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GSTM1, GSTT1, GSTP1, GSTA1 and colorectal cancer risk: A comprehensive meta-analysis

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ABSTRACT

Glutathione S-transferases (GSTs) catalyse reactions between glutathione and lipophilic compounds with electrophilic centres, leading to neutralisation of toxic compounds, xenobiotics and products of oxidative stress. Controversy exists about whether GST polymorphisms (GSTM1 null/present genotype, GSTT1 null/present genotype, GSTP1 Ile105Val and GSTA1*A/*B) represent risk factors for colorectal cancer. This meta-analysis aims to examine the associations between the above-mentioned polymorphisms and colorectal cancer risk. Forty-four studies were eligible for GSTM1 (11,998 colorectal cancer cases, 17,552 controls), 34 studies for GSTT1 (8596 cases, 13,589 controls), 19 studies for GSTP1 (5421 cases, 7671 controls) and four studies for GSTA1 polymorphism (1648 cases, 2039 controls). Pooled odds ratios (ORs) were appropriately derived from fixed-effects or random-effects models. Separate analyses were conducted on Caucasian and Chinese populations. Where appropriate, sensitivity analysis concerning the deviation of genotype frequencies in controls from the Hardy–Weinberg equilibrium was performed. GSTM1 null allele carriers exhibited increased colorectal cancer risk in Caucasian populations (pooled OR = 1.150, 95% confidence interval (CI): 1.060–1.248, random effects); no significant association was detected for Chinese subjects (pooled OR = 1.025, 95% CI: 0.903–1.163, fixed effects). Similarly, GSTT1 null allele carriers exhibited increased colorectal cancer risk in Caucasian populations (pooled OR = 1.312, 95% CI: 1.119–1.538, random effects); the association in Chinese subjects was not significant (pooled OR = 1.068, 95% CI: 0.788–1.449, random effects). Concerning GSTP1 Ile105Val no significant associations were demonstrated in either race. GSTA1*A/*B polymorphism was not associated with colorectal cancer risk. GSTM1 and GSTT1 null genotypes confer additional risk for colorectal cancer in Caucasian populations.

1. Introduction

Cytosolic glutathione S-transferase (GST) comprises multiple isoenzymes; attention has been mainly drawn upon mu (GSTM), theta (GSTT), pi (GSTP) and alpha (GSTA) classes. GSTs catalyse reactions between glutathione and lipophilic compounds with electrophilic centres, leading to neutralisation of toxic compounds, xenobiotics and products of oxidative stress/reactive oxygen species.[1] Concerning the substrates of GSTs, it is worth mentioning that GSTM1 and GSTT1 participate in the deactivation of carcinogenic intermediates of polycyclic aromatic hydrocarbons present in tobacco (reviewed in Bolt and colleagues[2] and Raimondi and colleagues[3]). Among the pretty distinct functions mediated

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by GSTP1, glutathione peroxidase activity towards lipid peroxides, and high sensitivity to active oxygen species are worth mentioning (reviewed in Gao and colleagues[4]).

GSTM1, GSTT1, GSTP1 and GSTA1 have been found polymorphic in the population.[5,6] The so-called 'null' GSTM1 and GSTT1 genotypes have demonstrated well-established functional relevance, i.e. reduced enzyme activity, which in turn seems to denote impaired ability to detoxify carcinogens, a state possibly conferring increased cancer risk.[1] As recently reviewed,[7] the prevalence of GSTT1 null status ranges from 20% in Caucasians to 60% among Asians, whereas approximately 50% of humans (ranging from 22% in Africa to 62% in Europe) are GSTM1 null.

Given their biochemical function, their association between the above-mentioned GST genes and colorectal cancer risk has been thoroughly examined. Specifically, studies have assessed the effect of GSTM1 and GSTT1 null phenotype, GSTP1 Ile105Val G allele and GSTA1*B allele, which all denote putatively status of lower GST activity.

All the aforementioned have been reflected upon the meta-analytical level albeit with significant potential for improvement. Concerning GSTT1 null genotype in colorectal cancer, a meta-analysis has recently appeared[8]; careful examination of the studies included therein reveals, however, that nine otherwise eligible case-control studies[9–17] have not been taken into account. Similarly, the most recent meta-analysis[4] on GSTP1 Ile105Val polymorphism and colorectal cancer has not included two case-control studies[18,19] and has, on the other hand, included two studies with overlapping populations,[20,21] which seem particularly crucial and influential as they exhibit extremely low odds ratios at the meta-analysis of the recessive model.

Regarding the GSTM1 null genotype-colorectal cancer interplay, four meta-analyses have been published between 2001 and 2005[22–25]; to our knowledge, no meta-analysis has appeared thereafter on the topic. It is worth mentioning that the larger among the published meta-analyses has included 20 case-control studies on GSTM1[24]; more than 20 additional relevant studies have appeared thereafter, doubling the amount of the existing data. In addition, it is worth noting that the above-mentioned meta-analyses had not reached unanimity in their conclusions; concerning GSTM1 null genotype, Houlston and Tomlinson,[22] de Jong and colleagues[23] and Chen and colleagues[25] did not support any association, whereas Ye and Parry[24] detected a borderline finding in Caucasians. It is also worth reporting that no meta-analysis on GSTA1 has been published till now.

Under the light of the above, the need for a comprehensive, up-to-date meta-analysis on GSTM1, GSTT1, GSTP1 and GSTA1 genotypes seems evident. Given that the effect of GST polymorphisms may be modified by race,[4,8,26] this meta-analysis has adopted separate analyses for Chinese and Caucasian populations.

2. Materials and methods

2.1. Trial identification

Eligible articles were identified by a search of MEDLINE bibliographical database for the period up to July 2009 (last search: July 20, 2009) using combinations of the following keywords: 'glutathione', 'GSTM1', 'GSTT1', 'GSTP1', 'GSTA1', 'polymorphism', 'genotype', 'colon cancer', 'colorectal cancer', 'rectal cancer' and 'rectum'. In addition, we checked all the references of relevant reviews and eligible articles that our search retrieved. Language restrictions were not used and two investigators (KPE and TNS), working independently, searched the literature and extracted data from each eligible case-control study.

