Interleukin-1β and Interleukin-1 Receptor Antagonist Gene Polymorphisms and Gastric Cancer: A Meta-analysis

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Abstract

Background: **Polymorphisms of *interleukin-1B* (*IL1B*) and its receptor antagonist (*IL1RN*) genes have been inconsistently associated with gastric cancer risk. We examined these associations by performing meta-analyses.**

Materials and Methods: **Twenty-five studies testing the association between *IL1B* and/or *IL1RN* gene polymorphisms and gastric cancer were examined: 14 studies of *IL1B-511*, 14 studies of *IL1B-31*, 8 studies of *IL1B+3954*, and 23 studies of *IL1RN*. Overall and ethnicity-specific summary odds ratios and corresponding 95% confidence intervals for gastric cancer associated with these polymorphisms were estimated using fixed- and random-effects models. Heterogeneity and publication bias were evaluated.**

Results: ***IL1B-511T* and *IL1RN**2 were associated with gastric cancer risk in Caucasians, but not in Asians. For *IL1B-511T*, the association in Caucasians was stronger when intestinal-subtype and noncardia gastric cancer cases were examined. A nonsignificant trend was observed between *IL1B-31C* and gastric cancer in Caucasians. No significant association of *IL1B+3954T* and gastric cancer risk was detected. Studies with better methodologic characteristics reported stronger effects. There was no evidence of publication bias.**

Conclusion: ***IL1B-511T* is associated with gastric cancer susceptibility in Caucasians. The meta-analyses suggest that the conflicting results among studies may be explained by variation in allele frequencies among the ethnic groups and variation in tumor types, as well as by the methodologic quality of the studies.** (Cancer Epidemiol Biomarkers Prev 2006;15(9):1674–87)

Introduction

In 2002, 1.9 million cancer cases worldwide, representing 17.8% of all cancers, were estimated to be associated with infectious agents, with 5.5% of all cancers attributable to *Helicobacter pylori* (*H. pylori*) infection (1). Considering that chronic inflammation promotes the development of many gastrointestinal malignancies (2), a number of polymorphisms in genes related to the inflammatory response have been investigated as factors predisposing to gastric cancer (3). The most extensively studied are those encoding interleukin-1β (IL-1β) and its receptor antagonist (IL1Ra; refs. 4-6).

IL-1β is a proinflammatory cytokine induced by *H. pylori* infection and is a powerful inhibitor of gastric acid secretion. Its effects promote hypochlorhydria, favoring further colonization of *H. pylori* and a more severe gastritis. Over decades, gastric atrophy and adenocarcinoma may develop (7). Three single nucleotide polymorphisms (SNP) of the *IL1B* gene have been most frequently evaluated for association with gastric cancer: C-T base transitions at positions −511 and +3954 and a T-C base transition at position −31 (8-10). The SNPs at −31 and −511 are in near-complete linkage disequilibrium (4).

IL-1 receptor antagonist is an anti-inflammatory protein that modulates the effects of IL-1β (11). The *IL1RN* gene contains an 86 bp variable number of tandem repeats (VNTR) polymorphism in intron 2. Five different alleles have been described, with two to six repeats (12).

The presence of *IL1B-511T*, *IL1B-31C*, *IL1B+3954T*, or *IL-1RN**2 alleles has been associated with gastric cancer risk in some reports (4, 13-15), but not in others (16, 17). Therefore, we did meta-analyses to find sources of variation in the reports.

Materials and Methods

Search Strategy and Study Selection. We searched for observational studies published from January 2000 (when the first association between *IL1B* gene polymorphisms and gastric cancer was published; ref. 4) to September 2005 using PubMed software to search Medline (U.S. National Library of Medicine, Bethesda, MD). Searching was done by two independent reviewers (M.C.C. and B.G.S.). Combinations of the keywords gastric cancer, IL-1, *IL-1B*, *IL1RN*, *IL1B-511*, *IL1B-31*, *IL1B+3954*, association, polymorphisms, SNP, odds ratio (OR), gene, and allele were used. References cited in the selected articles were also considered.

Two investigators (M.C.C. and M.B.P.) independently reviewed the articles and extracted the data; discrepancies were resolved through discussion. Studies testing the association between *IL1B* (*−31*, *−511*, and *+3954*) and/or *IL1RN* gene polymorphisms and gastric cancer were included if all the following conditions were met: (*a*) the study assessed the association between gastric cancer and at least one of the polymorphisms; (*b*) the study population included subjects with and without gastric cancer; (*c*) the study reported ORs or data for their calculation; and (*d*) the study was published in English or Spanish.

Supplemental information regarding sample description for El-Omar et al. (4, 18) and Yang et al. (19) was taken from cited references (20-22), respectively. Additional information about

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genotype distribution was obtained by contacting the authors (see Acknowledgments). In two overlapping reports from Mexico (23, 24), we retained the one with the largest sample size (24). We omitted results from one study due to an extreme departure from the well-established, near-complete linkage disequilibrium between *IL1B-31C* and *IL1B-511T* (results for other polymorphisms were included; ref. 25). Although the *IL1B-511* SNP was assessed by Rocha et al. (26), the data could not be analyzed because the numerical results were not published. In addition, we omitted one study with an insufficiently described control group (27). Thus, this analysis included 25 studies in 27 populations (4-6, 13, 14, 16-19, 24-26, 28-40).

Because gastric adenocarcinoma accounts for >95% of all gastric tumors (41), we assumed that only patients with adenocarcinomas were recruited in studies lacking tumor type information; therefore, in our meta-analyses, ''gastric cancer'' refers to gastric adenocarcinoma alone.

