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Association between *IL6* -174G/C and cancer: A meta-analysis of 105,482 individuals

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Abstract. Interleukin-6 (IL6) is a pleiotropic inflammatory cytokine, which is implicated in the development and progression of several types of cancer. The -174G/C polymorphism of the *IL6* gene controls serum levels of IL6 and may be associated with cancer risk, but the results from the published studies on the association between this polymorphism and cancer risk are conflicting. A comprehensive meta-analysis was conducted to assess the association of *IL6* -174G/C with cancer risk. Studies were identified by searches of MEDLINE and HuGE Published Literature databases, with no restrictions. An eligible 83 articles involving 44,735 cancer patients and 60,747 controls were included. Combined odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of the association between the *IL6* -174 G/C polymorphism and cancer risk. Potential sources of heterogeneity were explored by meta-regression and sensitivity analysis. Overall, the *IL6* -174G/C polymorphism was not significantly associated with cancer risk. However, cancer risk was increased for individuals with the CC genotype compared to those carrying the GG genotype in African populations (OR=1.83, 95% CI 1.26-2.67, P=0.002), but not in Caucasian populations (OR=1.00, 95% CI 0.92-1.08, P=0.938). The present meta-analysis provides the first evidence of the ethnic-specific association of the *IL6* -174G/C polymorphism with cancer risk. Further investigations with a large number of cases and controls are required to confirm the associations between this polymorphism and cancer in Africans.

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Introduction

Clinical and epidemiological studies suggest that chronic inflammation predisposes individuals to different types of cancer, and inflammatory molecules promote the proliferation of malignant cells (1,2). The connection between inflammation and cancer is mediated by several mechanisms, including genetic and epigenetic alterations, that generate an inflammatory microenvironment that further reinforces the development of cancer (3). Moreover, functional polymorphisms of inflammatory cytokine genes are associated with cancer susceptibility (4-6).

Interleukin-6 (IL6) is a pleiotropic inflammatory cytokine that is important for immune responses, cell survival, proliferation and apoptosis (7). Elevated expression of IL6 and its major effector, signal transducer and activator of transcription-3 (STAT3), have been implicated in different stages of tumor development, including initiation, promotion, malignant conversion, invasion and metastasis (8-12). The best characterized genetic variants of *IL6* is a G-to-C substitution at position -174, upstream of the transcription start site, which has been reported to influence IL6 levels *in vitro* and *in vivo* (13,14). Elucidation of an association, if any, between this polymorphism and cancer risk would support the hypothesis that genetic variants in *IL6*, resulting in aberrant IL6 expression, play a role in cancer development.

Individual studies and previously published meta-analyses regarding the association of *IL6* -174G/C with cancer susceptibility (15,16) enrolled too few subjects to provide conclusive

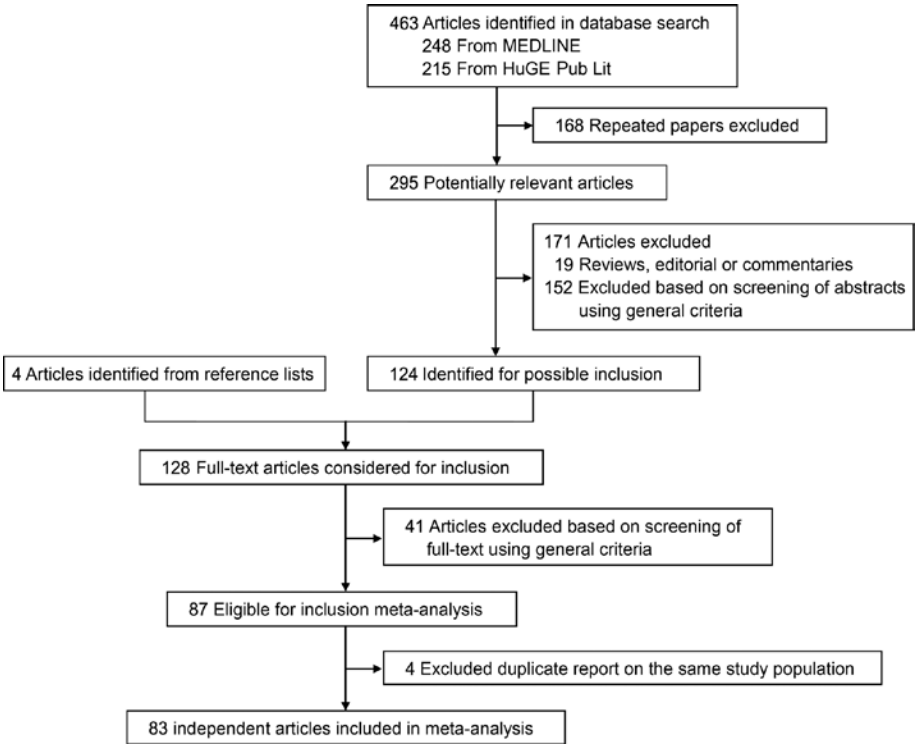

Figure 1. Flow diagram of the study selection for the meta-analysis.

evidence for or against an association of this polymorphism with cancer risk. The aim of this study was to assess the association of *IL6* -174G/C polymorphism with cancer risk by conducting a comprehensive meta-analysis of all eligible case-control studies.

