RESEARCH ARTICLE

The -765G>C Polymorphism in the Cyclooxygenase-2 Gene and Digestive System Cancer: a Meta-analysis

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Abstract

Background: Published data regarding associations between the -765G>C polymorphism in cyclooxygenase-2 (*COX-2*) gene and digestive system cancer risk have been inconclusive. The aim of this study was to comprehensively evaluate the genetic risk of the -765G>C polymorphism in the *COX-2* gene for digestive system cancer. Materials and Methods: A search was performed in Pubmed, Medline (Ovid), Embase, CNKI, Weipu, Wanfang and CBM databases, covering all studies until Feb 10, 2014. Statistical analysis was performed using Revman5.2. Results: A total of 10,814 cases and 16,174 controls in 38 case-control studies were included in this meta-analysis. The results indicated that C allele carriers (GC+CC) had a 20% increased risk of digestive system cancer when compared with the homozygote GG (odds ratio (OR)=1.20, 95% confidence interval (CI), 1.00-1.44 for GC+CC *vs* GG). In the subgroup analysis by ethnicity, significant elevated risks were associated with C allele carriers (GC+CC) in Asians (OR = 1.46, 95% CI=1.07-2.01, and *p*=0.02) and Africans (OR=2.12, 95% CI=1.57-2.87, and *p*< 0.00001), but not among Caucasians, Americans and mixed groups. For subgroup analysis by cancer type (GC+CC *vs* GG), significant associations were found between the -765G>C polymorphism and higher risk for gastric cancer (OR=1.64, 95% CI=1.03-2.61, and *p*=0.04), but not for colorectal cancer, oral cancer, esophageal cancer, and others. Regarding study design (GC+CC *vs* GG), no significant associations were found in then population-based case-control (PCC), hospital-based case-control (HCC) and family-based case-control (FCC) studies. Conclusions: This meta-analysis suggested that the -765G>C polymorphism of the *COX-2* gene is a potential risk factor for digestive system cancer in Asians and Africans and gastric cancer overall.

Keywords: Cyclooxygenase-2 - digestive system cancer - meta-analysis - polymorphism

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Introduction

Digestive system cancer is a sophisticated disease that results from complex interactions between environmental factors, genetic variants, and gene-environment interactions (Berlau et al., 2004; Pharoah et al., 2004; Yaghoobi et al., 2004). *Helicobacter pylori (H. pylori)* infection, environmental tobacco smokes and lifestyle represent the most important exogenous risk factors (Cook et al., 2010). Although these factors have been documented to influence the risk of cancer, not all individuals develop the disease, even though they are exposed in the same environment. This indicates that genetic differences, such as variants, may contribute to cancer pathogenesis. Therefore, numerous published studies have focused on the association of genetic variants with cancer susceptibility. And among them, cyclooxygenase (COX) gene has been extensively studied.

The COX gene, mapped to chromosome 1q25.2-q25.3 in human, is 8.3 kb in size, contains 10 exons and produces an mRNA of 4.6 kb, which encodes a constitutive isoenzyme (*COX-1*) and an inducible isoenzyme (*COX-2*) (Tazawa et al., 1994). COX-1 is constitutively expressed and is involved in the homeostasis of various physiological functions (Dubois et al., 1998), while *COX-2*, known as rate-limiting enzyme produced during the production of prostaglandins, is often undetectable in normal tissue, whereas in tumor tissue specimens its expression is observably higher (Harrison et al., 1994; Seibert et al., 1994; Wu et al., 1996; Bakhle et al., 2001; Cao et al., 2002; Wang et al., 2007). This gene is polymorphic, and a large number of single nucleotide polymorphisms (SNPs) have been identified, such as -765G>C (reference SNP ID, rs20417), -1195G>A (rs689466), -8473T>C (rs5275), -1759G>A (rs3218625), -202C>T (rs2745557), and -1290A>G (rs689466). Among all of these polymorphisms, the -765G>C polymorphisms in *COX-2* gene were the most widely studied for their implication in cancer risk. Several meta-analyses investigating this -765G>C polymorphism of *COX-2*

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gene with digestive system cancer risks were performed; however, the results were inconsistent for different ethnicity and cancer types (Dong et al., 2010; Liu et al., 2010; Wang et al., 2013; Yan et al., 2013). Therefore, we conducted this meta-analysis on all eligible case-control studies to estimate the overall digestive system cancer risk associated with this polymorphism.

Materials and Methods

Selection of studies

A systematic literature search of the Pubmed, Medline (Ovid), Embase, CNKI, Weipu database, Wanfang database and CBM database was carried out to identify studies involving association between the -765G>C polymorphism of *COX-2* gene and digestive system cancer risk (updated on Feb 10, 2014). The search terms were used as follows: (cyclooxygenase-2 or *COX-2* or PTGS2) in combination with (polymorphism or variant or mutation) and (cancer or carcinoma or neoplasm). The search results were limited to English and Chinese languages. Studies included in our meta-analysis met the following inclusion criteria: (1) evaluation of the -765G>C polymorphism of *COX-2* gene and digestive system cancer risk, (2) the design had to be a case-control design published in a journal, (3) genotype distributions in both cases and controls were available for estimating an odds ratio with 95% confidence interval (CI) and P value, and (4) genotype distributions in control group should be consistent with Hardy-Weinberg equilibrium (HWE). Studies were excluded if one of the following existed: (1) no controls, (2) genotype frequencies or number not reported, and (3) abstracts, reviews. For duplication or overlapping publications, the studies with larger number of cases and controls or been published latest were included.