The following information was recorded for each study: first author, year of publication, age (minimum and maximum values or mean), sex, ethnicity, country and region of origin, matching variables, sources of controls, evidence of Hardy-Weinberg equilibrium (HWE), variables for which statistical adjustment was done, number of cases and controls by genotypes, histopathologic subgroups, tumor location subgroups, genotyping techniques, genotyping quality control measures, and testing of gene- and environment-gene interactions. Unless otherwise indicated, ethnicity was assumed to correspond to the geographic region where the subjects were selected. If an article presented results stratified by sex, data were combined (17). When two regions were reported in the same article (25, 30), the information of each area was analyzed separately.

Quality Score Assessment. Methodologic quality was independently assessed by three reviewers (M.C.C., B.G.S., and M.B.P.), according to a set of predefined criteria (Addendum 1), based on the scale of Thakkinstian et al. (42). Disagreements were resolved by consensus. Scores ranged from 0 (lowest) to 10 (highest). Reports scoring <5 were classified as ''low quality,'' and those ≥5 as ''high quality.''

Statistical Analysis

Pooled Frequencies of the Putative Risk Alleles. Ethnic-specific frequencies of all the risk alleles in controls were estimated by the inverse variance method (Appendix of ref. 42). A Q test for heterogeneity was done for each ethnic group. Under the null hypothesis of no difference in effect across studies, the Q statistic is χ^2-distributed with degrees of freedom (*df*) equal to the number of studies minus 1.

Meta-analysis. For the controls in each study, for each *IL1B* polymorphism, we calculated HWE by the χ^2 goodness of fit test, with 1 *df*.

ORs and 95% confidence intervals (95% CI) were estimated for each polymorphism, using the log(OR) and the corresponding SE values for the meta-analyses. The estimated ORs were as follows:

- For *IL1B-511* SNP: T/T versus C/C (OR_1), C/T versus C/C (OR_2), and T/T versus C/T (OR_3).
- For the *IL1B-31* SNP: C/C versus T/T (OR_1), T/C versus T/T (OR_2), and C/C versus T/C (OR_3).
- For the *IL1B+3954* SNP: T carriers versus C/C.
- For the *IL1RN* VNTR: 2/2 versus L/L (OR_1), L/2 versus L/L (OR_2), and 2/2 versus L/2 (OR_3). L represents any long allele (allele 1, 3, 4, or 5).

A dominant genetic model was assumed for *IL1B+3954* polymorphism. For *IL1B-511*, *IL1B-31*, and *IL1RN* (analyzed as biallelic loci) polymorphisms, the following algorithm (43) was used to determine the most appropriate genetic model:

- Recessive model: if $OR_1 = OR_3 \neq 1$ and $OR_2 = 1$.
- Dominant model: if $OR_1 = OR_2 \neq 1$ and $OR_3 = 1$.
- Overdominant model: if $OR_2 = 1/OR_3 \neq 1$ and $OR_1 = 1$.
- Codominant model: if $OR_1 > OR_2 > 1$ and $OR_1 > OR_3 > 1$ (or $OR_1 < OR_2 < 1$, and $OR_1 < OR_3 < 1$).

Because the studies by Zhang et al. (14), Sakuma et al. (31), Chang et al. (32), Chen et al. (36), and Zeng et al. (in the low-risk region; ref. 25) conducted in Asian populations had cells with no counts, we added 1 for each cell for these studies to determine the genetic model. However, based on the reported increased gastric cancer risk of *IL1RN**2 subjects (6, 28, 36), a dominant genetic model was also considered.

To explore sources of heterogeneity across studies, we did stratified and logistic meta-regression analyses. We examined the following study characteristics: ethnicity (Caucasians, Asians, and Hispanics), matching by age and sex (matched versus unmatched studies), sample size (<200, 200-400, and >400 subjects), type of controls (blood donors/healthy subjects/nongastroenterology patients, gastroenterology patients, and population-/neighbor-based sample), quality score (low versus high), genotyping techniques (RFLP analysis, confronting two pair primer analysis, Taqman, denaturing high-performance liquid chromatography, single-strand conformational polymorphism analysis, and sequencing), and other variables used to create the quality score (Addendum 1).

Using the indicated genetic model to collapse the three genotypes into two groups, the pooled estimate of risk was obtained using both fixed-effects (Mantel-Haenszel) and random-effects (44) models. If there was no obvious heterogeneity, the fixed-effects model was used to estimate the summary gene effect; otherwise, the random-effects model was used. In the absence of between-study heterogeneity, the methods provide almost identical results.

Meta-analysis of Subgroups. Studies including information on histologic subtype (Lauren's classification; ref. 45) or tumor location allowed us to explore the effect of *IL1B-511T*, *IL1B-31C*, and *IL1RN**2 polymorphisms on intestinal- and diffuse-subtype and noncardia gastric cancer using all controls. Atypical or mixed cases analyzed separately from the two histologic main groups were not included in the analyses. Data were insufficient for such analyses for the *IL1B+3954* SNP. Few studies tested the associations with cardia gastric cancer.

Evaluation of Publication Bias. For each polymorphism, publication bias was evaluated by the Begg's and Egger's funnel plot asymmetry tests (46, 47).

Statistical analysis was done with Stata, version 9 (Stata Corporation, College Station, TX). $P < 0.05$ was considered statistically significant, except for heterogeneity, Egger's and Begg's tests, where a level of 0.10 was used.