2.2. Eligible studies and data abstraction

All case-control studies with any sample size examining the association between colorectal cancer and GSTM1 null genotype, GSTT1 null genotype, GSTP1 Ile105Val polymorphism and GSTA1*A/*B polymorphism were considered eligible for this meta-analysis. For each of the eligible case-control studies the following data were collected: journal name, year of publication, inclusion and exclusion criteria, characteristics of the included studies (in relation to the source of cases and controls), method of ascertainment of the diagnosis, stage of colorectal cancer, demographic characteristics of the population being studied, prevalence of meaningful risk factors (smoking, alcohol consumption and family history of colorectal cancer) in cases and controls, frequencies of genotypes in cases and controls for each of the above-mentioned genotypes/polymorphisms, adjusted odds ratios (ORs) and factors which ORs were adjusted for.

2.3. Statistics

Based on the genotype frequencies in cases and controls, crude odds ratios (ORs) as well as their standard errors (SEs) were calculated. The ORs pertained to (i) null genotype carriers versus present (positive) genotype carriers concerning GSTM1, (ii) null genotype carriers versus present (positive) genotype carriers concerning GSTT1, (iii) regarding GSTP1 Ile105Val, heterozygous (AG, Ile/Val) carriers versus AA subjects; homozygous (GG, Val/Val) carriers versus AA; dominant model, i.e. GG and AG grouped together versus AA (Ile/Ile) subjects; and recessive model, i.e. GG versus AG and AA grouped together, (iv) regarding GSTA1 polymorphism: heterozygous (GSTA1*A/*B) versus GSTA1*A/*A subjects; homozygous (GSTA1*B/*B) carriers versus GSTA1*A/*A; dominant model, i.e. *A/*B and *B/*B grouped together versus GSTA1*A/*A subjects; and recessive model, i.e. *B/*B versus GSTA1*A/*A and *A/*B grouped together. In case of zero cells, an appropriate continuity correction (addition of 0.5) was implemented.[27] Apart from the analysis on crude ORs, the adjusted ORs from the individual studies were also appropriately entered into the meta-analytical models in an attempt to minimise the effect of confounding factors.

Similarly to the algorithm implemented in our previously published meta-analyses[26–31] separate analyses were performed on Chinese and Caucasian populations. Specifically, the term 'Caucasian' pertained to Indo-european populations, while the term 'Chinese' essentially encompassed Chinese, Korean, Japanese and Thai studies. This classification was adopted following the published data derived from physical anthropology and the study of human evolution.[32,33]

The fixed-effects model (Mantel–Haenszel method) and the random effects (DerSimonian Laird) model were used to calculate the pooled OR. Between-study heterogeneity and between-study inconsistency were assessed by using Cochran Q statistic and by estimating I^2, respectively.[34] In case no significant heterogeneity was detected, the fixed-effects model was chosen. Evidence of publication bias was determined using Egger's formal statistical test[35] and by visual inspection of the funnel plot. For the interpretation of Egger's test, statistical significance was defined as $p < 0.1$. Meta-analysis was performed using the STATA 'metan' command.

In addition, meta-regression was performed to assess whether odds ratio (OR) was associated with publication year. The exponentiated coefficient is provided, since the dependent variable in the meta-regression model is log(OR). Meta-regression was performed with the '*metareg*' STATA command.

Moreover, regarding GSTP1 Ile105Val and GSTA1 polymorphisms, sensitivity analysis was performed excluding studies whose allele frequencies in controls exhibited a significant deviation from the Hardy–Weinberg equilibrium (HWE), given that the deviation may denote bias.[36] For the assessment of the deviation from HWE, the appropriate goodness-of-fit chi-square test was performed.[36,37] For the evaluation of the goodness-of-fit chi-square test, statistical significance was defined as $p < 0.05$. As far as GSTM1 and GSTT1 null genotypes are concerned, tests for deviation from Hardy–Weinberg Equilibrium could not be performed on control subjects, given that the individual studies have not made the distinction between null/non-null heterozygotes and non-null/non-null homozygotes. Analyses were conducted using STATA 10.0 (STATA Corp. College Station, TX, United States of America).

3. Results

3.1. *Eligible studies*

Fig. 1 graphically illustrates the trial flow chart. Of the 1325 abstracts retrieved through the search criteria, 1249 were irrelevant, three articles[38–40] were conducted exclusively on colorectal adenomas, eleven articles[10,21,41–49] were excluded because they were conducted on overlapping populations with other eligible studies (these excluded articles represent smaller studies performed on subsets of larger eligible studies), three studies[50–52] were excluded given that they have not included controls in their study design, six articles[5,6,22–25] were reviews/meta-analyses, and two studies[53,54] were excluded due to other reasons (one was a pooled analysis on unpublished data,[53] whereas the other was excluded due to the reporting reasons,[54] i.e. no reporting of the relevant genotype frequencies). As a result, 51 case-control articles were included in this meta-analysis.[9–20,55–93] The characteristics of the eligible studies are presented in Table 1.

Forty-four case-control studies[9,11,13–17,55–59,61–75,77–88,90–93] were included in the meta-analysis on GSTM1 genotype (11,998 colorectal cancer cases, 17,552 controls, i.e. 31 studies

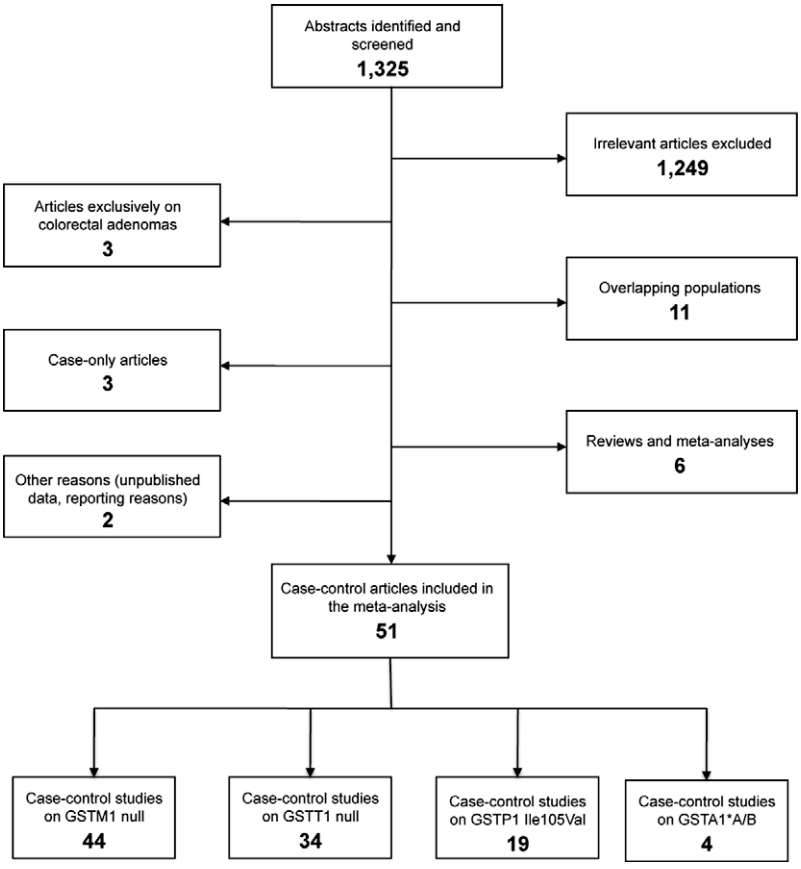

Fig. 1 – Study flow chart explaining the selection of the 51 eligible case-control articles included in the meta-analysis.

