0.91, 1.03	0.33
Hospital controls	2	1644	1606	1.05	0.91, 1.22	0.21
Codominant: Val/Val versus Ile/Ile	17	3065	4376	0.88	0.77, 1.01	0.16
Codominant: Val/Ile versus Ile/Ile	17	4659	6353	1.06	0.98, 1.14	0.19
Dominant (Val/Ile + Val/Val) versus Ile/Ile	17	5121	7035	1.02	0.94, 1.10	0.29
Recessive: Val/Val versus (Val/Ile + Ile/Ile)	17	5121	7035	0.86	0.76, 0.98	0.13

a Fixed effects (Mantel–Haenszel) model.

4. Discussion

In 1998, Harris and colleagues[44] first examined the association between the *GSTP1* gene polymorphism and the risk of colorectal cancer. After that, the case-control studies provided controversial results. In 2005, Chen and colleagues[59] carried out a meta-analysis (four comparisons dealing with *GSTP1*, involving 612 colorectal cancer patients and 755 controls) and demonstrated no significant association with *GSTP1* Ile105Val polymorphism and colorectal cancer risk. However, it is based on relatively small studies with insufficient statistical power to detect a small change in risk.

Based on cumulated evidence, we performed an updating meta-analysis on 17 studies with 5281 cases and 7176 controls. Therefore a larger sample size and increased statistical power could be obtained. The major finding of our meta-analysis demonstrates that the *GSTP1* polymorphism is unlikely to be a major risk factor for susceptibility to colorectal cancer, and it is consistent with that of a previous meta-analysis. In race specific analysis, the summary OR was 1.00 (95%CI: 0.93–1.06) for Caucasians and 0.95 (95%CI: 0.83–1.09) for Asians. This may suggest that there is nothing to do with the race for the *GSTP1* (Val) and colorectal cancer susceptibility. When stratified analysis was performed to different sources of controls, the overall OR for healthy and hospital is 0.97 (95%CI: 0.91–1.03) and 1.05 (95%CI: 0.91–1.22), respectively. A sensitivity analysis was also performed. After excluding the studies deviated from HWE[46,52,58] the overall OR is 0.99 (95% CI: 0.93–1.05) by fixed effects, which implied that Val may be no significance association with colorectal cancer.

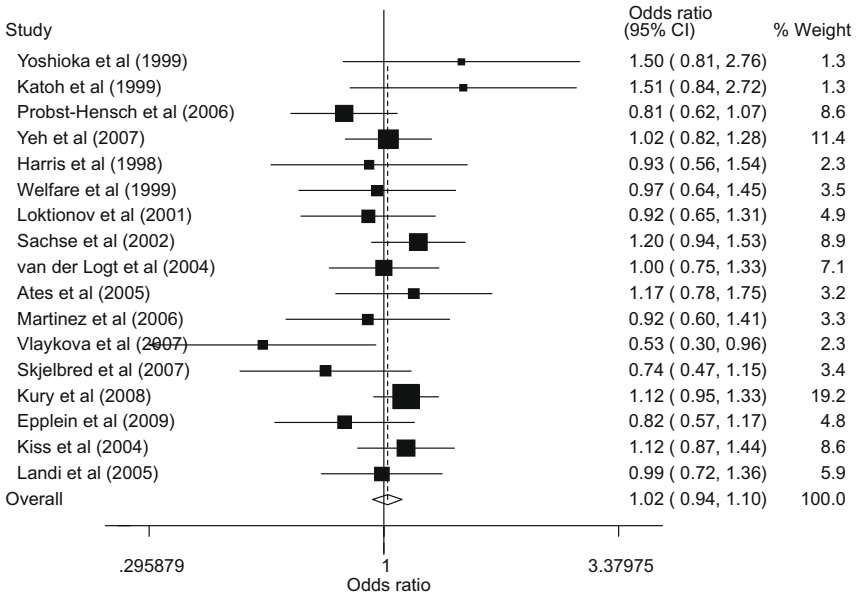

Fig. 3 – Meta-analysis with a fixed-effect model for the association between colorectal cancer risk and the *GSTP1* Ile105Val polymorphism (dominant model, Val/Ile + Val/Val versus Ile/Ile). Values of OR > 1, implied an increased risk for colorectal cancer with the combined genotype. Otherwise, figure set up as per Fig. 2.