Results

Characteristics of Studies. Twenty-five studies examined the relationship between *IL1B* and/or *IL1RN* gene polymorphisms and gastric cancer risk and fit the criteria. Data from 14 studies, including 2,953 cases and 3,350 controls, were available for the meta-analysis of *IL1B-511* SNP; 2,616 cases and 4,230 controls for *IL1B-31* SNP (14 studies); 1,299 cases and 2,298 controls for *IL1B+3954* SNP (8 studies); and 3,901 cases and 6,449 controls for *IL1RN* VNTR (23 studies). Characteristics of the studies are given in Addendum 2. Ten studies were conducted in Asia, 10 in Europe, 1 in North America, and 4 in Central and South America.

Results of HWE analysis for controls were reported in 20 studies. Three studies did not report it (5, 36, 40), one study tested cases only (17), and one mentioned the test, but the results were not presented (25). Our calculations provided an

estimate of HWE that differed from that presented for four studies: for *IL1B-31* and *IL1B-511* in Chang et al. (32), for *IL1B-511* in El-Omar et al. (18), for *IL1B-511* in Gatti et al. (17), and for *IL1B-511* in Hartland et al. (38). We did *IL1B-511* and *IL1B-31* meta-analyses including and excluding studies deviating from HWE and obtained similar results. Deviation from HWE may signal problems in genotyping (48, 49) or selection bias in controls and/or population stratification (50). It can also alter the assumed type I error rate (51, 52). For these reasons, and for brevity, we present results excluding studies deviating from HWE. Studies omitted due to departure from HWE were Gatti et al. (ref. 17; for *IL1B-511*), El Omar et al. (for *IL1B-511*; ref. 18), Chang et al. (for *IL1B-511* and *IL1B-31*; ref. 32), and Hartland et al. (*IL1B-511*; ref. 38).

The studies differed in the extent of characterization of the tumors. Twenty-one reports mentioned histologic confirmation of gastric cancer cases, but the remaining four used the diagnosis from medical records (or tumor registries) or did not clarify the source of diagnosis (17, 26, 30, 31). Regarding tumor location, 12 studies included cases from more than one gastric site (three of them presented stratified analyses), eight included only noncardia cases, and six did not report the location. Five studies did not report the histologic subgroup (Addendum 2). Analyses stratified by subtype and/or genotype distributions in each subtype were presented in 12 reports (5, 6, 13, 16, 17, 29, 30, 32, 35-37, 39).

Most of the reports presented demographic information for cases and controls. In 10 studies (5, 13, 25, 26, 30, 32, 35, 36, 38, 39), the mean age of controls and cases differed (younger controls). Age and sex matching was described in nine studies and five reported matching by ethnicity (Addendum 2). One study was controlled by *H. pylori* status *a priori* using only *H. pylori*–negative subjects (6). Eleven of the 25 reports adjusted for potential confounders (including *H. pylori* and/or *cagA* status; refs. 5, 19, 24-26, 28, 29, 31, 32, 34, 36). Six studies reported screening controls by endoscopic examination to exclude gastric cancer at study entry (5, 24, 33, 34, 36, 40). Five studies recruited population- or neighbor-based controls (4, 18, 19, 28, 34) and eight recruited blood donors as controls (Addendum 2). All studies used PCR-based methods for genotyping. The most commonly used method to assess *IL1B* SNPs was RFLP. Eight studies (13, 18, 19, 28, 29, 35, 37, 40) reported genotyping quality control measures (positive and negative controls, an alternative genotyping assay to validate the data and/or repetition of a portion of samples in the genotyping). Only three studies mentioned use of blinding the case-control status of samples while genotyping (19, 28, 40).

Six studies tested for combinations of alleles and *H. pylori* genotype (5, 19, 24, 25, 29, 36). Sicinschi et al. (29) showed a multiplicative interaction between *IL1B-31* and *CagA* status using intestinal-subtype gastric cancer cases. Figueiredo et al. (5) tested the interaction and combination between *IL1B-511* and *H. pylori* genotypes (*cagA* and *vacA*). In that study, the multiplicative interaction was negative, but the combination showed significant results. Twelve studies evaluated the effect of the number of risk alleles and/or combination of them, reporting significant results in selected subgroups (5, 6, 13, 16, 18, 19, 28-30, 35, 36, 39).

Frequencies of the Putative Risk Alleles in Control Populations

IL1B-511T. Fourteen studies estimated the T-allele frequency (Table 1), one of them reporting allele frequencies for two populations (30). Of the 15 populations, 8 were Caucasian and 7 were Asian. There was no marked heterogeneity across the studies in Caucasians or across studies in Asians ($P > 0.10$). Pooled allele T frequencies were: 0.33 (95% CI, 0.31-0.34) in Caucasians and 0.51 (95% CI, 0.49-0.53) in Asians ($P < 0.001$ for the difference in proportions).

IL1B-31C. Fourteen studies (Table 2) reported the genotypes of the *IL1B-31* SNP in controls: five studies in Caucasians, six in Asians, and three in Hispanics. The studies in Caucasians

Table 1. Genotype frequencies in gastric cancer cases and controls from the 14 studies (15 populations) included in the analysis of *IL1B-511* polymorphism