Table 1 – Characteristics of the eligible studies.

Study	Country	Ethnicity	Polymorphisms studied	Source and ascertainment of cases	Source of controls	Method	Tumour Stage	Prevalence of risk factors in cases	Prevalence of risk factors in controls	Factor taken into account during OR adjustment
Strange[55]	UK	Caucasian	GSTM1	Individuals with histologically confirmed adenocarcinomas who attended the Surgery Clinic	Post-mortem individuals who died primarily from cardiovascular disease and had no evidence of cancer	Starch gel electrophoresis	N/A	N/A	N/A	N/A
Zhong[56]	UK	Caucasian	GSTM1	Individuals with histologically confirmed diagnosis from one hospital	Randomly selected from two hospitals and a group of volunteers	PCR	N/A	N/A	N/A	N/A
Chenevix-Trench[93]	Australia	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed adenocarcinomas	Unselected and geriatric healthy individuals without cancer or family history of cancer	PCR	N/A	N/A	N/A	N/A
Deakin[92]	UK	Caucasian	GSTM1, GSTT1	Individuals who attended one hospital (ANC)	Unrelated healthy individuals from the same hospital	PCR	N/A	N/A	N/A	N/A
Guo[91]	China	Asian	GSTM1	Individuals with histologically confirmed adenocarcinomas at one hospital	Individuals matched for race, place of birth, sex and age, were recruited from healthy screening programs	PCR	N/A	N/A	N/A	N/A
Katoh[90]	Japan	Asian	GSTM1, GSTT1	Consecutive histologically confirmed adenocarcinoma cases presenting at two hospitals and one medical centre	Individuals recruited from regular medical health check-ups, without gastrointestinal symptoms and previous or current diagnosis of cancer	Multiplex PCR	N/A	N/A	smoking 56.3%	N/A
Harries[18]	UK	Caucasian	GSTP1	Individuals attending chemotherapy, radiotherapy or surgical clinics at one hospital (ANC)	Randomly selected healthy individuals from the Clinical Biochemistry Department of the same hospital	PCR	N/A	N/A	N/A	N/A
Harris[89]	Australia	Caucasian	GSTP1	Individuals with histologically confirmed diagnosis	Randomly selected healthy individuals	PCR-RFLP	N/A	N/A	N/A	N/A
Gertig[88]	USA	98% Caucasian	GSTM1, GSTT1	Nested case-control study using incident cases from the Physicians' Health Study (ANC)	Individuals matched for age	PCR	N/A	smoking 63.5%	smoking 63.8%	BMI, physical activity, alcohol use
Lee[87]	Singapore	Asian	GSTM1	Individuals with histologically confirmed diagnosis who attended the Surgery Clinic	Hospital patients with no history of neoplasms ascertained through a clinical chemistry	PCR	N/A	N/A	N/A	N/A
Slattery[86]	USA	Mixed (91.4% Caucasian, 4.2% African/American, 4.4% Hispanic)	GSTM1	English-speaking mentally competent patients without previous colorectal cancer, aged between 30 and 79 years old, without history of FAP, ulcerative colitis, or Crohn's disease identified from three Cancer Registries in Utah, Northern California, and Minnesota (ANC)	Individuals randomly selected from Health Care Administration lists, random-digit dialing, driver license lists and memberships lists matched for gender and age	PCR	N/A	N/A	N/A	age, energy intake, BMI, long-term physical activity, dietary fibre, usual number of cigarettes smoked
Abdel-Rahman[85]	Egypt	Caucasian	GSTM1, GSTT1	Individuals without history of prior chemotherapy or radiotherapy, recruited from three hospitals (ANC)	Age-matched healthy individuals who were friends of other cancer patients	PCR	N/A	family history of CRC 4.5%	family history of CRC 2%	N/A

Gawronska-Szklarz[84]	Poland	Caucasian	GSTM1	Individuals with histologically confirmed adenocarcinomas without HNPCC	Healthy individuals matched for age and sex	PCR	N/A	N/A	N/A	N/A
Katoh[20]	Japan	Asian	GSTP1	Consecutive histologically confirmed adenocarcinoma cases presenting at two hospitals and one medical centre	Individuals recruited from regular medical health check-ups, without previous or current diagnosis of cancer	PCR-RFLP	N/A	N/A	N/A	gender, age
Welfare[83]	UK	Caucasian	GSTM1, GSTT1, GSTP1	Individuals with histologically confirmed diagnosis at one hospital	Age- and sex-matched community individuals identified from the records of the general practitioner	Multiplex PCR	N/A	N/A	N/A	N/A
Zhang[82]	Sweden	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed adenocarcinomas at the Department of Pathology in two hospitals	Randomly selected healthy individuals without gastrointestinal diseases or history of tumours	Multiplex PCR	Duke: 7(A), 37(B), 27(C), 7(D)	N/A	N/A	N/A
Kiss[81]	Hungary	Caucasian	GSTM1	Individuals with histologically confirmed diagnosis at one hospital	Healthy individuals who participated in a health status examination survey matched for age and sex	PCR	N/A	N/A	N/A	N/A
Butler[80]	Australia	Caucasian	GSTM1, GSTT1	Individuals who attended the Department of Gastroenterology in one hospital, without FAP or HNPCC (ANC)	Individuals matched for gender and attending the blood bank of the hospital for blood donation	PCR-RFLP	N/A	N/A	N/A	age
Loktionov[79]	UK	Caucasian	GSTM1, GSTT1, GSTP1	Individuals with histologically confirmed diagnosis without family history of CRC or multiple cancer who attended the Surgery Clinic	Individuals with a normal flexible sigmoidoscopy	PCR	Duke: 37(A), 79(B), 83(C), 7(D)	smoking 18.8%	smoking 15%	gender, age, smoking status
Saadat[78]	Iran	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed adenocarcinomas	Healthy individuals matched for age and sex	PCR	N/A	N/A	N/A	N/A
Laso[77]	Spain	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed diagnosis who attended the Surgery Clinic	Individuals without a history of malignancy recruited from the Trauma Service	Multiplex PCR	Duke: 4(A), 96(B), 67(C), 69(D)	smoking 40%	smoking 34.8%	N/A
Sachse[9]	UK	Caucasian	GSTM1, GSTT1, GSTP1	Individuals identified through ICD-9, without FAP, inflammatory bowel disease, ulcerative colitis, diverticular disease or previous malignancy (ANC)	Individuals without history of previous cancer, matched for age, sex, and general practioner	TaqMan	Duke: 57(A), 162(B), 148(C1), 25(C2)	N/A	N/A	N/A
Sgambato[10]	Italy	Caucasian	GSTM1, GSTT1	Individuals who attended the Oncology Clinic (ANC)	Healthy subjects visiting the hospital for routine blood tests without personal and/or family history of cancer	PCR	N/A	N/A	N/A	N/A
Sweeney[76]	USA	Caucasian	GSTA1	Individuals with histologically confirmed diagnosis	Individuals matched for race, geographic region, age and sex	PCR-RFLP	N/A	N/A	N/A	age, sex, geographic region, race, current smoking, education, total meat consumption