Materials and methods

This meta-analysis was performed according to the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA statement) (17) and the reporting meta-analysis of observational studies in epidemiology (MOOSE) (18).

Data sources and study selection. To identify all studies on the association between the *IL6* -174G/C polymorphism and cancer risk, we conducted a systematic search of the literature published before April 2011 using the MEDLINE database and the HuGE Published Literature database (HuGE Pub Lit) (19) with no restrictions. For MEDLINE, keywords 'IL-6' OR 'IL 6' OR 'IL6' OR 'interleukin-6' OR 'interleukin 6' AND 'polymorphism' AND 'cancer' were used; For HuGE Pub Lit, keywords 'IL6' AND 'cancer' were used for searching eligible studies. In addition, a manual review of references from primary or review articles was screened to trace additional relevant studies. Studies were included if they had a case-control design and the available frequency of three genotypes regarding the *IL6* -174G/C polymorphism. Of the studies with overlapping data, we selected the ones with the largest number of subjects.

Data extraction. Three investigators independently extracted data and reached a consensus on all items. The following data were extracted from each study: the first author's last name, publication year, ethnicity of the subjects, cancer type, study design (retrospective case-control or prospective cohort study), and numbers of genotyped cases and controls with GG, GC or CC genotypes. Ethnic group was defined as African, Caucasian or 'mixed', including more than one ethnic category. Studies investigating more than one type of cancer with overlapping or same controls were regarded as individual data sets only in subgroup analyses by cancer type.

Statistical analysis. Hardy-Weinberg equilibrium (HWE) analysis for the frequencies of GG, GC and CC genotypes among controls in each study was assessed using Pearson's Chi-square test. The strength of the association between *IL6* -174G/C polymorphism and cancer risk was measured by odds ratio (OR) with its 95% confidence interval (CI). The pooled ORs for *IL6* -174G/C genotypes CC, GC and C allele carriers (CC or GC) against GG genotype were calculated, respectively. The significance of the pooled OR was determined by the Z-test and $P<0.05$ was considered statistically significant. Subgroup analysis was performed using stratification by study character, cancer type, ethnicity and study design, respectively. If a cancer type contained less than three independent individual studies, it was categorized into the 'other types' group.

Testing for heterogeneity among studies was performed by a Chi-square-based Q-test (20). Since Q-test is poor at detecting true heterogeneity, heterogeneity was considered significant for $P<0.10$ rather than $P<0.05$ (21). Additionally, the magnitude of the between-study heterogeneity was also assessed by I^2, which can be calculated from the basic results of a typical meta-analysis as $I^2 = 100\% \times (Q-df)/Q$, ranges form 0 to 100%, and is typically considered low for $I^2<25\%$,

Table I. Characteristics of all studies included in the meta-analysis.

Author	Year	Ethnicity	Cancer type	Study design[a]	Cases			Controls			P-value[b]
					GG	GC	CC	GG	GC	CC	
Snoussi	2005	African	Breast cancer	Retrospective	199	98	8	150	46	4	0.830
Vishnoi	2007	African	Gallbladder cancer	Retrospective	97	25	2	153	44	3	0.936
Ahirwar	2008	African	Bladder cancer	Retrospective	86	24	26	130	56	14	0.027
Kesarwani	2008	African	Prostate cancer	Retrospective	102	84	14	103	87	10	0.120
Upadhyay	2008	African	Esophageal cancer	Retrospective	135	28	5	131	62	8	0.845
Badr El-Din	2009	African	Brain tumor	Retrospective	6	27	12	5	87	6	0.000
Gangwar	2009	African	Cervical cancer	Retrospective	107	36	17	142	51	7	0.372
Foster	2000	Caucasian	Kaposi sarcoma	Retrospective	61	44	10	44	55	27	0.214
Hulkkonen	2000	Caucasian	Leukemia	Retrospective	8	13	14	81	201	118	0.785
Zheng	2000	Caucasian	Multiple myeloma	Retrospective	22	36	15	33	69	26	0.357
Martinez-Escribano	2002	Caucasian	Melanoma	Retrospective	14	26	2	20	26	2	0.071
El-Omar	2003	Caucasian	Esophageal and gastric cancer	Retrospective	88	91	34	83	98	28	0.913
Howell	2003	Caucasian	Melanoma	Retrospective	48	79	34	79	101	44	0.258
Hwang	2003	Caucasian	Gastric cancer	Retrospective	19	9	2	22	8	0	0.399
Landi	2003	Caucasian	Colorectal cancer	Retrospective	133	180	48	145	133	33	0.761
Yakupova	2003	Caucasian	Multiple myeloma	Retrospective	23	33	13	37	53	12	0.286
Campa	2004	Caucasian	Lung cancer	Retrospective	64	111	68	55	105	47	0.818
Cozen	2004	Caucasian	Lymphoma	Retrospective	41	37	8	25	39	14	0.858
Gazouli	2004	Caucasian	Kaposi sarcoma	Retrospective	10	4	1	11	22	7	0.482
Smith	2004	Caucasian	Breast cancer	Retrospective	57	67	20	79	101	44	0.258
Vasku	2004	Caucasian	Lymphoma	Retrospective	19	35	9	36	46	23	0.259
Basturk	2005	Caucasian	Renal cell carcinoma	Retrospective	15	10	0	27	13	9	0.007
Campa	2005	Caucasian	Lung cancer	Retrospective	629	954	412	615	993	374	0.448
Cordano	2005	Caucasian	Lymphoma	Retrospective	134	197	77	106	184	59	0.167
Festa	2005	Caucasian	Basal cell carcinoma	Retrospective	57	126	58	62	130	68	0.993
Hefler	2005	Caucasian	Breast cancer	Retrospective	78	139	52	91	105	31	0.935
Mazur	2005	Caucasian	Multiple myeloma	Retrospective	11	31	12	16	28	6	0.239
Seifart	2005	Caucasian	Lung cancer	Retrospective	47	52	17	90	107	46	0.163
Balasubramanian	2006	Caucasian	Breast cancer	Retrospective	170	244	83	168	235	87	0.759
Gonzalez-Zuloeta Ladd	2006	Caucasian	Breast cancer	Prospective	55	86	30	1,286	1,733	632	0.246
Gunter	2006	Caucasian	Colorectal cancer	Retrospective	79	90	35	83	81	26	0.385
Kamangar	2006	Caucasian	Gastric cancer	Prospective	21	54	27	51	58	43	0.004
Lan	2006	Caucasian	Lymphoma	Retrospective	211	231	68	241	264	85	0.358
Michaud	2006	Caucasian	Prostate cancer	Prospective	170	223	91	230	293	90	0.832
Morgan	2006	Caucasian	Aneurysm	Retrospective	40	40	6	867	1,358	495	0.360
Nogueira de Souza	2006	Caucasian	Cervical cancer	Retrospective	24	32	0	148	102	3	0.001
Rothman	2006	Caucasian	Lymphoma	Retrospective	1,277	1,658	564	1,097	1,470	499	0.860