Data extraction

Two independent reviewers (FZ and CY) collected the data and reached a consensus on all items. In case of disagreement, a third author (HZ) would assess these articles. A standardized data form was used and included: first author's name, year of publication, original country, ethnicity, cancer type, study design, total number of cases and controls, genotyping method, and genotype distribution in cases and controls.

Statistical analysis

Odds ratios (ORs) with 95% CI was used to assess the strength of association between the *COX-2*-765G>C polymorphism and digestive system cancer risk. We first estimated with the risk of genetic model (CC+GC *vs* GG), and then estimated the risk of (C *vs* G) model. The pooled OR was calculated by a fixed-effects model or a random-effects model according to the heterogeneity. Heterogeneity was checked by a χ^2-based Q statistic and P <0.10 was considered statistically significant. If the result was $p>0.10$, OR was pooled according to the fixed-effect model; otherwise, the random effect model was used. The statistical significance of OR was analyzed by Z test, and $p<0.05$ was considered as statistically significant. To evaluate the ethnicity-specific, cancer type-specific, study design-specific effects, we performed stratification analyses on ethnicity, cancer type, and study design. For the subgroup analysis by ethnicity, the study populations were stratified into four groups: Asians, Caucasians, Americans, Africans, Mixed (if it was difficult to discriminate the ethnicity of participants according to the data presented, the study was termed "Mixed"). Subgroup analysis by cancer type were preformed if one cancer type contained three and more than three individual studies (if 1 cancer type was investigated <3 individual case-control studies, then it was combined into the group of "others"). In addition, subjects were categorized into different classifications according to study design: population-based case-control study (PCC), hospital-based case-control study (HCC), and family-based case-control study (FCC).

Sensitivity analysis was also performed by sequence excluding individual study to check the robustness of the result (Zhang et al., 2010). The possible publication bias was examined visually in a Begg's funnel plot and the degree of asymmetry was tested by Egger's test (Begg et al., 1994; Egger et al., 1997). HWE was tested by Pearson's χ^2 test (Zhang et al., 2010). Statistical analysis was performed using Reman5.2 software (The Cochrane Collaboration, www.cochrane.org).

Results

Study inclusion and characteristics

The initial search identified 194 studies from the selected electronic databases (Figure 1). After reading the titles and abstracts, 95 potential articles were included for full-text view. After reading the full texts, 50 studies were excluded for being irrelevant to digestive system cancer risk and *COX-2* gene. Therefore, 45 full-text articles remained for data extraction. 1 articles were excluded for not present usable data (Kamal et al., 2012), An additional 3 articles were excluded for repeat or overlapping studies (Zhang et al., 2005; Zhang et al., 2006; Zhang et al., 2011). Therefore, a total of 41 case-control studies published in 40 articles were identified. However, the control group genotypes in 4 case-control studies were not consistent with HWE (KX et al., 2008; Akkiz et al., 2011; Talar-Wojnarowska et al., 2011; Kamal et al., 2012), and these