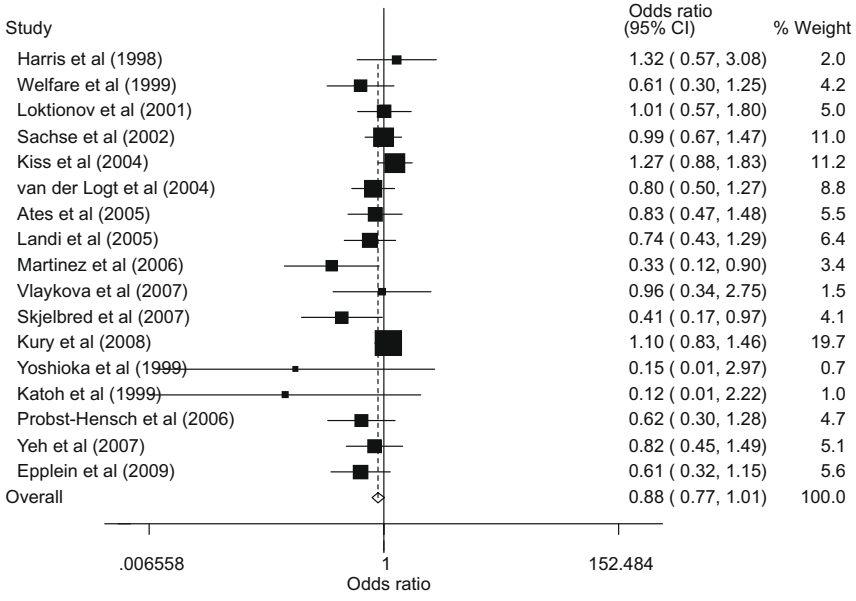

Fig. 4 – Meta-analysis with a fixed-effect model for the association between colorectal cancer risk and the *GSTP1* Ile105Val polymorphism (codominant model, Val/Val versus Ile/Ile). Values of OR > 1, implied an increased risk for colorectal cancer with the Val/Val genotype. Otherwise, figure set up as per Fig. 2.

Sporadic colorectal cancer is a consequence of multiple risk factors, and the interaction between environmental and genetic factors is generally accepted. Glutathione S-transferases (GSTs), have been reported to encode phase II metabolising enzymes, GSTs detoxify potential mutagens by conjugation to glutathione. A as a result, the potential carcinogens are eliminated.[9] *GSTP1*, a member of GSTs, a polymorphic Ile105-Val (resulting from an A to G substitution at base 1 578) has been found to modify the enzyme's activity and affinity for electrophilic substrates[13] resulting in lower enzyme activity to variety of electrophilic molecules.[5,9] Thus, the altered metabolic activity of these enzymes may influence susceptibility to cancer, including colorectal cancer.

Cancer susceptibility might be resulted from differences in the expression of metabolic enzymes.[60] Most of the human metabolic enzymes are genetically polymorphic, and these

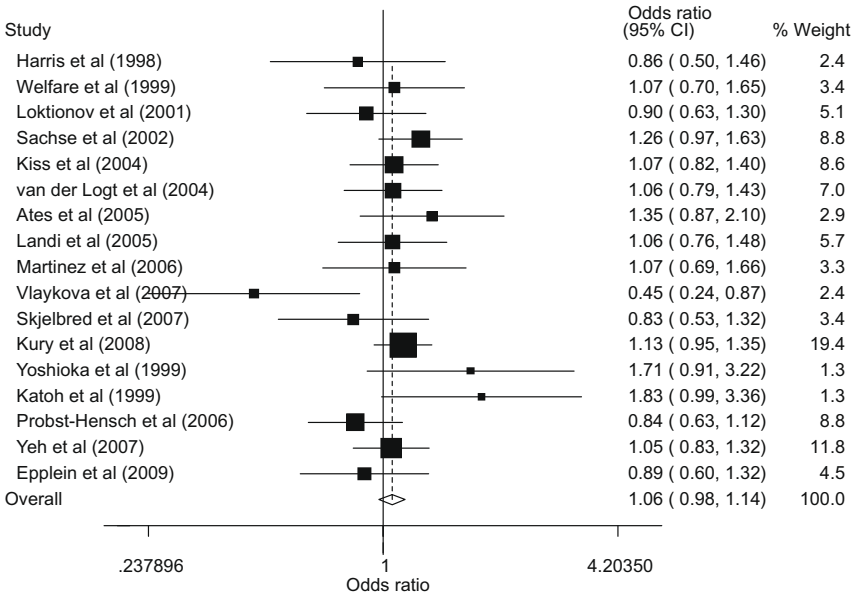

Fig. 5 – Meta-analysis with a fixed-effect model for the association between colorectal cancer risk and the *GSTP1* Ile105Val polymorphism (codominant model, Val/Ile versus Ile/Ile). Values of OR > 1, implied an increased risk for colorectal cancer with the Val/Ile genotype. Otherwise, figure set up as per Fig. 2.