(*continued on next page*)

Table 1 – *continued*

Study	Country	Ethnicity	Polymorphisms studied	Source and ascertainment of cases	Source of controls	Method	Tumour Stage	Prevalence of risk factors in cases	Prevalence of risk factors in controls	Factor taken into account during OR adjustment
Tiemmersma[75]	Netherlands	Caucasian	GSTM1	Identified through the Netherlands Cancer registry and the municipal registries of three Dutch towns (ANC)	Randomly selected individuals from the same registries, matched for gender, age and centre	Multiplex PCR	N/A	smoking 70.6%	smoking 69.8%	N/A
Ye[74]	UK	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed diagnosis at one hospital	Unrelated healthy individuals from the same hospital	PCR-RFLP	N/A	N/A	N/A	N/A
Zhu[73]	China	Chinese (Han)	GSTM1, GSTT1	Histologically confirmed sporadic adenocarcinoma cases, Department of Oncology, Zhongnan hospital, University of Wuhan	Unrelated healthy individuals from the same hospital, matched for age and gender	PCR	N/A	N/A	N/A	N/A
Nascimento[72]	Brazil	Mixed (90% Caucasian, 10% African-American)	GSTM1, GSTT1	Individuals with histologically confirmed diagnosis at one hospital without inflammatory diseases, adenomatous polyposis or a family history of CRC	Blood donors from the same hospital without gastrointestinal symptoms or previous cancer	Multiplex PCR	TNM: 47 (I or II), 48 (III or IV)	smoking 41.2%	N/A	gender, age
Slattery[71]	USA	Mixed (82% Caucasian, 4.1% African-American, 7.6% Hispanic, 4.6% Asian, 0.7% Native American and 1% multiple ethnicities)	GSTM1	English-speaking mentally competent patients without previous colorectal cancer, aged between 30 and 79 years old, without history of FAP, ulcerative colitis, or Crohn's disease identified from two Cancer Registries in Utah and Northern California (ANC)	Individuals randomly selected from Health Care Administration lists, driver license lists and memberships lists matched for gender and age	PCR	N/A	smoking 53.5%	smoking 48.1%	age, physical activity level, alcohol intake, body size
van der Hel[11]	Netherlands	Caucasian	GSTM1, GSTT1	Individuals identified from a population-based screening program ('DOM' program) (ANC)	Randomly selected individuals from a population-based screening program	Multiplex PCR	N/A	smoking 34.9%	smoking 31%	age, 'DOM' cohort
Kiss[70]	Hungary	Caucasian	GSTM1, GSTT1, GSTP1	Individuals with histologically confirmed diagnosis without FAP, HNPCC and ulcerative colitis	Cancer-free individuals from in- or outpatient wards and volunteers for health status examination, matched for age, gender, smoking habit and red meat consumption	PCR-RFLP	N/A	N/A	N/A	N/A
van der Logt[69]	Netherlands	Caucasian	GSTM1, GSTT1, GSTP1, GSTA1	Individuals who attended the Department of Gastroenterology and General Surgery of one University Hospital (ANC)	Individuals recruited by advertisement in a local paper	PCR-RFLP	Duke: 7(A), 103(B), 81(C), 102 (D)	N/A	N/A	gender, age
Ates[68]	Turkey	Caucasian	GSTM1, GSTT1, GSTP1	Individuals with histologically confirmed adenocarcinomas at two hospitals	Selected among healthy people with no history of cardiovascular disease, cancer, chronic degenerative neurological disease, COPD, hepatitis, diabetes, hypertension, atopy, autoimmune diseases, or allergies	Real-Time PCR	N/A	smoking 50.3%	smoking 41.2%	gender, age