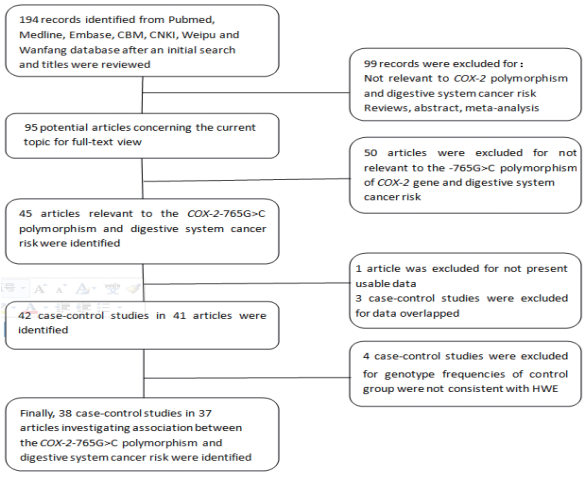

Figure 1. Flow Diagram of Included/Excluded Studies

studies were excluded. Thus, a final total of 38 case-control studies in 37 articles which met our inclusion criteria were identified, including 10814 cases and 16174 controls. Among 38 cases and controls, there were 20 case-controls of Asians (Hamajima et al., 2001; Koh et al., 2004; Liu et al., 2006; Guo et al., 2007; Tan et al., 2007; Chiang et al., 2008; Lin et al., 2008; Saxena et al., 2008; Ueda et al., 2008; Xing et al., 2008; Xu et al., 2008; Tang et al., 2009; Upadhyay et al., 2009; Zhao et al., 2009; Mittal et al., 2010; He et al., 2011; Zhang et al., 2011; Chang et al., 2012; Li et al., 2012; Shin et al., 2012), 11 of Caucasians (Cox et al., 2004; Pereira et al., 2006; Hou et al., 2007; Moons et al., 2007; Sitarz et al., 2008; Andersen et al., 2009; Hoff et al., 2009; Iglesias et al., 2009; Kristinsson et al., 2009; Pereira et al., 2010; Daraei et al., 2012), 4 of Americans (Ulrich et al., 2005; Gunter et al., 2006; Gong et al., 2009; Thompson et al., 2009), and 1 of Africans (Bye et al., 2011), and 2 of Mixed (Bye et al., 2011; Wang et al., 2012). 9 investigated gastric cancer (Liu et al., 2006; Pereira et al., 2006; Hou et al., 2007; Saxena et al., 2008; Sitarz et al., 2008; Tang et al., 2009; Zhang et al., 2011; Li et al., 2012; Shin et al., 2012), 16 investigated colorectal cancer (Hamajima et al., 2001; Cox et al., 2004; Koh et al., 2004; Ulrich et al., 2005; Gunter et al., 2006; Tan et al., 2007; Xing et al., 2008; Ueda et al., 2008; Andersen et al., 2009; Gong et al., 2009; Hoff et al., 2009; Iglesias et al., 2009; Thompson et al., 2009; Pereira et al., 2010; Daraei et al., 2012; Wang et al., 2012), 3 investigated oral cancer (Chiang et al., 2008; Lin et al., 2008; Mittal et al., 2010), 6 investigated esophageal cancer (Guo et al., 2007; Moons et al., 2007; Kristinsson et al., 2009; Upadhyay et al., 2009; Bye et al., 2011), and 4 investigated pancreatic cancer and hepatocellular carcinoma (HCC) (Xu et al., 2008; Zhao et al., 2009; He et al., 2011; Chang et al., 2012). Of these articles, 18 studies were performed in HB (Hamajima et al., 2001; Cox et al., 2004; Gunter et al., 2006; Pereira et al., 2006; Guo et al., 2007; Chiang et al., 2008; Lin et al., 2008; Saxena et al., 2008; Xing et al., 2008; Xu et al., 2008; Hoff et al., 2009; Iglesias et al., 2009; Upadhyay et al., 2009; Mittal et al., 2010; Pereira et al., 2010; He et al., 2011; Chang et al., 2012; Shin et al., 2012), 19 studies were performed in PB (Ulrich et

Table 1. Characteristics of the Studies Included in Meta-analysis

First author	Year	Country	Ethnicity	Cancer type	Study design	No. (Cases/Controls)	Genotyping method
Pereira et al	2006	Portugal	Caucasians	Gastric	HCC	73/ 210	PCR-RFLP
Liu et al	2006	China	Asians	Gastric	PCC	247/427	DHPLC
Hou et al	2007	Poland	Caucasians	Gastric	PCC	290/409	TaqMan
Saxena et al	2008	India	Asians	Gastric	HCC	62/241	PCR-RFLP
Sitarz et al	2008	Netherlands	Caucasians	Gastric	PCC	241/100	PCR-sequence
Tang et al	2009	China	Asians	Gastric	PCC	100/105	PCR-RFLP
Zhang et al	2011	China	Asians	Gastric	PCC	323/944	PCR—RFLP
Li et al	2012	China	Asians	Gastric	PCC	296/319	PCR—RFLP
Shin et al	2012	Korea	Asians	Gastric	HCC	100/100	PCR-RFLP
Hamajima et al	2001	Japan	Asians	Colorectal	HCC	148/241	PCR-CTPP
Cox et al	2004	Spain	Caucasians	Colorectal	HCC	220/257	TaqMan
Koh et al	2004	Singapore	Asians	Colorectal	PCC	310/1177	TaqMan
Ulrich et al	2005	America	Americans	Colorectal	PCC	494/584	PCR-RFLP
Gunter et al	2006	America	Americans	Colorectal	HCC	210/196	PCR-RFLP
Tan et al	2007	China	Asians	Colorectal	PCC	1000/1300	PCR-RFLP
Xing et al	2008	China	Asians	Colorectal	HCC	137/199	PCR-RFLP
Ueda et al	2008	Japan	Asians	Colorectal	PCC	455/1051	PCR-RFLP
Gong et al	2009	America	Americans	Colorectal	PCC	162/211	PCR-RFLP
Iglesias et al	2009	Spain	Caucasians	Colorectal	HCC	284/123	PCR-RFLP
Thompson et al	2009	America	Americans	Colorectal	PCC	421/479	TaqMan
Andersen et al	2009	Denmark	Caucasians	Colorectal	PCC	359/765	TaqMan
Hoff et al	2009	Netherlands	Caucasians	Colorectal	HCC	326/369	PCR-RFLP
Pereira et al	2010	Portugal	Caucasians	Colorectal	HCC	117/256	PCR-RFLP
Daraei et al	2012	Iran	Caucasians	Colorectal	PCC	110/120	PCR-RFLP
Wang et al	2012	Multicenter	Mixed	Colorectal	FCC	305/359	PCR-RFLP
Chiang et al	2008	China	Asians	Oral	HCC	178/205	PCR-RFLP
Lin et al	2008	China	Asians	Oral	HCC	297/280	PCR-RFLP
Mittal et al	2010	India	Asians	Oral	HCC	176/96	PCR-RFLP
Guo et al	2007	China	Asians	Esophageal	HCC	1026/1270	PCR-RFLP
Moons et al	2007	Netherlands	Caucasians	Esophageal	PCC	140/495	PCR-RFLP
Upadhyaya et al	2009	India	Asians	Esophageal	HCC	174/216	PCR-RFLP
Kristinsson et al	2009	Netherlands	Caucasians	Esophageal	PCC	222/236	PCR-RFLP
Bye et al (A)	2011	South Africa	Africans	Esophageal	PCC	347/462	TaqMan
Bye et al (M)	2011	South Africa	Mixed	Esophageal	PCC	190/422	TaqMan
Xu et al	2008	China	Asians	Pancreatic	HCC	283/566	PCR-RFLP
Zhao et al	2009	China	Asians	Pancreatic	PCC	393/786	PCR-RFLP
He et al	2011	China	Asians	HCC*	HCC	300/300	PCR-RFLP
Chang et al	2012	China	Asians	HCC*	HCC	298/298	PCR-RFLP