Table I. Continued.

Author	Year	Ethnicity	Cancer type	Study design[a]	Cases			Controls			P-value[b]
					GG	GC	CC	GG	GC	CC	
Theodoropoulos	2006	Caucasian	Colorectal cancer	Retrospective	111	76	35	64	86	50	0.055
Vogel	2006	Caucasian	Breast cancer	Prospective	108	167	86	98	177	86	0.728
Wang	2006	Caucasian	Lymphoma	Retrospective	486	474	174	393	410	138	0.068
Berkovic	2007	Caucasian	GEP-NETs	Retrospective	25	44	11	69	75	18	0.724
Brenner	2007	Caucasian	Glioma	Retrospective	222	332	100	319	503	211	0.621
Deans	2007	Caucasian	Gastro-oesophageal cancer	Retrospective	71	83	43	79	101	44	0.258
Duch	2007	Caucasian	Multiple myeloma	Retrospective	28	22	2	35	23	2	0.442
Gatti	2007	Caucasian	Gastric cancer	Retrospective	42	13	1	23	27	6	0.642
Gonullu	2007	Caucasian	Breast cancer	Retrospective	15	17	6	14	3	7	<0.001
Litovkin	2007	Caucasian	Breast cancer and uterine leiomyoma	Retrospective	44	64	25	30	39	9	0.490
Oliveira	2007	Caucasian	Osteosarcoma	Retrospective	9	23	32	10	68	82	0.405
Purdue	2007	Caucasian	Lymphoma	Retrospective	177	245	91	157	210	90	0.194
Slattery	2007	Caucasian	Colorectal cancer	Retrospective	952	1,043	355	728	897	347	0.015
Talseth	2007	Caucasian	Colorectal cancer	Retrospective	36	58	24	25	53	22	0.542
Vogel	2007	Caucasian	Basal cell carcinoma	Prospective	65	176	63	89	157	69	0.988
Vogel	2007	Caucasian	Colorectal cancer	Prospective	98	168	89	204	364	185	0.371
Zanke	2007	Caucasian	Colorectal cancer	Retrospective	381	557	195	373	539	213	0.461
Colakogullari	2008	Caucasian	Lung cancer	Retrospective	10	29	5	27	22	9	0.222
Crusius	2008	Caucasian	Gastric cancer	Prospective	78	122	43	415	517	206	0.044
Ennas	2008	Caucasian	Leukemia	Retrospective	17	16	6	64	43	5	0.506
Fontanella	2008	Caucasian	Aneurysm	Retrospective	144	157	34	66	71	19	0.989
Gu	2008	Caucasian	Melanoma	Prospective	69	106	32	69	102	33	0.646
Kury	2008	Caucasian	Colorectal cancer	Retrospective	363	489	171	435	504	182	0.079
Vairaktaris	2008	Caucasian	Oral cancr	Retrospective	42	102	18	90	60	6	0.298
Vogel	2008	Caucasian	Lung cancer	Prospective	105	202	96	204	361	179	0.437
Wilkening	2008	Caucasian	Colorectal cancer	Prospective	79	163	61	162	297	121	0.481
Aladzsity	2009	Caucasian	Multiple myeloma	Retrospective	37	43	17	36	49	14	0.681
Birmann	2009	Caucasian	Multiple myeloma	Prospective	21	46	10	52	82	28	0.655
Cherel	2009	Caucasian	Breast cancer	Retrospective	102	131	60	29	58	25	0.695
Moore	2009	Caucasian	Prostate cancer	Prospective	191	485	281	196	401	250	0.152
Ozgen	2009	Caucasian	Papillary thyroid carcinoma	Retrospective	21	14	7	143	171	26	0.009
Pierce	2009	Caucasian	Prostate cancer	Prospective	82	101	32	864	848	306	0.000
Talar-Wojnarowska	2009	Caucasian	Pancreatic cancer	Retrospective	13	19	9	22	19	9	0.191
Tsilidis	2009	Caucasian	Colorectal cancer	Prospective	68	93	39	113	170	71	0.627
Vasku	2009	Caucasian	Colorectal cancer	Retrospective	22	46	32	22	47	31	0.601
Wang	2009	Caucasian	Prostate cancer	Prospective	91	116	43	84	128	40	0.448