Landi[67]	Spain	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed adenocarcinomas at one hospital	Randomly enrolled patients without cancer or any other chronic disease, matched for sex and age	APEX and oligonucleotide microarray	N/A	smoking 46.8%, family history of CRC 11.7%	smoking 44.1%, family history of CRC 3.8%	gender, age
Rajagopal[12]	UK	Caucasian	GSTT1	Individuals with histologically confirmed adenocarcinomas at one University Hospital	Individuals unrelated to cases without malignant and/or inflammatory conditions	PCR	Duke: 31(A), 158(B), 122(C), 42(D)	N/A	N/A	N/A
Sun[19]	Sweden	Caucasian	GSTP1	Individuals with histologically confirmed adenocarcinomas at one hospital	Individuals with no history of any cancer	PCR-RFLP	N/A	N/A	N/A	N/A
Fan[66]	China	Chinese	GSTM1, GSTT1	Individuals with histologically confirmed diagnosis in Regional Hospitals (Zhejiang province, Jiashan county)	General Survey in Zhejiang province, Jiashan county	PCR-RFLP	N/A	N/A	N/A	gender, age
Huang[65]	USA	39.6% African, 60.4% Caucasian	GSTM1, GSTT1	Identified through the North Carolina Central Cancer Registry (ANC)	Individuals matched for race, age and sex	Multiplex PCR	N/A	smoking 61.7%, alcohol 31.4%, family history of CRC 19.9%	smoking 58.5%, alcohol 32.4%, family history of CRC 8.7%	gender, age and offset terms
Little[64]	UK (Scotland)	Caucasian	GSTM1, GSTT1	Individuals with histologically confirmed diagnosis	Individuals matched for age and sex	PCR	N/A	alcohol 37%, family history of CRC 19%	alcohol 41%, family history of CRC 9%	gender, age, family history of CRC, aspirin use, NSAIDs use, physical activity
Martinez[63]	Spain	Caucasian	GSTM1, GSTT1, GSTP1, GSTA1	Individuals with histologically confirmed diagnosis at one hospital	Unrelated healthy individuals recruited among medical and nursery staff and students	Multiplex PCR	N/A	N/A	N/A	N/A
Probst-Hensch[13]	Singapore	Asian	GSTM1, GSTT1, GSTP1	Individuals with histologically confirmed diagnosis from the Singapore Cancer Registry	Individuals who donated blood or/and buccal cells without history of CRC	TaqMan	TNM: 151 (I or II), 139 (III or IV)	smoking 27%, alcohol 18%, family history of CRC 2%	smoking 41%, alcohol 22%, family history of CRC 4%	gender, age, education, year of recruitment, dialect group
Skjelbred[14]	Norway	Caucasian	GSTM1, GSTT1, GSTP1	Individuals identified in the NORCCAP screening group based on flexible sigmoidoscopy examination and patients operated at hospitals	Individuals identified in the NORCCAP study, with normal results at flexible sigmoidoscopy screening	Multiplex PCR	N/A	smoking 61.5%, alcohol 71.5%	smoking 53.3%, alcohol 78.2%	gender, age, smoking dose, alcohol consumption, total meat consumption and total fruit, berry and vegetable
Yeh[62]	Taiwan	Asian	GSTM1, GSTT1, GSTP1	Patients recruited from one specific hospital with histologically confirmed adenocarcinomas, excluding familial adenomatous polyposis, HNPCC, inflammatory bowel disease and other malignancie.	Age- and sex-matched individuals recruited from health examinations without colorectal diseases, history of other cancers and family history of CRC	PCR-RFLP	N/A	N/A	N/A	gender, age, education

(*continued on next page*)

Table 1 – *continued*

Study	Country	Ethnicity	Polymorphisms studied	Source and ascertainment of cases	Source of controls	Method	Tumour Stage	Prevalence of risk factors in cases	Prevalence of risk factors in controls	Factor taken into account during OR adjustment
Yoshida[61]	Japan	Asian	GSTM1	Patients recruited at two specific hospitals (ANC)	Healthy subjects without cancer recruited at two hospitals	PCR-RFLP	N/A	smoking 39.4%	smoking 50.4%	gender, age, smoking habit
Vlaykova[60]	Bulgaria	Caucasian	GSTP1	Surgical patients with adenocarcinomas identified by the accepted protocols in Bulgaria for surgical interventions and obtaining of human biopsies	Normal volunteers without indications for CRC, not related to cases and attending the regular annual prophylactic examinations	PCR-RFLP	TNM: 8 (I), 53 (II), 13 (III), 8 (IV)	N/A	N/A	N/A
Cotterchio[15]	Canada	Caucasian	GSTM1, GSTT1	Histologically confirmed incident colorectal cancer cases aged from 20 to 74 years identified through the Ontario Cancer Registry	Population-based controls randomly selected and frequency-matched, within sex and 5-year age groups, identified from a list of residential telephone numbers	TaqMan	N/A	N/A	N/A	gender, age
Csejtei[16]	Hungary	Caucasian	GSTM1, GSTT1	Histologically confirmed adenocarcinoma cases who had an operation for an adenocarcinoma in two hospitals	Age- and sex-matched healthy individuals from hospitals' archive data	PCR	Duke: 14(A), 34(B), 46 (C), 8(D)	N/A	N/A	N/A
Kury[59]	France	Caucasian	GSTM1, GSTT1, GSTP1, GSTA1	Histologically confirmed cases aged >40 years old, recruited in regional hospitals and clinics with a personal history of CRC, excluding those suspected of having a familial form of CRC	Age- and sex-matched healthy individuals from two regional Health Examination Centres, without a familial history of CRC or polyps	TaqMan	N/A	N/A	N/A	gender, age
Epplein[58]	America	Mixed (41.6% Japanese American, 17.9% African-American, 19.1% Latino, 15% Caucasian, 6.4% Native Hawaiian)	GSTM1, GSTT1, GSTP1	Identified through tumour registries of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute (ANC)	Individuals who contributed blood to the biorepository and remain alive and free of CRC at the age of the case's diagnosis	TaqMan	N/A	smoking 60%, family history of CRC 10%	smoking 53%, family history of CRC 8%	age at blood draw, hours of fasting before blood draw, processed meat, ethanol, obesity, history of colorectal cancer screening
Matakova[17]	Slovakia	Caucasian	GSTM1, GSTT1, GSTP1	Individuals with histologically confirmed diagnosis who attended the Surgery Clinic and Oncology Centre	Heathy volunteers from the same geographic region with the cases, matched for age, gender and ethnicity	PCR	N/A	N/A	N/A	N/A
Zupa[57]	Italy	Caucasian	GSTM1	Individuals with adenocarcinomas who attended the Oncology Clinic (ANC)	Healthy subjects visiting the hospital for routine blood tests without personal and/or family history of cancer	PCR	N/A	N/A	N/A	N/A

Abbreviations: ANC, ascertainment not clarified; FAP, familial adenomatous polyposis; HNPCC, hereditary non-polyposis colorectal cancer; CRC, colorectal cancer; ICD, international classification of diseases; COPD, chronic obstructive pulmonary disease; NORCCAP, NORwegian colorectal cancer prevention study; N/A, not available.

on Caucasian populations,[9,11,14–17,55–57,59,63–65,67–70,74,75,77–85,88,92,93] eight studies on Chinese populations[13,61,62,66,73,87,90,91], four studies on mixed populations[58,71,72,86] and one study on an African-American population[65]). Thirty-four case-control studies[9–17,58,59,62–66,68–70,72–74,77–80,82,83,85,88,90,92,93] were eligible concerning GSTT1 genotype (8596 cases, 13,589 controls, i.e. 26 studies on Caucasian populations,[9–12,14–17,59,63–65,68–70,74,77–80,82,83,85,88,92,93] five studies on Chinese populations,[13,62,66,73,90] two studies on mixed populations[58,72] and one study on an African-American population[65]). Nineteen case-control studies[9,13,14,17–20,58–60,62,63,67–70,79,83,89] were included regarding GSTP1 polymorphism (5421 cases, 7671 controls, i.e. 15 studies on Caucasian populations,[9,14,17–19,59,60,63,67–70,79,83,89] three studies on Chinese populations[13,20,62] and one study on mixed populations[58]) and four case-control studies[59,63,69,76] on GSTA1 polymorphism (1648 cases, 2039 controls, all of Caucasian origin). Evidently, the sum of studies surpasses the number of eligible articles, as one or more studies were presented per article.