*HCC, hepatocellular carcinoma; population-based case-control study (PCC), hospital-based case-control study (HCC), and family-based case-control study (FCC)

al., 2005; Liu et al., 2006; Hou et al., 2007; Moons et al., 2007; Tan et al., 2007; Sitarz et al., 2008; Ueda et al., 2008; Andersen et al., 2009; Gong et al., 2009; Kristinsson et al., 2009; Tang et al., 2009; Thompson et al., 2009; Zhao et al., 2009; Bye et al., 2011; Zhang et al., 2011; Daraei et al., 2012; Li et al., 2012), 1 in FB (Wang et al., 2012). Different genotyping methods were used, including TaqMan, Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), DHPLC (PCR-based denaturing high-performance liquid chromatography). The characteristics of each case-control study are listed in Table 1. Genotype and allele distributions for each case-control study are shown in Table 2.

Quantitative data synthesis

All studies: As shown in Figure 2, the heterogeneity of (CC+ GC *vs* GG) for all 38 studies was assessed and the value of χ^2 was 259.68 with 37 degrees of freedom and $p<0.00001$ in a random-effects model. Additionally, the I-square, which is another index of the test of heterogeneity, was 86%, suggesting a moderate heterogeneity. Thus, we chose the random-effects model to synthesize the data. Overall, OR was 1.20 (95% CI=1.00-1.44), and the test for overall effect Z value was 2.01 ($p=0.04$) for (CC+GC *vs* GG) genetic model. The results suggested the C allele carriers (CC+GC) may have a 20% increased risk compared with the homozygote GG. Summary results for all comparisons are presented in Table 3.

Subgroup analyses: In the subgroup analysis by ethnicity (GC+CC *vs* GG, Figure 3), the analysis was stratified into four subgroups: Asians (6303 cases and 10121 controls), Caucasians (2382 cases and 3340 controls), Americans (12867 cases and 1470 controls), Africans (347 cases and 422 controls), and Mixed (495 cases and 821 controls). Significantly increased risks were found among Asians (OR=1.46, 95% CI=1.07-2.01, and $p=0.02$) and Africans (OR=2.12, 95% CI=1.57-2.87, and $p<0.00001$), but not among Caucasians (OR=1.01, 95% CI=0.83-1.22, and $p=0.96$), Americans (OR=0.99, 95% CI=0.84-1.17, and $p=0.93$), and Mixed (OR=0.63, 95%

Table 2. Distribution of *COX-2*-765G>C Genotype and Allele among Digestive System Cancers and Controls