First author, year of publication (reference)	Country, region	Ethnic group	Controls age*	*n*	Controls, genotypes (*n*)				Allele T frequency†	Cases, genotypes (*n*)			
					CC	CT	TT	T carriers‡		CC	CT	TT	T carriers‡
Zhang, 2005 (14)	China, northwestern	Asian	35-73	320	43	71	52	123	0.53	34	78	42	120
Perri, 2005 (30)	Italy, south (low gastric cancer prevalence)	Caucasian	18-70§	232	68	64	14	78	0.32	34	44	8	52
Perri, 2005 (30)	Italy, north (high gastric cancer prevalence)	Caucasian	18-65§	314	89	99	28	127	0.36	48	37	13	50
Ruzzo, 2005 (6)	Italy, Central	Caucasian	35-80	238	45	48	7	55	0.31	53	58	27	85
Sakuma, 2005 (31)‖	Japan	Asian	ND	243	25	56	22	78	0.49	35	71	34	105
Lu, 2004 (34)	China, northern	Asian	59.1 (9.4)¶	550	67	163	70	233	0.51	72	125	53	178
Glas, 2004 (35)	Germany	Caucasian	18-68§	233	65	58	22	80	0.35	33	35	20	55
Yang, 2004 (19)	China, high-risk area	Asian	61.5 (10.1)¶	538	57	136	65	201	0.52	70	158	52	210
Chen, 2004 (36)	Taiwan	Asian	17-83§	306	34	93	37	130	0.51	24	87	31	118
Lee, 2004 (37)	South Korea, Seoul	Asian	18-91	764	95	208	130	338	0.54	62	180	89	269
Machado, 2003 (39)	Portugal, northern	Caucasian	18-64§	593	137	129	40	169	0.34	90	171	26	197
Wu, 2003 (40)	Taiwan-China	Asian	60.7 (13.4)¶	450	61	124	45	169	0.47	69	106	45	151
Figueiredo, 2002 (5)	Portugal, Porto	Caucasian	24-62§	357	61**	60**	15**	75	0.33	69**	127**	25**	152
Machado, 2001 (13)	Portugal, northern	Caucasian	19-61§	370	100	87	31	118	0.34	50	85	17	102
El-Omar, 2000 (4)	Poland, Warsaw	Caucasian	ND	795	217	166	46	212	0.30	127	170	69	239

NOTE: The studies are presented in decreasing order based on the date of publication.
Abbreviation: ND, not described.
*Minimum and maximum values in years.
†In control groups.
‡T carriers: CT and TT genotypes.
§Controls are younger than the cases.
‖The number of subjects in each genotype was estimated based on the reported proportions.
¶Mean in years (SD).
**Additional information provided by the authors.

Table 2. Genotype frequencies in gastric cancer cases and controls from 14 studies included in the analysis of *IL1B-31* polymorphism

First author, year of publication (reference)	Country, region	Ethnic group	Controls age*	*n*	Controls, genotypes (*n*)				Allele C frequency†	Cases, genotypes (*n*)			
					CC	TC	TT	C carriers‡		CC	TC	TT	C carriers‡
Palli, 2005 (28)	Italy, central	Caucasian	55.5 (7.0)§	731	58	252	236	310	0.34	20	81	84	101
Rocha, 2005 (26)	Brazil, south	Mixed ethnic background	33.8 (10)§,‖	702	111	263	162	374	0.45	29	90	47	119
Zhang, 2005 (14)	China, northwestern (high-risk area)	Asian	35-73	320	33	78	55	111	0.43	36	80	38	116
Ruzzo, 2005 (6)	Italy, central	Caucasian	35-80	238	11	51	38	62	0.37	26	58	54	84
Garza-Gonzalez, 2005 (24)	Mexico, north	Hispanic	18-92	278	70	102	43	172	0.56	13	48	2	61
Zambon, 2004 (33)	Italy	Caucasian	18-94	773	76	274	294	350	0.33	9	50	70	59
Lu, 2004 (34)	China, northern	Asian	59.1 (9.4)§	550	87	149	64	236	0.54	60	121	69	181
Glas, 2004 (35)	Germany	Caucasian	18-68‖	233	22	58	65	80	0.35	14	41	33	55
Yang, 2004 (19)	China (high-risk area)	Asian	61.5 (10.1)§	538	72	126	60	198	0.52	61	136	83	197
Lee, 2004 (37)	South Korea, Seoul	Asian	18-91	764	130	212	91	342	0.55	89	182	60	271
Gatti, 2004 (17)	Brazil, north	Hispanic	Same as cases	112	10	34	12	44	0.48	17	25	14	42
Lee, 2003 (16)	South Korea, Seoul	Asian	28-74	362	40	94	38	134	0.51	47	93	50	140
Wu, 2003 (40)	Taiwan-China	Asian	60.7 (13.4)§	450	42	125	63	167	0.45	47	102	71	149
El-Omar, 2000 (4)	Poland, Warsaw	Caucasian	ND	795	46	164	219	210	0.30	66	172	128	238

NOTE: The studies are presented in decreasing order based on the date of publication.
Abbreviation: ND, not described.
*Minimum and maximum values in years.
†In control groups.
‡C carriers: CT and CC genotypes.
§Mean in years (SD).
‖Controls are younger than the cases.

showed homogeneity ($P > 0.10$), and the pooled frequency of allele C was 0.33 (95% CI, 0.31-0.34). There was heterogeneity across the studies in Asians ($P < 0.10$), and the pooled frequency of allele C was 0.50 (95% CI, 0.47-0.54). Studies in Hispanics were also heterogeneous ($P < 0.10$), and the pooled frequency of allele C was 0.50 (95% CI, 0.42-0.57).

IL1B+3954T. Eight studies (in nine populations, Table 3) investigated the *IL1B+3954* polymorphism in controls: four of them in Caucasians, three in Asians, and one in Hispanics. There was no marked heterogeneity among studies in Caucasians ($P > 0.10$), and the pooled frequency of allele T was 0.23 (95% CI, 0.21-0.25). Studies in Asians also appeared homogeneous ($P > 0.10$), and the pooled frequency was 0.05 (95% CI, 0.04-0.06). The frequency of allele T in the study conducted in Hispanics was 0.08 (95% CI, 0.06-0.10).