Table I. Continued.

Author	Year	Ethnicity	Cancer type	Study design[a]	Cases			Controls			P-value[b]
					GG	GC	CC	GG	GC	CC	
Cacev	2010	Caucasian	Colorectal cancer	Retrospective	64	70	26	68	75	17	0.582
Guey	2010	Caucasian	Bladder cancer	Retrospective	470	438	109	450	495	120	0.356
Jakubowska	2010	Caucasian	Breast and ovarian cancer	Retrospective	135	227	102	73	144	73	0.907
MARIE-GENICA Consortium	2010	Caucasian	Breast cancer	Retrospective	986	1,571	585	1,774	2,671	1,036	0.586
Schonfeld	2010	Caucasian	Breast cancer	Retrospective	274	408	156	379	487	211	0.017
Giannitrapani	2011	Caucasian	Hepatocellular carcinoma	Retrospective	63	36	6	51	37	10	0.402
Grimm	2011	Caucasian	Cervical cancer	Retrospective	55	51	25	85	96	27	0.990
Bushley	2004	Mixed	Ovarian cancer	Retrospective	5	34	143	9	46	163	0.020
Dossus	2010	Mixed	Prostate cancer	Prospective	3,594	3,218	1,125	3,832	3,402	1,274	<0.001
Dossus	2010	Mixed	Breast cancer	Prospective	2,847	2,523	820	3,707	3,324	1,035	<0.001
Ognjanovic	2010	Mixed	Colorectal cancer	Retrospective	173	74	22	357	136	43	<0.001

[a]Retrospective case-control or prospective cohort study. [b]P-value for Hardy-Weinberg equilibrium analysis among controls. References provided upon request.

modest for 25-50% and large for >50% (22). Meta-regression was carried out to investigate whether statistical heterogeneity between the results of the multiple studies was related to one or more characteristics of the studies (23). To identify the studies that led to significant heterogeneity, sensitivity analysis for between-study heterogeneity was implemented by the sequential algorithm proposed by Patsopoulos *et al* (24). Whenever the P-value of the Q-test was >0.10, the summarized OR estimate of each study was calculated by the fixed-effects model (Mantel-Haenszel method) (25). Otherwise, the random-effects model (DerSimonian and Laird method) was used (26). Funnel plots were used to examine whether the results of a meta-analysis may have been affected by publication bias (27). A modified version of Egger's test proposed by Harbord, Egger and Sterne was implemented to test funnel plot asymmetry (28). All statistical analyses were performed using Stata statistical software (Stata/SE version 10.1 for Windows; Stata Corp, College Station, TX, USA).

Results

Characteristics of the included studies. The detailed steps of our literature search are described in Fig. 1. Eighty-three independent articles that met the inclusion criteria were included in the final analysis. Of these articles, one study provided data on breast and prostate cancer using independent controls (29), therefore each group in this article was treated as an independent study in our meta-analysis. The characteristics of the included studies are summarized in Table I. Overall, the present meta-analysis is based on a total of 105,482 participants, including 44,735 cancer patients and 60,747 controls. The studies were published between April 2000 and March 2011. Seventeen studies were conducted with a prospective cohort design, and 67 were conducted with a retrospective case-control design. Approximately two-thirds of cases and controls (29,019 cases and 42,120 controls) were from 73 studies involving Caucasian populations, a fraction of the data (1,138 cases and 1,299 controls) from seven studies involving African populations, and nearly one-third of the data (14,578 cases and 17,328 controls) from four studies involving 'mixed' populations. As shown in Table I, there were two studies from Dossus *et al* involving Caucasians, African-Americans, Asians, Latinos and native Hawaiians; one study from Ognjanovic *et al* involving Caucasians, Asians and Hawaiians; and one study from Bushley *et al* involving Caucasians, Asians and other populations. In the controls, the frequency of the rare C allele among controls varied considerably between Caucasians and Africans (0.417±0.052 and 0.207±0.097, respectively; P<0.001). A significant deviation from HWE was noted in two studies in Africans, nine in Caucasians and four in mixed populations (Table I).