Author	Cases (n)			Controls (n)				Cases (n)		Controls (n)	HWE[a] for control
	CC	GC	GG	CC	GC	GG	C	G	C	G	P
Pereira et al	5	32	36	13	67	130	42	104	93	327	0.28
Liu et al	0	27	220	0	43	384	27	467	43	811	0.27
Hou et al	10	70	210	11	110	288	90	490	132	686	0.9
Saxena et al	19	29	14	8	62	171	67	57	78	402	0.42
Sitarz et al	8	57	176	9	32	59	73	409	50	150	0.14
Tang et al	9	34	57	5	24	76	52	148	34	176	0.11
Zhang et al	35	0	288	41	0	903	70	576	82	1806	0.46
Li et al	2	53	241	1	43	275	57	535	45	593	0.62
Shin et al	0	18	82	0	10	90	18	182	10	190	0.6
Hamajima et al	0	8	140	0	11	230	8	288	11	471	0.023
Cox et al	11	59	150	10	77	170	81	359	97	417	0.73
Koh et al	NA	37*	273	NA	110*	1067	NA	NA	NA	NA	NA
Ulrich et al	10	140	344	20	159	405	160	828	199	969	0.37
Gunter et al	5	54	151	3	52	141	64	356	58	334	0.46
Tan et al	0	81	919	0	63	1237	81	1919	63	2537	0.37
Xing et al	1	17	119	1	29	169	19	255	31	367	0.84
Ueda et al	0	15	440	0	62	989	15	895	62	2040	0.32
Gong et al	9	45	108	12	72	127	63	261	96	326	0.67
Iglesias et al	13	99	172	4	43	76	125	443	51	195	0.48
Thompson et al	11	119	291	15	121	343	141	701	151	807	0.29
Andersen et al	9	83	267	13	186	566	101	617	212	1318	0.61
Hoff et al	10	75	241	8	112	249	95	557	128	610	0.26
Pereira et al	2	38	77	7	83	166	42	192	97	415	0.37
Daraei et al	5	67	38	9	58	53	77	143	76	164	0.20
Wang et al	11	87	207	10	111	238	109	501	131	587	0.49
Chiang et al	0	42	136	0	39	166	42	314	39	371	0.13
Lin et al	NA	104*	193	NA	173*	107	NA	NA	NA	NA	NA
Mittal et al	6	78	92	6	49	41	90	262	61	131	0.08
Guo et al	0	96	930	0	55	1215	96	1956	55	2485	0.43
Moons et al	7	41	92	4	107	384	55	225	115	875	0.24
Upadhyay et al	4	69	101	11	57	148	77	271	79	353	0.09
Kristinsson et al	7	62	153	6	73	157	76	368	85	387	0.47
Bye et al (A)[b]	80	167	100	122	230	110	327	367	474	450	0.94
Bye et al (M)[b]	34	75	81	44	183	195	143	237	271	573	0.91
Xu et al	0	28	255	0	24	542	28	538	24	1108	0.61
Zhao et al	0	36	357	0	30	756	36	750	30	1542	0.59
He et al	4	65	231	1	27	272	73	527	29	571	0.59
Chang et al	0	36	262	0	48	250	36	560	48	548	0.13

[a]HWE: Hardy-Weinberg equilibrium; [b](A) Afrians, [b](M), Mixed; *Mumbers of GC+CC; NA, not available

Table 3. Stratified Analysis of the *COX-2-765G>C* Polymorphism on Cancer Risk

Variables	CC+GC *vs* GG				C *vs* G☆			
	N✪	Cases/controls	OR (95% CI)	*p**	N	Cases/controls	OR (95% CI)	*p**
Total	38	10814/16174	1.20 (1.00, 1.44)	0.04	36	20624/29422	1.23 (1.06, 1.42)	0.006
Total[a]	36	10207/14717	1.24 (1.05, 1.48)	0.01				
Subgroup by ethnicity								
Asians	20	6303/10121	1.46 (1.07, 2.01)	0.02	18	11392/17326	1.57 (1.19, 2.06)	0.001
Caucasians	11	2382/3340	1.01 (0.83, 1.22)	0.96	11	4974/6670	1.00 (0.85, 1.18)	0.97
Americans	4	1287/1470	0.99 (0.84, 1.17)	0.93	4	2574/2940	0.97 (0.84, 1.12)	0.71
Afrians	1	347/422	2.12 (1.57, 2.87)	<0.00001	1	694/924	0.85 (0.69, 1.03)	0.10
Mixed[b]	2	495/821	0.63 (0.29, 1.37)	0.24	2	990/1562	1.12 (0.86, 1.46)	0.39
Subgroup by cancer type								
Gastric	9	1732/2855	1.64 (1.03, 2.61)	0.04	9	3674/5698	1.55 (0.97, 2.49)	0.07
Colorectal	16	5058/7687	1.01 (0.89, 1.14)	0.92	15	9496/13020	1.00 (0.90, 1.11)	0.96
Oral	3	651/581	0.66 (0.29, 1.50)	0.32	2	708/602	0.95 (0.56, 1.63)	0.86
Esophageal	6	2099/3101	1.32 (0.77, 2.25)	0.32	6	4198/6202	1.31 (0.96, 1.78)	0.09
Others[c]	4	1274/1950	1.89 (0.96, 3.72)	0.06	4	2548/3900	1.85 (0.97, 3.51)	0.06
Subgroup by study design								
HCC	18	4409/5423	1.27 (0.92, 1.76)	0.15	17	8434/10274	1.34 (1.02, 1.75)	0.04
PCC	19	6100/10392	1.17 (0.94, 1.46)	0.16	18	11580/18430	1.16 (0.97, 1.39)	0.09
FCC	1	305/359	0.93 (0.67, 1.29)	0.67	1	610/718	0.97 (0.74, 1.29)	0.86

✪number of case-control studies; *P value for Q-test. ☆Due to that the number of case and control for GC and CC genotype in Koh et al and Lin et al were available, therefore, the comparisons of C *vs* G did not include the two studies. [a]All studies excluding the studies without HWE. [b]If it was difficult to discriminate the ethnicity of participants according to the data presented, the study was termed "mixed". [c]Cancers studied if 1 cancer type was investigated by<3 individual case-control studies, then it was combined and termed "others". population-based case-control study (PCC), hospital-based case-control study (HCC), and family-based case-control study (FCC).