3.2. *Pooled effects of genotypes*

At the overall analysis, the null GSTM1 genotype was associated with increased colorectal cancer risk (pooled OR = 1.114, 95% confidence interval (CI): 1.040–1.194, random effects). The association seemed confined to Caucasian populations (pooled OR = 1.150, 95% CI: 1.060–1.248, random effects, Fig. 2a), as the association did not reach significance in the subset of Chinese studies (pooled OR = 1.025, 95% CI: 0.903–1.163, fixed effects, Fig. 2b) or in studies on mixed populations (pooled OR = 0.918, 95% CI: 0.829–1.015, fixed effects). The results remained practically unchanged when the adjusted ORs were entered in the meta-analysis model (in case the former were available in the individual studies). Specifically, at the overall analysis pooled OR = 1.098, 95% CI: 1.026–1.176, random effects; on Caucasian populations pooled OR = 1.147, 95% CI: 1.045–1.259, random effects; on Chinese populations pooled OR = 1.010, 95% CI: 0.889–1.147, fixed effects; on mixed populations pooled OR = 0.967, 95% CI: 0.855–1.094, random effects.

Similarly, at the overall analysis, the null GSTT1 genotype was associated with increased colorectal cancer risk (pooled OR = 1.202, 95% CI: 1.045–1.382, random effects). The association seemed confined to Caucasian populations (pooled OR = 1.312, 95% CI: 1.119–1.538, random effects, Fig. 3a), as the association did not reach significance in the subset of Chinese studies (pooled OR = 1.068, 95% CI: 0.788–1.449, random effects, Fig. 3b). No meta-analysis was performed on mixed populations, as solely two studies were performed on the latter. Once again, the results remained practically unchanged when the adjusted ORs were entered in the meta-analysis model. Specifically, at the overall analysis pooled OR = 1.187, 95% CI: 1.043–1.350, random effects; on Caucasian populations pooled OR = 1.297, 95% CI: 1.096–1.535, random effects; on Chinese populations pooled OR = 1.074, 95% CI: 0.775–1.487, random effects.

Regarding GSTP1 Ile105Val, the results of the meta-analysis are presented in detail in Table 2. No statistically significant associations were detected. The results of the recessive model at the overall analysis are depicted in Fig. 4. Interestingly, incorporation of the adjusted ORs for heterozygous and homozygous Ile105Val carriers resulted in the emergence of a positive association in heterozygous carriers of Caucasian origin (pooled OR = 1.096, 95% CI: 1.003–1.199, fixed effects); all other associations remained statistically not significant (data not shown for the reason of brevity). For the optimal interpretation of the finding, the reader is referred to the respective sensitivity analysis (see below).

Regarding GSTA1 status, no significant associations were demonstrated. Specifically, for heterozygous (GSTA1*A/*B) carriers pooled OR = 1.029, 95% CI: 0.889–1.190, fixed effects; for homozygous (GSTA1*B/*B) carriers pooled OR = 1.090, 95%

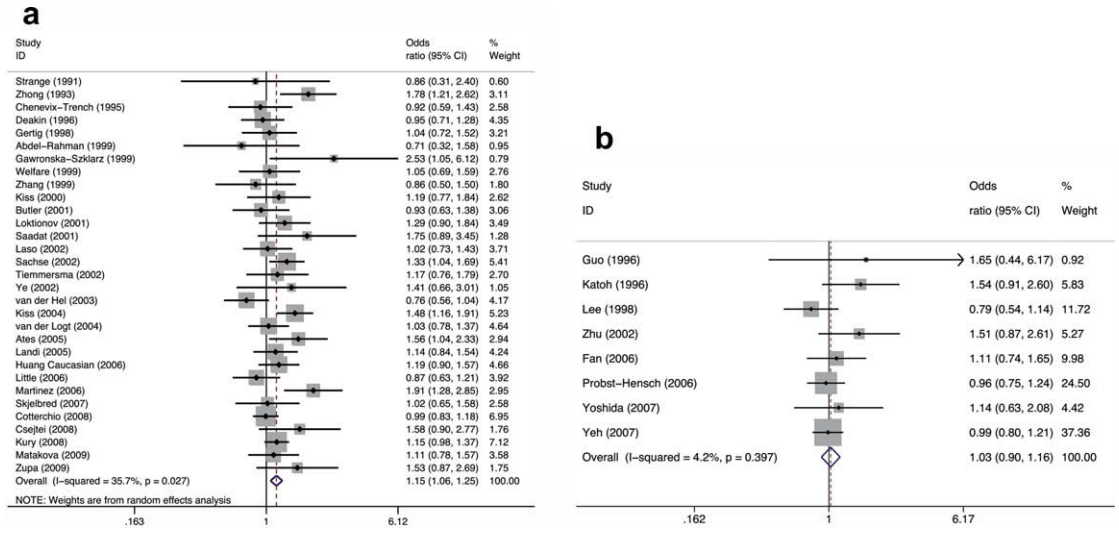

Fig. 2 – Forest plot for the overall association between null GSTM1 genotype and colorectal cancer risk for (a) Caucasian and (b) Chinese subjects. Each study is shown by the point estimate of the Odds Ratio (OR) (the size of the square is proportional to the weight of each study) and 95% confidence interval for the OR (extending lines); the pooled OR and 95% confidence interval have been appropriately derived from: (a) random and (b) fixed-effects models.