Test of heterogeneity. There was a significant heterogeneity in overall comparison of the CC genotype vs. GG genotype (P<0.001 and I^2=43.8%). The meta-regression showed that the strong heterogeneity could not be traditionally explained by cancer types, ethnicities or study designs (P=0.285, 0.129 and 0.306, respectively). Furthermore, the 15 studies that deviate from HWE showed similar heterogeneity with that of studies that were in HWE (Table II), suggesting that the remarkable

Table II. Summary of ORs and 95% CIs for the *IL6* -174G>C polymorphism and cancer risk and heterogeneity test for studies of each group.

Variables	No.[a]	Cases/controls	CC vs. GG			GC vs. GG			GC/CC vs. GG		
			OR (95% CI)[b]	P_Hc	I^2 (%)	OR (95% CI)[b]	P_H[c]	I^2 (%)	OR (95% CI)[b]	P_H[c]	I^2 (%)
Total	84	44,735/60,747	1.01 (0.95-1.08)	<0.001	43.8	1.01 (0.96-1.07)	<0.001	52.5	1.01 (0.96-1.07)	<0.001	57.0
Ethnicities											
African	7	1,138/1,299	1.83 (1.26-2.67)[e]	0.294	17.7	0.80 (0.55-1.16)	0.002	71.0	0.94 (0.67-1.31)	0.004	68.9
Caucasian	73	29019/42120	1.00 (0.92-1.08)	<0.001	43.8	1.02 (0.96-1.09)	<0.001	52.4	1.02 (0.96-1.08)	<0.001	58.2
Mixed	4	14,578/17,328	0.98 (0.92-1.05)	0.488	0.0	1.00 (0.96-1.05)	0.839	0.0	1.00 (0.95-1.04)	0.789	0.0
Cancer types[d]											
Breast cancer	13	12,640/20,281	1.01 (0.94-1.09)	0.374	7.2	1.06 (0.96-1.17)	0.033	46.4	1.05 (0.95-1.15)	0.043	44.3
Colorectal cancer	13	6,798/7,502	0.97 (0.82-1.14)	0.014	52.4	1.01 (0.89-1.13)	0.030	47.2	1.00 (0.87-1.13)	0.002	61.9
Prostate cancer	6	10,043/12,438	0.99 (0.91-1.07)	0.254	24.0	1.03 (0.97-1.09)	0.361	8.6	1.01 (0.96-1.07)	0.357	9.3
Lymphoma[d]	7	6,213/5,586	0.96 (0.86-1.07)	0.574	0.0	0.96 (0.89-1.04)	0.609	0.0	0.96 (0.89-1.03)	0.621	0.0
Multiple myeloma[d]	6	422/601	1.23 (0.82-1.83)	0.609	0.0	1.07 (0.80-1.41)	0.717	0.0	1.10 (0.84-1.44)	0.731	0.0
Lung cancer[d]	5	2,801/3,234	1.06 (0.92-1.23)	0.715	0.0	1.05 (0.83-1.33)	0.071	53.7	1.01 (0.90-1.13)	0.153	40.2
Gastric cancer[d]	5	554 /1,585	1.06 (0.78-1.44)	0.114	46.2	0.98 (0.55-1.73)	0.001	78.8	0.95 (0.54-1.76)	<0.001	80.4
Melanoma[d]	3	410/476	1.13 (0.75-1.68)	0.790	0.0	1.18 (0.88-1.58)	0.721	0.0	1.17 (0.88-1.54)	0.672	0.0
Cervical cancer	3	347/661	1.85 (1.12-3.08)[f]	0.320	12.2	1.11 (0.68-1.82)	0.068	62.7	1.22 (0.92-1.61)	0.195	38.7
Other types	28	4,723/9,403	1.10 (0.85-1.43)	<0.001	67.6	0.94 (0.79-1.11)	<0.001	67.1	0.97 (0.82-1.16)	<0.001	71.5
Study design											
Prospective	17	18,759/28,718	1.01 (0.95-1.07)	0.893	0.0	1.03 (0.98-1.07)	0.150	26.6	1.02 (0.98-1.06)	0.316	11.8
Retrospective	67	25,976/32,029	1.00 (0.91-1.11)	<0.001	51.9	0.98 (0.92-1.06)	<0.001	56.3	0.99 (0.92-1.06)	<0.001	61.9
HWE											
>0.05	69	26,067/36,098	1.01 (0.93-1.09)	<0.001	44.7	1.00 (0.94-1.07)	<0.001	50.8	1.00 (0.94-1.07)	<0.001	59.0
<0.05	15	18,668/24,649	1.02 (0.90-1.15)	0.043	42.3	1.06 (0.95-1.18)	0.001	61.4	1.04 (0.96-1.13)	0.019	48.3

[a]No. of comparisons. [b]Random-effect model was used when P-value for heterogeneity test was <0.10; otherwise, the fixed-effect model was used. [c]P-value of Q-test for heterogeneity. [d]All the studies were from Caucasian populations. [e]P=0.002. [f]P=0.017.