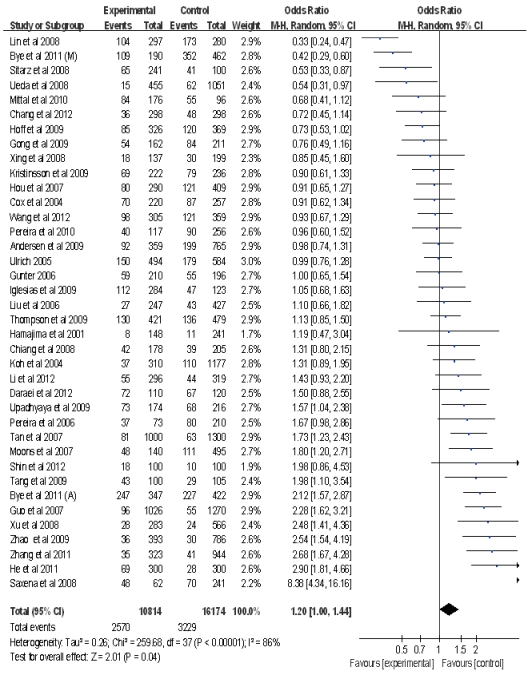

Figure 2. Meta-analysis with a Random-effects Model for the Association between Digestive System Cancer Risk and the *COX-2-765G>C* Polymorphism (GC+CC *vs* GG)

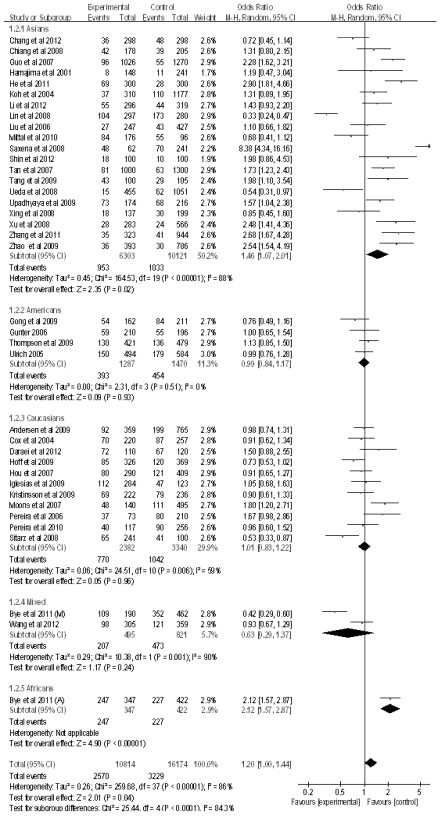

Figure 3. Meta-analysis with a Random-effects Model for the Association between Digestive System Cancer Risk and the *COX-2-765G>C* Polymorphism (GC+CC *vs* GG): Subgroup Analysis by Ethnicity

CI=0.29-1.37 and *p*=0.24). Thus, the Asians and Africans C carriers (CC+GC) may have higher risk of digestive system cancer than others ethnicity. Summary results of other genetic comparisons are listed in Table 3. In the subgroup analysis by cancer type (GC+CC *vs* GG, Figure 4), the analysis was stratified into five subgroups: gastric cancer (1732 cases and 2855 controls), colorectal cancer (5058 cases and 7687 controls), oral cancer (651cases and 581 controls), esophageal cancer (2099 cases and 3101 controls), and others (1274 cases and 1950 controls). Significantly increased risks were found among gastric cancer (OR=1.64, 95% CI=1.03-2.61, and *p*=0.04), but not among colorectal cancer (OR=1.01, 95% CI=0.89-1.14, and *p*=0.92), oral cancer (OR=0.66, 95% CI=0.29-1.50, and *p*=0.32), esophageal cancer (OR=1.32, 95% CI=0.77-

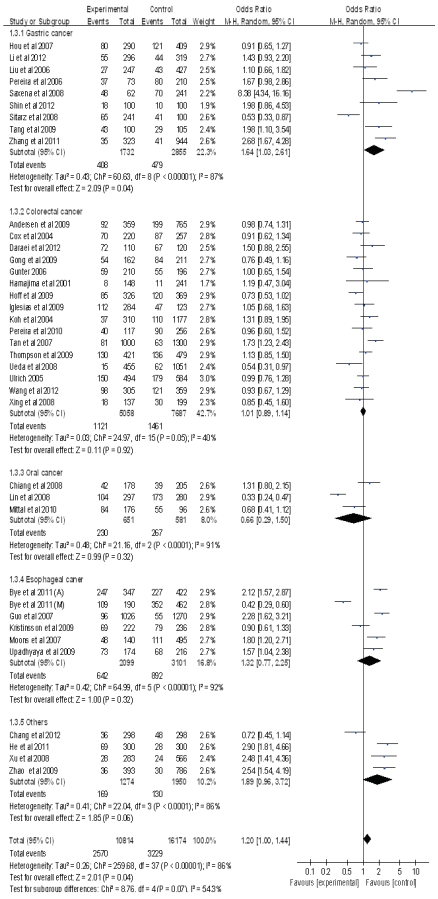

Figure 4. Meta-analysis with a Random-effects Model for the Association between Digestive System Cancer Risk and the *COX-2-765G>C* Polymorphism (GC+CC *vs* GG): Subgroup Analysis by Cancer Type