*IL1RN*2.* To estimate the pooled frequency of allele 2 of *IL1RN* VNTR, data were used from control groups in 23 studies (Table 4), including 25 populations. The 12 studies in Caucasians showed homogeneity ($P > 0.10$). The pooled frequency of allele 2 was 0.27 (95% CI, 0.26-0.28). The pooled frequency among Asians was 0.06 (95% CI, 0.04-0.08; nine populations) and this estimate was heterogeneous ($P < 0.10$).

Table 3. Genotype frequencies in gastric cancer cases and controls from the eight studies (nine populations) included in the analysis of *IL1B+3954* polymorphism

First author, year of publication (reference)	Country, region	Ethnic group	Controls, age*	*n*	Controls, genotypes (*n*)				Allele T frequency†	Cases, genotypes (*n*)			
					CC	CT	TT	T carriers‡		CC	CT	TT	T carriers‡
Palli, 2005 (28)	Italy, Central	Caucasian	55.5 (7.0)§	731	331	182	33	215	0.23	114	57	14	71
Sicinschi, 2005 (29)	Mexico, South	Hispanic	28-81‖	399	223	35	4	39	0.08	115	18	4	22
Zhang, 2005 (14)	China, North-western	Asian	35-73	320	158	8	0	8	0.02	114	40	0	40
Sakuma, 2005 (31)¶	Japan	Asian	ND	243	93	10	0	10	0.05	113	27	0	27
Glas, 2004 (35)	Germany	Caucasian	18-68**	233	87	53	5	58	0.22	59	26	3	29
Hartland, 2004 (38)	United Kingdom, Newcastle upon Tyne	Caucasian	18-72**	345	178	97	11	108	0.21	28	27	4	31
Zeng, 2003 (25)	China, low prevalence region	Asian	21.2 (1.35)§,¶,**	276	164	28	0	28	0.07	77	7	0	7
Zeng, 2003 (25)	China, high prevalence region	Asian	21.8 (1.95)§,¶,**	255	152	17	0	17	0.05	77	8	1	9
El-Omar, 2000 (4)	Poland, Warsaw	Caucasian	ND	795	242	158	29	187	0.25	212	140	14	154

NOTE: The studies are presented in decreasing order based on the date of publication.
Abbreviation: ND, not described.
*Minimum and maximum values in years.
†In control groups.
‡T carriers: CT and TT genotypes.
§Mean in years (SD).
‖Additional information provided by the authors.
¶The number of subjects in each genotype was estimated based on the reported proportions.
**Controls are younger than the cases.

Table 4. Genotype frequencies in gastric cancer cases and controls from the 23 studies (25 populations) included in the analysis of *IL1RN* VNTR polymorphism

First author, year of publication (reference)	Country, region	Ethnic group	Controls, age*	*n*	Controls, genotypes (*n*)†				Allele 2 frequency‡	Cases, genotypes (*n*)†			
					LL	L2	22	2 carriers§		LL	L2	22	2 carriers§
Palli, 2005 (28)	Italy, central	Caucasian	55.5 (7.0)‖	722	300	194	43	237	0.26	80	96	9	105
Sicinschi, 2005 (29)	Mexico, south	Hispanic	58.5 (12.3)‖	520	158	117	73	190	0.38	73	65	34	99
Rocha, 2005 (26)	Brazil, south	Mixed ethnic background	33.8 (10)‖,¶	702	375	140	21	161	0.17	104	56	6	62
Zhang, 2005 (14)	China, northwestern (high-risk area)	Asian	35-73	320	130	31	5	36	0.12	135	19	0	19
Perri, 2005 (30)	Italy, south (low gastric cancer prevalence)	Caucasian	18-70¶	232	79	53	14	67	0.28	58	23	5	28
Perri, 2005 (30)	Italy, north (high gastric cancer prevalence)	Caucasian	18-65¶	314	111	85	20	105	0.29	55	35	8	43
Ruzzo, 2005 (6)	Italy, central	Caucasian	35-80	238	63	30	7	37	0.22	72	56	10	66
Sakuma, 2005 (31)**	Japan	Asian	ND	243	90	13	0	13	0.06	126	13	1	14
Chang, 2005 (32)	South Korea, Seoul	Asian	21-72¶	668	405	29	0	29	0.03	214	20	0	20
Garza-Gonzalez, 2005 (24)	Mexico, north	Hispanic	18-92	226	83	93	25	118	0.36	13	11	1	12
Zambon, 2004 (33)	Italy	Caucasian	18-94	773	349	229	66	295	0.28	76	47	6	53
Lu, 2004 (34)	China, northern	Asian	59.1 (9.4)‖	550	249	46	5	51	0.09	216	32	2	34
Glas, 2004 (35)	Germany	Caucasian	18-68¶	233	73	61	11	72	0.29	29	8	51	59
Chen, 2004 (36)	Taiwan	Asian	17-83¶	306	155	9	0	9	0.03	120	21	1	22
Hartland, 2004 (38)	United Kingdom, Newcastle upon Tyne	Caucasian	18-72¶	348	151	113	25	138	0.28	29	28	2	30
Gatti, 2004 (17)	Brazil, north	Hispanic	Same as cases	112	29	24	3	27	0.27	20	32	4	36
Zeng, 2003 (25)	China, low-risk area	Asian	21.2 (1.35)‖,¶	276	177	13	2	15	0.04	75	9	0	9
Zeng, 2003 (25)	China, high-risk area	Asian	21.8 (1.95)‖,¶	255	163	4	2	6	0.02	76	9	1	10
Machado, 2003 (39)	Portugal, northern	Caucasian	18-64¶	577	155	123	28	151	0.29	132	88	51	139
El-Omar, 2003 (18)	United States (New Jersey, Connecticut, and western Washington State)	Mixed, Caucasians mainly	30-79††	398	121	76	13	89	0.24	83	60	45	105
Lee, 2003 (16)	South Korea, Seoul	Asian	28-74	362	151	20	1	21	0.06	171	18	1	19
Wu, 2003 (40)	Taiwan-China	Asian	60.7 (13.4)‖	450	198	30	2	32	0.07	188	31	1	32
Figueiredo, 2002 (5)	Portugal, Porto	Caucasian	24-62¶	358	77	48	11	59	0.26	119	71	32	103
Machado, 2001 (13)	Portugal, northern	Caucasian	19-61¶	372	116	84	20	104	0.28	81	47	24	71
El-Omar, 2000 (4)	Poland, Warsaw	Caucasian	ND	795	237	153	39	192	0.27	156	117	93	210