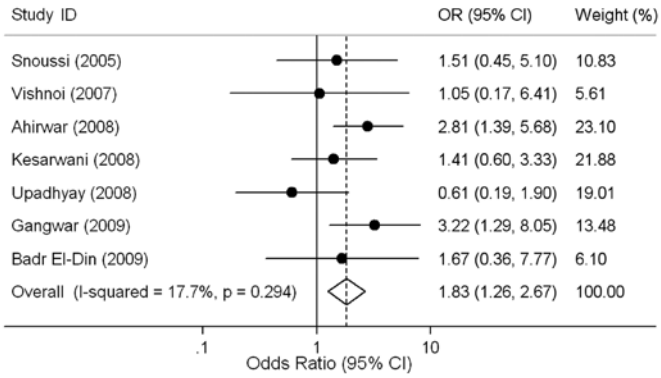

Figure 2. Risk of cancer for *IL6* -174 CC vs. GG genotype in African population. The circles and horizontal lines correspond to the study-specific odds ratio (OR) and 95% confidence interval (CI). The combined ORs and their 95% CIs are indicated by the diamonds.

heterogeneity among the overall analysis was not due to the variability of the control quality. Therefore, we carefully assessed the association of the *IL6* -174G/C polymorphism with cancer risk in several subgroups, and carried out sensitivity analyses of between-study heterogeneity to detect studies that have remarkable influence on homogeneity.

For ethnic-specific subgroup analysis, no heterogeneity was detected within African population studies (P=0.294), but there were significant heterogeneity within Caucasian population studies (P<0.001). Sensitivity analysis of between-study heterogeneity revealed that five studies (30-34) mainly contributed to the heterogeneity within Caucasian population studies. After performing cancer type-specific analyses, we found no heterogeneity in studies of breast, prostate, lung, gastric cancer, lymphoma, multiple myeloma, melanoma and cervical cancer (Table II). However, there was strong heterogeneity for colorectal cancer, which was due to one Caucasian study (32).

Quantitative data synthesis. Overall, the CC genotype was not significantly associated with cancer risk when compared to the GG genotype (OR=1.01, 95% CI 0.95-1.08, P=0.698). Ethnic-specific ORs showed that cancer risk was increased for individuals carrying the CC genotype compared to those with the GG genotype in African populations (OR=1.83, 95% CI 1.26-2.67, P=0.002; Fig. 2), but not in Caucasian populations (OR=1.00, 95% CI 0.92-1.08, P=0.938; Table II). After excluding the studies (30-34) responsible for heterogeneity, we found that Caucasian individuals carrying the CC genotype had no remarkable effect on risk of cancer compared to GG genotype individuals (OR=1.02, 95% CI 0.97-1.07, P=0.561) with no significant between-study heterogeneities (P=0.196, I^2=12.6%). Although there were nine data sets in which the genotype distribution did not follow HWE, the corresponding meta-analysis was qualitatively similar with or without excluding them.

Subsequently, we stratified the association between the *IL6* -174G/C polymorphism and cancer risk by cancer types. When compared to individuals with the GG genotype, those with the CC genotype were associated with increased risk of cervical cancer (OR=1.85, 95% CI 1.12-3.08, P=0.017), but not with that of other types of cancer (Table II). Furthermore, no