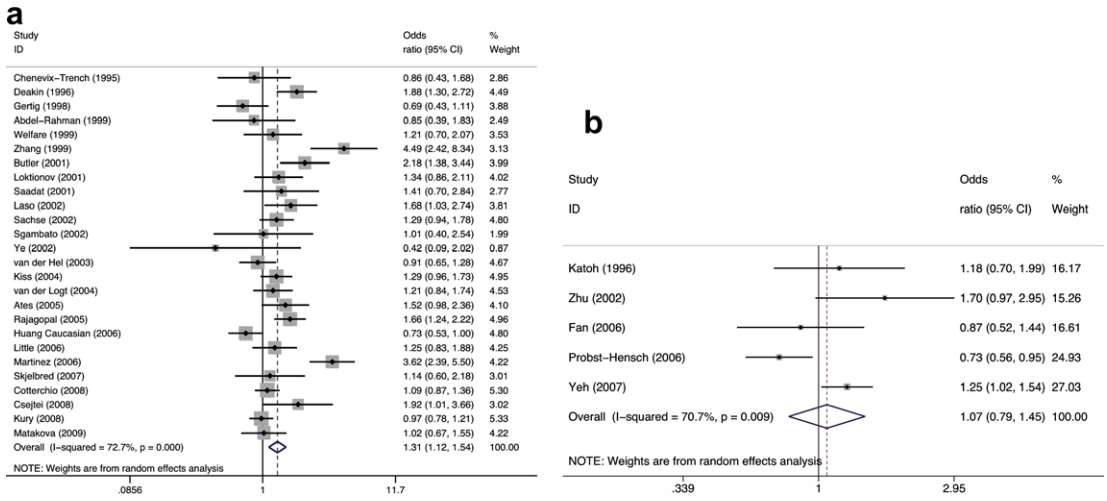

Fig. 3 – Forest plot for the overall association between null GSTT1 genotype and colorectal cancer risk for (a) Caucasian (random effects) and (b) Chinese subjects (random effects).

CI: 0.898–1.322, fixed effects; for the dominant model pooled OR = 1.044, 95% CI: 0.911–1.198, fixed effects and for the recessive model pooled OR = 1.173, 95% CI: 0.872–1.578, random effects. Subgroup analysis was not feasible as all studies were conducted on Caucasian populations. The results remained practically unchanged when the adjusted ORs were entered in the meta-analytical model; specifically, for heterozygous (GSTA1*A/*B) carriers pooled OR = 1.038, 95% CI: 0.896–1.204, fixed effects; for homozygous (GSTA1*B/*B) carriers pooled OR = 1.210, 95% CI: 0.837–1.749, random effects.

3.3. *Meta-regression and publication bias*

Meta-regression with publication year did not point to any association between publication year and the effect of GSTM1 null genotype (exponentiated coefficient = 1.005, 95% CI: 0.988–1.023, $p = 0.551$), or GSTT1 null genotype (exponentiated coefficient = 0.977, 95% CI: 0.938–1.018, $p = 0.266$). Similarly, meta-regression did not reveal any association between publication year and the effect of GSTP1 Ile105Val status at any analysis (data not shown for reasons of brevity). Concerning GSTA1 polymorphism status meta-regression was not performed due to the limited number of studies.[94]

Publication bias was significant in the meta-analysis for GSTM1 ($p = 0.005$), while it was not significant in the meta-analyses for GSTT1 ($p = 0.450$). With respect to GSTP1 publication bias was detected at the recessive model ($p = 0.095$), but not at any other analyses ($p = 0.833$ for heterozygous carriers; $p = 0.121$ for homozygous carriers and $p = 0.984$ for the dominant model). Similarly, concerning GSTA1 polymorphism status publication bias was detected at the recessive model ($p = 0.095$) but not at any other analyses ($p = 0.983$ for heterozygous carriers; $p = 0.159$ for homozygous carriers and $p = 0.544$ for the dominant model).

3.4. *Sensitivity analysis*

Regarding GSTP1 Ile105Val the examination of genotype frequencies in controls revealed significant deviation from HWE in three[20,58,68] of 19 studies. Specifically, the goodness-of-fit chi-square was as follows: chi2 (1) = 3.945, $p = 0.047$ for the study by Katoh and colleagues[20]; chi2 (1) = 10.618, $p = 0.001$ for the study by Ates and colleagues[68]; chi2 (1) = 13.661, $p = 0.0002$ for the study by Epplein and colleagues.[58] After the exclusion of the three studies significantly departing from HWE the results based on crude ORs remained practically unchanged. Specifically, at the overall analysis the pooled ORs were as follows: 1.050 (0.945–1.166, random effects) for heterozygous carriers, 0.939 (0.782–1.127, random effects) for homozygous carriers, 1.025 (0.922–1.138, random effects) for the dominant model and 0.936 (0.823–1.065, fixed effects) for the recessive model. Similarly no significant modifications were noted in race-specific analyses (data not shown for reasons of brevity). Importantly, the result on heterozygous Caucasian Ile105Val carriers, which had been derived from the incorporation of adjusted ORs, disappeared after the exclusion of the three studies significantly departing from HWE (pooled OR = 1.071, 95% CI: 0.950–1.208, fixed effects).

With respect to GSTA1 polymorphism status, no sensitivity analysis was needed as no significant deviation from HWE was demonstrated among controls of the included studies.

4. Discussion

The principal message of this meta-analysis is the establishment of a positive association between GSTM1 and GSTT1 null genotypes and colorectal cancer risk. The effects of GSTM1 and GSTT1 null genotypes tend indeed to be confined solely in Caucasians; importantly, this meta-analysis does not confirm the link between GSTT1 null genotype and colorectal cancer in East Asian populations suggested by Liao and colleagues.[8] Noticeably also, this meta-analysis does not confirm the isolated protective effect at the recessive model that Gao and colleagues[4] observed. These discrepancies can be attributed to the more comprehensive and elaborate approach in the present meta-analysis, leading to the inclusion

Table 2 – Pooled ORs by race for heterozygous, homozygous carriers, dominant and recessive model for the association between GSTP1 Ile105Val polymorphism and colorectal cancer. All pooled ORs were derived from fixed-effects models except for cells marked with [R] (random-effects model).