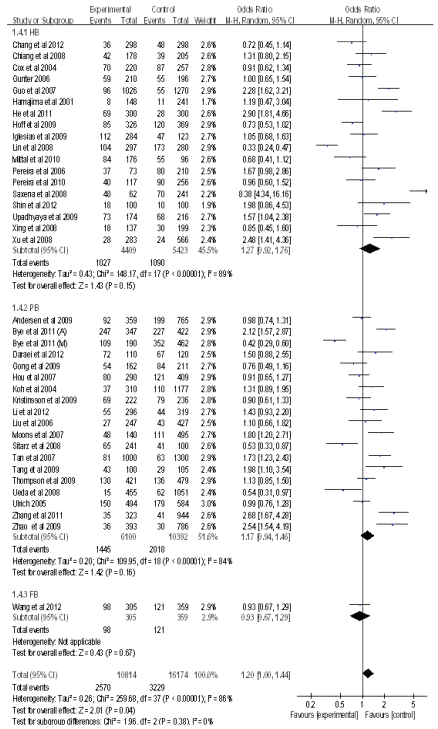

Figure 5. Meta-analysis with a Random-effects Model for the Association between Digestive System Cancer Risk and the *COX-2-765G>C* Polymorphism (GC+CC *vs* GG): Subgroup Analysis by Study Design

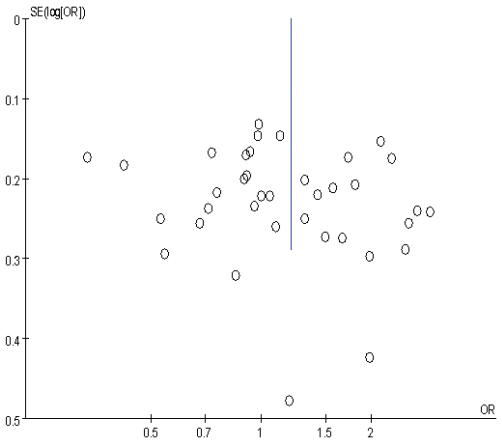

Figure 6. Begg's Funnel Plot for Publication Bias in Selection of Studies on the *COX-2-765G>C* Polymorphism (GC+CC *vs* GG)

2.25, and p=0.32), and others (OR=1.89, 95% CI=0.96-3.72, and p=0.06). Thus, the gastric cancer C carriers (CC+GC) may have a higher risk than others cancer type. Summary results of other genetic comparisons are listed in Table 3. For subgroup analysis by study design (GC+CC *vs* GG, Figure 5), no significant association between the -765G>C polymorphism of *COX-2* gene and digestive system cancer risk was found in HCC (4409 cases and 5423 controls: OR=1.27, 95% CI=0.92-1.76, and p=0.15), PCC (6100 cases and 10392 controls: OR = 1.20, 95% CI = 0.99-1.46, and P = 0.16), or FCC (305 cases and 359 controls: OR = 0.93, 95% CI = 0.67-1.29, and p=0.67). Thus, the polymorphism may not increase cancer risk in different study design. Summary results of other genetic comparisons are listed in Table 3.

Sensitivity analysis

In order to assess the stability of the results of the meta-analysis, we performed a sensitivity analysis through sequentially excluded individual studies. After sequentially excluding each case-control study, statistically similar results were obtained for (GC+CC *vs* GG) (all P values were <0.05), suggesting the stability of this meta-analysis (data not shown).

Publication bias

The publication bias was assessed by Begg's funnel plot and Egger's test. The graphical funnel plot of 38 studies of the -765G>C polymorphism of *COX-2* gene appeared to be asymmetrical in the (CC+GC *vs* GG) (Figure 6), which suggested that the combined ORs were not stable, possible explanation may be that probably due to limited number of eligible studies included. Therefore, more studies with large sample size were required to minimize the likelihood of bias.

Discussion

COX-2, known as prostaglandin-endoperoxide synthase 2 (PTGS2), is a rate-limiting enzyme only expressed by various stimulus such as growth factors, cytokines, mitogens (Harrison et al., 1994; Seibert et al., 1994; Wu et al., 1996; Jongthawin et al., 2012).

Evidence suggests that *COX-2* plays an key role in the carcinogenesis pathway, such as in the inhibition of apoptosis, tumor growth, angiogenesis, invasion and metastasis (Leahy et al., 2000; Tatsuguchi et al., 2004; Wang et al., 2007; Gu et al., 2012; Huang et al., 2013; Saad et al., 2013), which are all crucial to many cancers, especially those belonging to the gastrointestinal tract, such as oral cancer (Chiang et al., 2008), gastric cancer (Liu et al., 2006), esophageal cancer (Kristinsson et al., 2009; Upadhyay et al., 2009), and colorectal cancer (Cox et al., 2004).