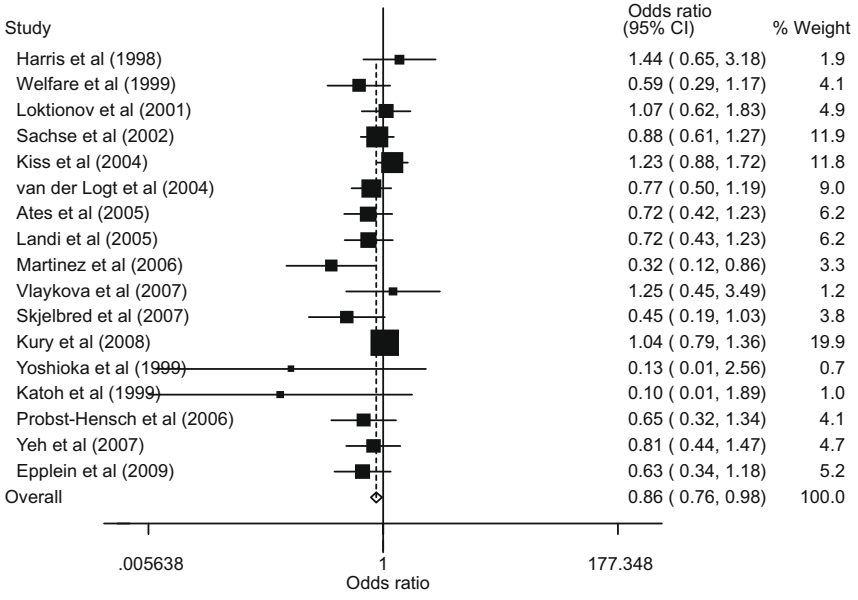

Fig. 6 – Meta-analysis with a fixed-effect model for the association between colorectal cancer risk and the *GSTP1* Ile105Val polymorphism (recessive model, Val/Val versus Val/Ile + Ile/Ile). Values of OR > 1, implied an increased risk for colorectal cancer with the combined genotype. Otherwise, figure set up as per Fig. 2.

polymorphisms may affect the enzyme activity or inducibility. Individuals carrying some 'high-risk' alleles have a strikingly increased risk for colorectal cancer.[51] Some genotypes of metabolic enzymes might be useful prognostic biomarkers for colorectal cancer.[48] Our result supports the hypothesis that even *GSTP1* polymorphism which did not have a significant influence on the risk of colorectal cancer, in certain still unknown circumstances or in not yet determined interactions, also slightly contribute to the modulation of the final risk. Thus *GSTP1* genotype of metabolic enzymes might not be a useful prognostic biomarker for CRC.

Possible limitations of the study have to be considered in interpreting the results.

First, there are probably other potential sources of heterogeneity, but because some factors were evaluated in only one or two studies, we were unable to explore them further in subgroup analyses or meta-regression. For example, age is a modifying factor of genotype expression,[61] but only one study[42] reported genotype colorectal cancer tumour associations stratified by age groups. Second, *GSTP1* variants show substantial variations in prevalence based environmental factors,[52] cigarette smoking, alcohol intake and dietary factors

(red meat intake, etc.) have been implicated as risk factors for developing colorectal cancer. Since the formatted data in these studies were not enough for pooling analysis, the interaction between other exposures and *GSTP1* (Ile versus Val) on colorectal cancer risk could not be evaluated in our study.

In addition, the funnel plot shows significant evidence of publication bias. The following were the probable reasons: First, only published studies were included in the meta-analysis; therefore a potential publication bias may have occurred. It is known that positive results usually have a greater probability of being published, and even though unpublished studies are generally of lesser quality than published ones.[62] Also, Meta-analysis of case-control studies is vulnerable to biases and confounding issues inherent in the original articles; therefore, study quality assessment and evaluation of heterogeneity are crucial, and there were two studies that were not in HWE balance. As the pooled analysis was based on a subset of published studies from the meta-analysis that tended to report no associations or weak associations, some statistically significant associations have undoubtedly occurred by chance, and caution is required in their interpretation.

Given the results of this meta-analysis, this study has not confirmed previous suggestions of a role for *GSTP1* polymorphisms in colorectal cancer susceptibility; however, whether they may act in synergy with other genes or environmental factors is the question for future studies.

5. Laboratory tests

The detailed methods used for determining the genotypes of *GSTP1* Ile105Val polymorphism are described in each article. All the studies included in the present analyses used genomic DNA extracted from peripheral blood. Most studies used PCR for genotyping, except for 4 studies that used Taqman Assay[42,49,57,58] and 1 study that used Oligonucleotide microarray and arrayed primer extension technique (APEX).[53] In addition, five studies used PCR-RFLP[43,46,50,51,54] and two studies, respectively, employed Real-Time PCR[52] and Multiplex PCR.[55] Only one study clearly noted that the result of PCR (false-negative and false-positive) should be concerned.[53] Four studies mentioned specifically blinding of the personnel who performed the genotyping.[42,43,56,58] Two studies evaluated critically and discussed their results to avoid having a probability of false positives being bigger than a given expected rate with the false discovery rate (FDR) procedure.[42,53]

5.1. *Potential public health impact*

To date, there is insufficient evidence implicating the *GSTP1* Ile105Val polymorphism in the aetiology of colorectal cancer for population testing.

5.2. *Conclusions and implications for research*

In conclusion, this major finding of our meta-analysis has not confirmed previous suggestions of a role for *GSTP1* Ile105Val polymorphism in colorectal cancer susceptibility. At the moment, the potential public health impact of these results is minimal, since there is insufficient evidence implicating the *GSTP1* Ile105Val polymorphism in the aetiology of colorectal cancer for population testing to date. Therefore, to understand the mechanism of the colorectal cancer, SNP–SNP, gene–gene and gene–environment interactions should be taken more into account.

6. Conflict of interest statement

None declared.

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