Race	Heterozygous (AG versus AA)		Homozygous (GG versus AA)		Dominant model (GG and AG versus AA)		Recessive model (GG versus AA and AG)	
	OR (95%CI)	Test for heterogeneity	OR (95%CI)	Test for heterogeneity	OR (95%CI)	Test for heterogeneity	OR (95%CI)	Test for heterogeneity
Overall (n = 19)	1.066[R] (0.963–1.179)	p = 0.065	0.905[R] (0.762–1.075)	p = 0.078	1.028[R] (0.932–1.134)	p = 0.052	0.906 (0.801–1.024)	p = 0.137
Caucasian (n = 15)	1.084[R] (0.966–1.218)	p = 0.093	0.960[R] (0.797–1.156)	p = 0.088	1.054[R] (0.942–1.179)	p = 0.072	0.939 (0.824–1.070)	p = 0.133
Chinese (n = 3)	1.067[R] (0.779–1.462)	p = 0.073	0.699 (0.443–1.104)	p = 0.412	0.972 (0.823–1.148)	p = 0.137	0.705 (0.448–1.110)	p = 0.382

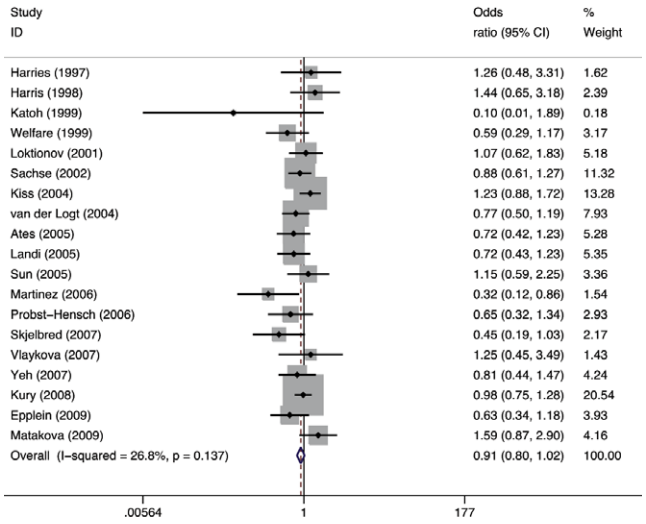

Fig. 4 – Forest plot for the overall association between the GSTP1 Ile105Val polymorphism and colorectal cancer risk following a recessive model for the overall analysis (fixed effects).

of additional nine studies (2636 cases, 5573 controls) on GSTT1 null genotype and two studies (225 cases, 410 controls) on GSTP1 Ile105Val.

Regarding race-specific effects the analogy that emerged between GSTM1 and GSTT1 is noteworthy; both genotypes do not appear to confer any additional risk in Chinese subjects. This pattern has also been observed in the context of breast cancer concerning GSTT1[28] null genotype. It should be declared, however, that our algorithm focused on Chinese studies, whereas the meta-analysis by Liao and colleagues[8] subclassified studies into 'Asian' and 'non-Asian'; as a result the study by Saadat and colleagues[78] performed on Iranian subjects (i.e. Indo-european origin) had been finally pooled with Chinese studies. The aforementioned pooling does not seem in line with data derived from physical anthropology, as the latter point to the fairly distinct genetic features of Indo-european and Chinese populations.[32,33] Given the relatively high odds ratio of the study by Saadat and colleagues,[78] as well as the omission of one study,[13] the results having pointed to the effects of GSTT1 null genotype in East Asians seem worth reconsidering and criticising. At any case, given that solely five studies have appeared on Chinese populations, further studies seem desirable for the establishment of more robust results.[94] At present, however, GSTM1 and GSTT1 seem to act as low-penetrance susceptibility genes for colorectal cancer solely in Caucasian populations.

Contrary to the effect mediated by GSTM1 and GSTT1 null genotypes, this meta-analysis does not support that either GSTA1*B or GSTP1 Ile105Val polymorphism is capable of conferring any additional colorectal cancer risk. Interestingly, the lack of association pertained to any possible categorisation of GSTA1-GSTP1 genotypes, i.e. allele carriers grouped together, homozygous and heterozygous examined separately. This may reflect differential physiological functions mediated by GST isoenzymes, as reviewer by Gao and colleagues[4]; this meta-analysis points to the need for biochemical studies explaining why GSTA1 and GSTP1 genotype variations seem

deprived of the cancer-predisposing features of GSTM1 and GSTT1 null variants. As mentioned above, it seems that the inclusion of the two additional studies renders the reported protective, recessive effects of GSTP1 Ile105Val questionable.

Noticeably, an aspect that points to the validity of the results presented in this meta-analysis is the fact that they persisted after performing a sensitivity analysis and after taking into account the adjusted ORs published in the individual studies, as an alternative approach to the calculation of crude ORs. Specifically, performing the meta-analysis without studies whose genotype frequencies in controls significantly departed from HWE, did not result in any substantial modification of results on crude ORs pertaining to GSTP1 Ile105Val. The sensitivity analysis has been performed due to the fact that deviation from HWE may point to methodological weaknesses, such as biased selection of subjects, genotyping errors or population stratification.[36] As a result, the sensitivity analysis further substantiates the point that for the time being, any protective recessive actions are questionable.

Comparing the results of the present meta-analysis with other recent meta-analyses on cancers of the gastrointestinal tract, it is worth mentioning that race-specific effects have been demonstrated in the case of the GSTT1 null genotype-gastric cancer links.[78,95] Specifically, similarly to the present results, both meta-analyses on gastric cancer have concluded that GSTT1 polymorphism confers additional risk in Caucasians, but not in Chinese population. This is of special importance, as a pattern arises, putting the stress on the effect of GSTT1 polymorphism in Caucasian populations. On the other hand, concerning GSTM1 polymorphism, the results of the present meta-analysis are in discrepancy with those in gastric cancer,[96] as the latter meta-analysis pointed to increased risk in Asian subjects.

A potential limitation of this meta-analysis is the fact that no gene-environment interactions have been examined. GSTM1 actively participates in the deactivation of carcinogenic intermediates of polycyclic aromatic hydrocarbons.[2] As the latter are present in tobacco smoke and in diet,[1] the tobacco-GST interaction has drawn considerable attention, since it might potentially implicate a variety of cancer types, including lung, bladder and colorectal cancer.[2] Nevertheless, a recent meta-analysis in the context of colorectal adenoma/cancer risk did not point to any significant tobacco-genotype interaction for GSTM1; solely a non-significant positive interaction between GSTT1 null genotype and smoking emerged only for colorectal adenoma risk.[3] Nevertheless, GSTs are multifunctional genes and possible gene-environment interactions are numerous. For instance, genotype-diet interactions may also exist; more pronounced effects of cruciferous vegetables in GSTM1 null carriers have been postulated, as GSTs augment the elimination of isothiocyanates from the body.[97]

In conclusion, this meta-analysis demonstrates that GSTM1 and GSTT1 null genotypes seem to be risk factors for colorectal cancer in Caucasians. On the contrary, according to the existing data neither GSTA1*B allele nor GSTP1 Ile105Val status seem to confer additional risk for colorectal cancer.

Conflict of interest statement

None declared.

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