NOTE: The studies are presented in decreasing order based on the date of publication.
Abbreviation: ND, not described.
*Minimum and maximum values in years.
†L represents any long allele (1, 3, 4, or 5).
‡In control groups.
§2 carriers: L2 and 22.
‖Mean in years (SD).
¶Controls are younger than cases.
**The number of subjects in each genotype was estimated based on the reported proportions.
†† Information taken from Gammon et al. (24).

There was also heterogeneity across studies in Hispanics and the pooled frequency of allele 2 was 0.29 (95% CI, 0.17-0.41; four populations).

Assessing Association between Gene Polymorphisms and Gastric Cancer Risk. We found no obvious publication bias for any of the polymorphisms (for all, Egger's test $P > 0.23$ and Begg's test $P > 0.41$).

***IL1B-511* Polymorphism and Gastric Cancer Risk.** From 14 studies in 15 populations (Table 1), the estimated OR_1, OR_2, and OR_3 were 1.15, 1.22, and 0.94, respectively. These estimates suggest a dominant effect of the T allele; therefore, C/T and T/T genotypes were combined and compared with C/C. Figure 1A presents the random-effects OR for this comparison. Individuals carrying the T allele have significantly higher gastric cancer risk compared with the individuals with the C/C genotype.

Ethnicity was identified as a source of heterogeneity ($P = 0.034$). Therefore, we did a stratified analysis (including 1,436 cases and 1,696 controls in Caucasians and 1,517 cases and 1,654 controls in Asians). As shown in Fig. 2A, no association was found between *IL1B-511T* and gastric cancer risk in Asians. In Caucasians, using either model, significantly increased risks were found (random-effects OR, 1.49; 95% CI, 1.20-1.85; Fig. 2B). Analyses stratified according to study characteristics were done. The following patterns with respect to the pooled OR emerged: (*a*) studies of higher quality reported stronger-effect estimates than studies of lower quality; (*b*) age- and sex-matched studies showed larger effect estimates than unmatched ones; (*c*) the larger the sample size, the larger the effect (Table 5). Genotyping technique was not related to heterogeneity.

Analyses stratified by histologic subtype were done, including eight populations (seven Caucasians and one Asian; refs. 5, 6, 13, 30, 35, 37, 39). For intestinal-subtype gastric cancer, the random-effects OR for *IL1B-511T* carriers versus the C/C genotype in Caucasians was 1.80 (95% CI, 1.27-2.56; Fig. 2C). A stronger effect was seen for high-quality studies (OR, 2.36; 95% CI, 1.80-3.09; P for heterogeneity = 0.90). There was no significant association for the diffuse-subtype gastric cancer in Caucasians.

A meta-analysis limited to noncardia gastric cancer (three studies in Caucasians and one in Asians; refs. 5, 31, 35, 39)

showed a significant association between *IL1B-511T* and noncardia gastric cancer risk in Caucasians using the C/C genotype as the reference (random-effects OR, 1.66; 95% CI, 1.29-2.13, $P_{heterogeneity} = 0.71$; Fig. 2D).

***IL1B-31* Polymorphism and Gastric Cancer Risk.** From 14 studies (Table 2), the estimated OR_1, OR_2, and OR_3 were 1.07, 1.06, and 0.97, respectively, suggesting a dominant effect of the C allele. Figure 1B shows the random-effects OR for *IL1B-31C* carriers compared with the T/T genotype and gastric cancer risk. Overall, a nonsignificant increase in gastric cancer risk for C carriers was observed. A meta-regression model showed an effect of ethnicity on the magnitude of the association, and because ethnicity was recognized as a source of heterogeneity in the *IL1B-511* meta-analysis, we stratified by this variable. The samples analyzed included 1,425 cases and 1,559 controls in Asians, 906 cases and 1,864 controls in Caucasians, and 285 cases and 807 controls in Hispanics.

There was heterogeneity across studies in Asians ($P = 0.07$; Table 5). *IL1B-31C* was not associated with increased gastric cancer risk in this ethnic group (random-effects OR for six studies was 0.91; 95% CI, 0.71-1.15). In Caucasians, the comparison between *IL1B-31C* carriers and the T/T genotype showed slightly increased risk (random-effects OR, 1.11; 95% CI, 0.74-1.67, $P_{heterogeneity} < 0.01$). Although the reasons for heterogeneity were not clear, nonsignificant associations were observed in some subgroups that were less heterogeneous. Under a random-effects model, the risk for gastric cancer in Hispanics was nonsignificantly higher in subjects carrying the *IL1B-31C* allele relative to the T/T genotype. Genotyping technique was not related to heterogeneity.