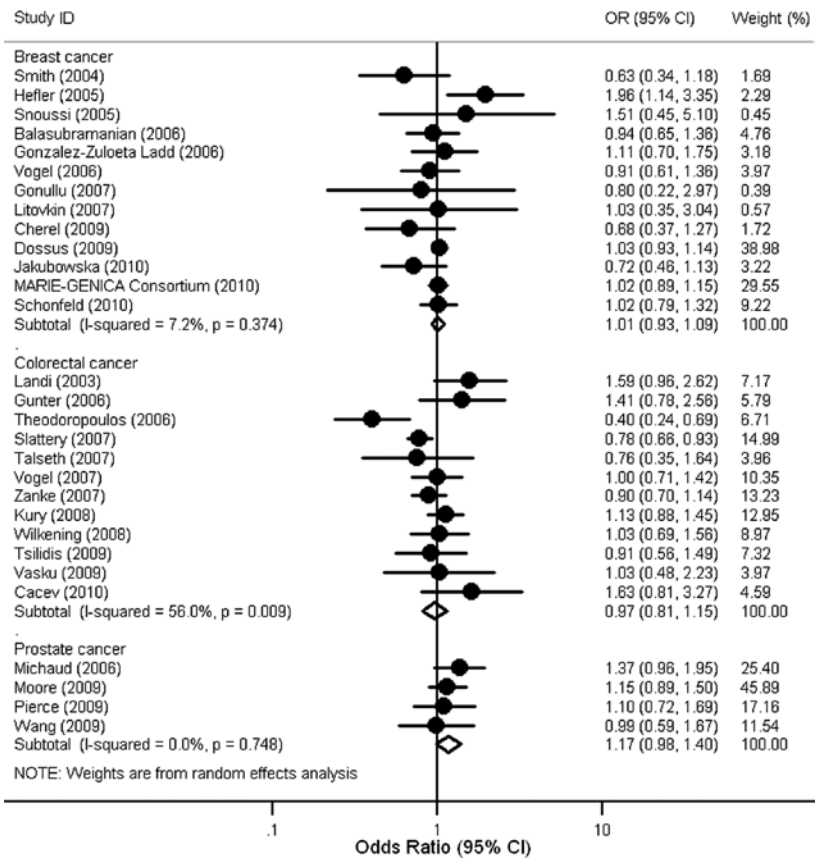

Figure 3. Risk of breast, colorectal and prostate cancer for *IL6* -174 CC vs. GG genotype in Caucasians. The circles and horizontal lines correspond to the study-specific odds ratio (OR) and 95% confidence interval (CI). The combined ORs and their 95% CIs are indicated by the diamonds for each type of cancer.

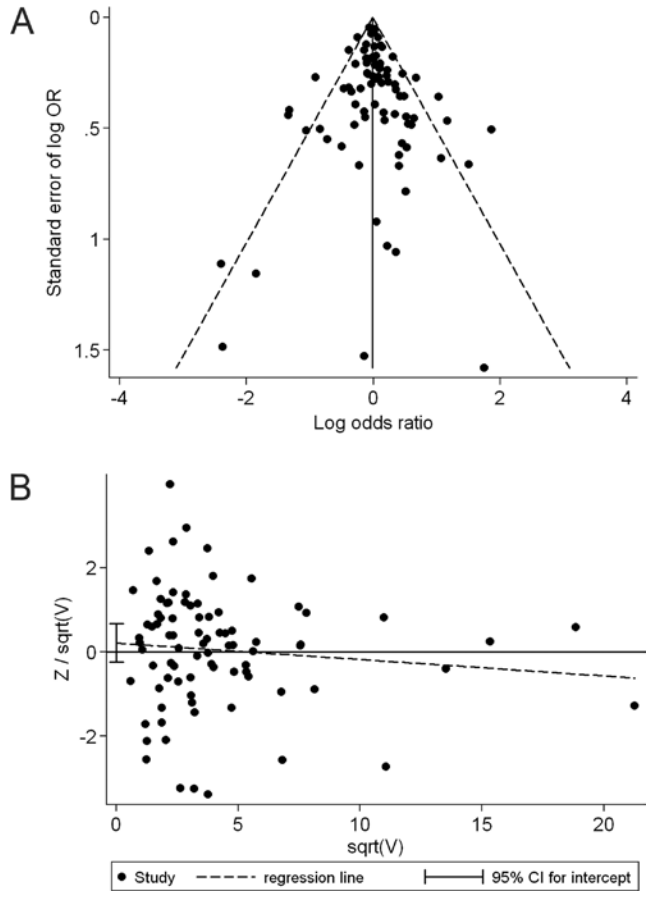

Figure 4. Publication bias test for the comparison of the CC genotype vs. GG genotype. (A) Funnel plot (with pseudo 95% percent confidence limits) for publication bias test. The natural logarithm of odds ratio (OR) and its standard error were used in the funnel plot. The points correspond to the log OR from individual trials, and the diagonal lines show the expected 95% confidence intervals (CIs) around the summary estimate. (B) Harbord's modified test for funnel plot asymmetry: regress Z/sqrt(V) on sqrt(V) -174G/C, where Z is efficient score and V is score variance.

significant association of the *IL6* -174G/C polymorphism with risk of breast, colorectal and prostate cancer was observed in individuals with Caucasian ancestry (Fig. 3). A few studies involving African populations did not allow us to perform subgroup analysis in Africans (Table I).

Lastly, we also assessed the ORs of cancer for individuals with the GC genotype or C allele carriers (GC or CC genotype) compared to those with the GG genotype, and found no significant association in overall and subgroup analyses (Table II).

Publication bias. The shape of the funnel plot did not reveal any evidence of obvious asymmetry in comparison of the CC genotype vs. GG genotype (Fig. 4A). Then, we used Harbord's test to provide statistical evidence of funnel plot symmetry, and the result did not show evidence of publication bias (t=0.91, P=0.366; Fig. 4B). Subgroup analyses by ethnicity and cancer type did not provide any evidence of publication bias (t=-1.59, P=0.172 for African populations; t=0.27, P=0.786 for Caucasian populations; t=-0.58, P=0.575 for breast cancer and t=1.02, P=0.329 for colorectal cancer). Similarly, no publication bias was detected when comparing the GC genotype to the GG genotype (t=0.18, P=0.861), the GC/CC genotype to the GG genotype (t=0.42, P=0.677) and in any comparison of the corresponding subgroup analyses.