Given the important roles of *COX-2* in cancer etiology, it is possible that genetic variations of the *COX-2* gene may affect the susceptibility to cancer development. Genetic variants, such as SNPs in the promoter region of the *COX-2* encoding gene, is the most extensively studied polymorphism, which features guanine (G) converting to cytosine (C) at position -765 bp of the promoter region, affecting transcription activity of -765G>C polymorphism of *COX-2* gene and its functional activity (Papafili et al., 2002; Szczeklik et al., 2004; Sitarz et al., 2008). To date, conclusions of the association of *COX-2*-765G>C polymorphism with digestive system cancer is still uncertain; thus, we performed a meta-analysis of 38 case-control studies, including 10814 cases and 16174 controls, to evaluate the associations between the *COX-2*-765G>C polymorphism and digestive system cancer risks. Considering the genetic background, cancer type and study design may affect the results of meta-analysis, subgroup analyses was performed by ethnicity, cancer type, and study design.

Our results showed that the *COX-2*-765G>C polymorphism was significantly associated with digestive system cancer risks in the (GC+CC *vs* GG) genetic model. In addition, in the (C *vs* G) model, we found significant associations between this polymorphism and digestive system cancer. These results indicated that this polymorphism may contribute to cancer risks. Consistent with the previous meta-analysis (Cao et al., 2010; Liang et al., 2011; Wang et al., 2013), we found significant increased risk -765G>C polymorphism with digestive system cancer, strongly suggesting that this polymorphism may contribute to digestive system cancer pathogenesis and help to explain individual differences of host susceptibility.

Considering the property of genetic background may affect the results of genetic association studies, we performed subgroup analysis by ethnicity. In this meta-analysis , we found that the variant C allele carriers (CC+GC) had increased risk of digestive system cancer in Asians and Africans, but not in Caucasians, Americans, and Mixed, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in. Thus, further studies are demanded to assess the effect of gene-environment interactions in different ethnicities and to validate these findings. In addition, significantly increased risks were found in Africans only one case-control study included, it may be due to chance because studies with small sample size may have insufficient statistical power to detect a slight effect. Therefore the results should be explained with great caution. When stratified separately according to cancer type, we found that this polymorphism was significantly associated with increased risks of gastric cancer. Possible explanation may be that differences in etiology may exist in difference cancer type. Subgroup analysis was also performed by study design, no significant increased risk of digestive system cancer was found among in HCC, PCC, and FCC subjects. Based on our study, it is worth noting that, selection bias was well avoided by performing rigorous and scientific inclusion criteria and exclusion criteria.

Heterogeneity is one of the important issues when performing meta-analysis. We found that heterogeneity between studies existed in overall comparisons. After subgroup analysis by ethnicity or cancer type, the heterogeneity was effectively removed or decreased among Americans and colorectal cancer, possible explanations may be that differences in genetic backgrounds and environmental exposures existed among different ethnicities, and differences in etiology may exist in difference cancer type. Another important factor contributing to heterogeneity was that homogeneity in either the case or control groups was uncertain. Ideally, all cases and controls should be matched for age, sex, and environmental exposures. In this meta-analysis, these issues could not all be explained precisely because of insufficient information for individual person.

Some limitations of this meta-analysis should be acknowledged when explaining our results. Firstly, all eligible studies were published reports written in English and Chinese indexed by the selected databases. It is possible that some potential published studies in other languages or unpublished studies could be missed, which might bias the results. Secondly, some studies were excluded due to lack of original data by email from the corresponding author, we could not evaluate the potential interactions between this polymorphism and digestive system cancer risks, which may lead to a selection bias. Thirdly, this meta-analysis included data from Asians, Caucasians, Americans, Africans and Mixed, no studies from Dutch populations; thus, our study may be applicable to these ethnic groups only. And the last, data were not stratified by other factors such as age, gender, family history, lifestyle variables, because insufficient information could be extracted from the primary publication. It is worth mentioning a study published by Saad et al (Saad et al., 2013), the study indicated potential value of PA extracted from rice bran in reducing colonic cancer risk in rats. Which may have implications for further medical research concerning digestive system cancer and personalized therapy for digestive system cancer patients.

In conclusion, To our knowledge, this is the most comprehensive meta-analysis conducted to date with respect to the associations between the -765G>C polymorphism of *COX-2* gene and digestive system cancer risks. Our results indicated the *COX-2*-765G>C polymorphism was significantly associated with increased risk of digestive system cancer, especially for Asians, Africans and gastric cancer. These results may have implications for further medical research concerning digestive system cancer and personalized therapy for

digestive system cancer patients. Regarding some limitations for this study, future large-scale studies will be needed to clarify the gene-gene and gene-environment interactions to better display the association between the -765G>C polymorphisms in *COX-2* gene and digestive system cancer risks.

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