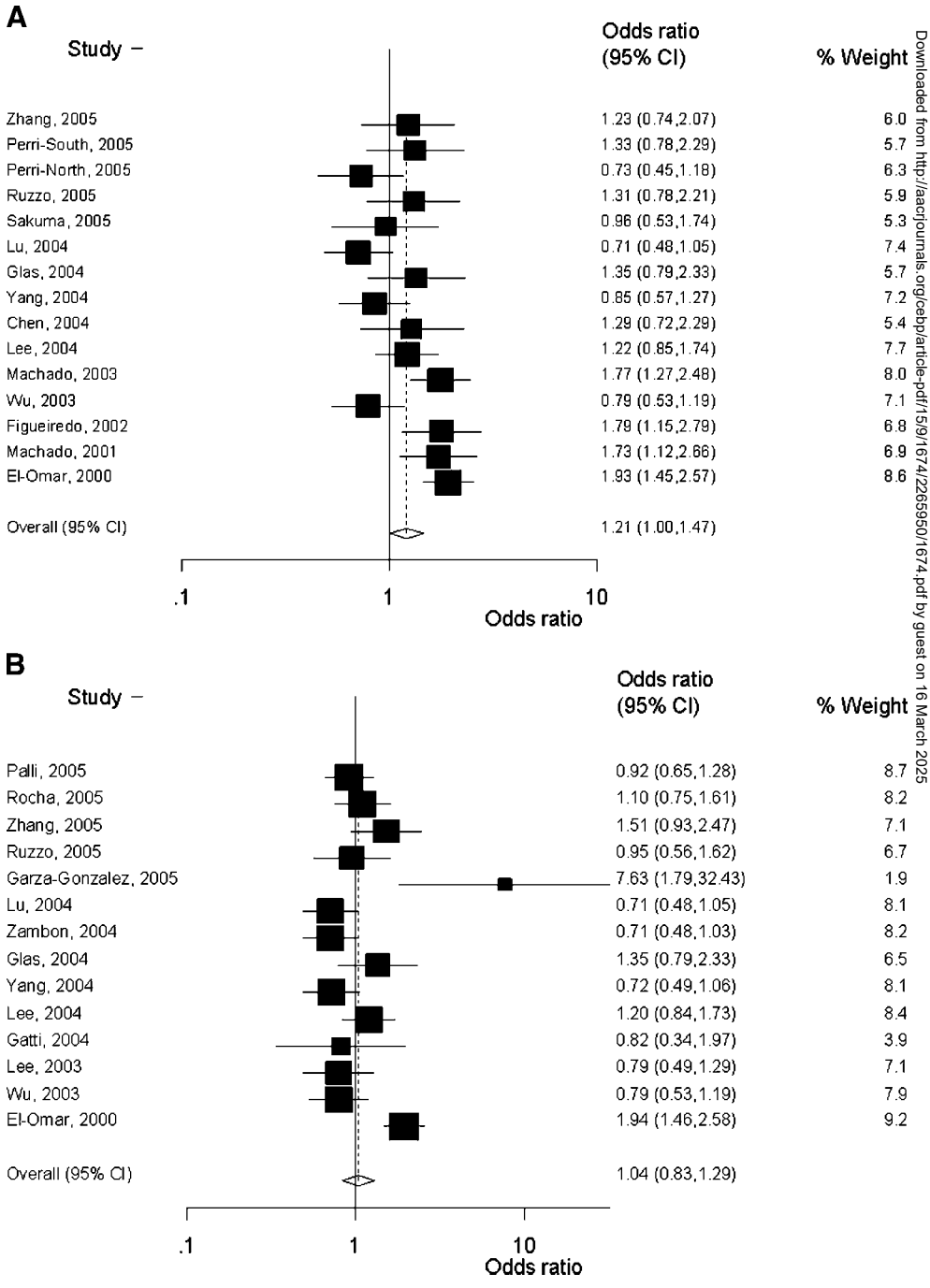

Figure 1. Random-effects ORs and 95% CIs of gastric cancer associated with *IL1B-511* and *IL1B-31* polymorphisms. ■, OR point estimates; *bars,* 95% CI. The studies are ordered by publication year. For each study, the size of the boxes is proportional to the weight that the study has in calculating the summary effect estimate, which is also displayed (◇). **A,** meta-analysis of *IL1B-511* polymorphism based on a dominant genetic model (T carriers versus C/C genotype). The *Q* statistic was 40.1, indicating the presence of heterogeneity ($P < 0.001$). **B,** meta-analysis of *IL1B-31* polymorphism based on a dominant genetic model (C carriers versus T/T genotype). The *Q* statistic was 44.3, indicating the presence of heterogeneity ($P < 0.001$).