Discussion

To the best of our knowledge, the present meta-analysis of 83 studies, involving 44,735 cases and 60,747 controls (counting every study's cases and controls only once), provides the most comprehensive assessment of an association of the *IL6* -174G/C polymorphism with cancer risk. It provides evidence that African individuals with the CC genotype have higher odds of cancer than individuals with the GG genotype; the findings of our meta-analysis do not show any association of the *IL6* -174G/C with cancer risk in Caucasian populations. These findings suggest an ethnic-specific effect of *IL6* -174G/C polymorphism on risk of cancer. The discrepancies among different populations suggest a possible role of ethnic differences in genetic backgrounds and the environment they lived in (35).

Recent studies have shown that IL6 and its major effector STAT3 play a role in the epigenetic switch from non-transformed epithelia to cancer cell (36,37). Elevated expression of IL6 via autocrine and paracrine mechanisms leading to subsequent chronic inflammation also exhibits a strong association with cancer (38-41). In the present study, we found that the *IL6* -174 CC genotype was significantly associated with increased risk of cervical cancer compared to the GG genotype. However, the smaller number of individuals genotyped in these studies precludes any formal conclusion. As compared to previous analyses based on substantially less data (15,16), the present analysis essentially shows null associations between *IL6* -174G/C and several common types of cancer, including breast, colorectal, prostate, lung, gastric cancer, lymphoma, multiple myeloma and melanoma.

Assessment of the between-study heterogeneity and identification of its sources are essential requirements in meta-analyses (23,42). In this study, we systematically examined the effect of *IL6* -174G/C on cancer risk across all reliable studies, and the results of the overall analysis showed a strong heterogeneous effect among the 83 studies. Given the fact that clinical characteristics of studies, including study population, design approach and type of cancer, are likely to be potential sources of heterogeneity, we first used meta-regression to detect whether any of the characteristics could explain the between-study variation. However, none of the potential sources considered were able to systematically explain the observed variation across studies. It seems likely that there exist more than one answer to the nature of overall heterogeneity. We, therefore, induced a new approach to perform sensitivity analysis of between-study heterogeneity (24), and detected that several studies with different clinical characteristics were responsible for the heterogeneity (30-34).

Apart from between-study heterogeneity, publication bias has also been recognized as a major concern in robust meta-analyses. Thereby, in this study, we used funnel plots to assess whether the studies included could be affected by publication bias. According to the recommendations by Sterne and Egger (43), the log OR and its standard error are used for the horizontal and vertical axis, respectively. No evidence of

publication bias was found when testing by a visual inspection of funnel plots. In support of this, Harbord's linear regression test confirmed the evidence of funnel plot symmetry across all constituent data sets.

Notably, social factors are believed to interact with genetic variants to govern complex human phenotypes (44,45). Cole *et al* recently demonstrated a strong interaction between the *IL6* -174G/C polymorphism and social environment factors, which may further affect the risk of inflammation-related disease (46). However, in the absence of the original data of the reviewed studies, our evaluation of potential interactions of gene-environment with cancer risk was limited. This may explain why previous genetic association studies and some subgroup analyses in our meta-analysis, especially the Caucasian studies, failed to show an association between the *IL6* -174G/C polymorphism and risk of cancer. Furthermore, two other polymorphisms (-6331T/C and -572G/C) and several haplotypes in the *IL6* promoter affect the transcriptional activity of *IL6* and may influence susceptibility to inflammation-related diseases (47-49). However, most studies included in our meta-analysis restricted their analysis to the *IL6* -174G/C polymorphism and few carried out the *IL6* haplotypic analysis on cancer susceptibility. It is difficult to estimate the role of a particular haplotype on cancer risk in the present meta-analysis.

Despite these limitations, our meta-analysis provides a leap in knowledge when compared to a previous study (15) that reviewed the association between the *IL6* -174G/C polymorphism and risk of cancer. First, our updated review is more comprehensive than the previous, as we identified 83 independent articles with a total of 105,482 individuals on the association of *IL6* -174G/C with cancer risk compared to 47 articles with 67,116 individuals in the previous report. Thus, our meta-analysis had significantly higher statistical power. Second, we noticed the potentially different roles of the *IL6* -174G/C polymorphism in the development of cancer among various populations, and found different associations of this polymorphism with cancer risk between Africans and Caucasians. Third, sensitivity analysis of heterogeneity was used to detect studies that were responsible for between-study heterogeneity (24). Fourth, we assessed the pooled effect of the *IL6* -174G/C polymorphism on cancer risk within or without the studies that did not follow HWE, and qualitatively similar results were found, suggesting that the estimations of our analyses are stable and convincing. Finally, for publication bias analysis, a modified method for testing funnel plot asymmetry was used (28), which maintains better control of the false-positive rate than the commonly used Egger's test. No publication bias was detected, suggesting that the pooled results should be unbiased.

In summary, the present meta-analysis provides evidence of the ethnic-specific association of the *IL6* -174G/C polymorphism with cancer risk. More sophisticated gene-environment interactions should be considered in future analyses, which may result in better understanding of the relevance between the *IL6* -174G/C polymorphism and risk of cancer. Moreover, this study reinforces the need to undertake investigations with very large number of cases and controls (including updated meta-analyses) to provide conclusive evidence for the associations between high-frequency genetic variants in low-penetrance genes and complex diseases, such as cancer.

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