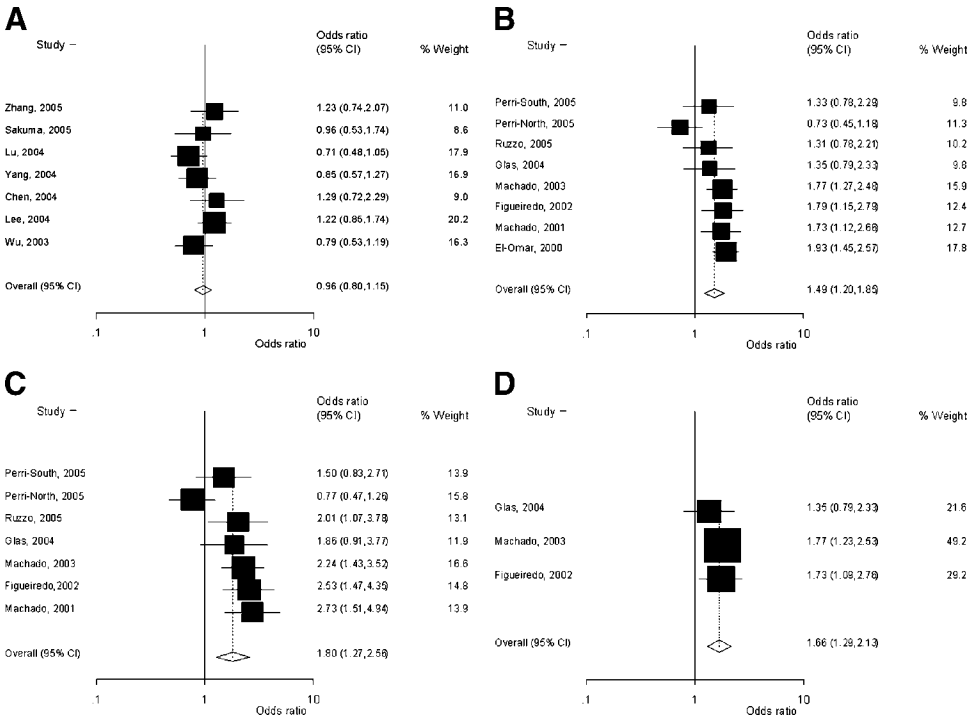

Figure 2. Random-effects ORs and 95% CIs of gastric cancer associated with *IL1B-511* polymorphism based on a dominant genetic model (T carriers versus C/C genotype). **A,** Asians, all gastric cancer cases ($P_{heterogeneity}$ = 0.306). **B,** Caucasians, all gastric cancer cases ($P_{heterogeneity}$ = 0.054). **C,** Caucasians, intestinal-subtype gastric cancer cases ($P_{heterogeneity}$ = 0.012). **D,** Caucasians, noncardia gastric cancer cases ($P_{heterogeneity}$ = 0.708).

Stratification by histologic subtype using the T/T genotype as the reference showed a moderate increase in intestinal-subtype gastric cancer among *IL1B-31C* carriers in Caucasians (random-effects OR, 1.61; 95% CI, 1.00-1.96; $P_{heterogeneity}$ = 0.37). We found no increased risk of diffuse-subtype gastric cancer. No significant association was observed in studies in Asians. Only one study in Hispanics (17) used the histologic subtype stratification for this SNP.

In a limited sample (one study in Asians, two in Hispanics, and two in Caucasians, refs. 16, 24, 26, 33, 35), we observed a strong but imprecise association between *IL1B-31T* and noncardia gastric cancer risk in Hispanics (random-effects OR, 2.54; 95% CI, 0.39-16.68). No association for noncardia gastric cancer was observed when studies in Caucasians were analyzed.

***IL1B+3954* Polymorphism and Gastric Cancer Risk.** Eight studies in nine populations (Table 3) investigated this association. Absence of subjects with the T/T genotype in four studies limited the data to estimate the pairwise differences and to choose the genetic model following the algorithm previously described. Based on the reported increased gastric cancer risk of the heterozygotes of

Table 5. Pooled ORs and 95% CIs of gastric cancer associated with *IL1B-511* and *IL1B-31* polymorphisms based on dominant genetic models

Group of analysis	*IL1B-511* (T carriers vs C/C genotype)				*IL1B-31* (C carriers vs T/T genotype)			
	*n**	$P_{heterogeneity}$	Method of estimation		*n**	$P_{heterogeneity}$	Method of estimation	
			Fixed–effects	Random–effects			Fixed–effects	Random–effects
			OR (95% CI)	OR (95% CI)			OR (95% CI)	OR (95% CI)
Total	15	<0.01	1.26 (1.13-1.40)	1.21 (1.00-1.47)	14	<0.01	1.04 (0.93-1.17)	1.04 (0.83-1.29)
Asians	7	0.31	0.95 (0.81-1.13)	0.96 (0.80-1.15)	6	0.07	0.90 (0.76-1.07)	0.91 (0.71-1.15)
Caucasians	8	0.05	1.56 (1.35-1.80)	1.49 (1.20-1.85)	5	<0.01	1.18 (1.00-1.40)	1.11 (0.74-1.67)
Quality score† <5	3	0.15	1.06 (0.79 1.43)	1.08 (0.71 1.63)	2	0.06	0.88 (0.64-1.20)	0.95 (0.51-1.79)
Quality score ≥5	5	0.81	1.77 (1.49 2.09)	1.77 (1.49 2.09)	3	<0.01	1.34 (1.09-1.63)	1.22 (0.71-2.10)
Matched‡	2	0.21	1.76 (1.37-2.27)	1.70 (1.19-2.42)	2	0.02	1.65 (1.28-2.12)	1.41 (0.71-2.82)
Unmatched	6	0.05	1.47 (1.22-1.76)	1.42 (1.08-1.87)	3	0.16	0.89 (0.71-1.12)	0.92 (0.66-1.26)
<200 subjects	0	—	—	—	0	—	—	—
200-400 subjects	6	0.10	1.35 (1.11-1.65)	1.34 (1.02-1.75)	2	0.36	1.13 (0.78-1.65)	1.13 (0.78-1.65)
>400 subjects	2	0.72	1.86 (1.50-2.31)	1.86 (1.50-2.31)	3	<0.01	1.19 (0.99-1.44)	1.09 (0.59-2.02)
Controls type§ 1	6	0.08	1.40 (1.16-1.68)	1.35 (1.03-1.77)	2	0.36	1.13 (0.78-1.65)	1.13 (0.78-1.65)
Controls type 2	1	—	—	—	1	—	—	—
Controls type 3	1	—	—	—	2	<0.01	1.41 (1.14-1.76)	1.34 (0.64-2.77)
Hispanics	—	—	—	—	3	0.03	1.17 (0.83-1.65)	1.52 (0.61-3.83)
Quality score † <5	—	—	—	—	1	—	—	—
Quality score ≥5	—	—	—	—	2	0.01	1.25 (0.86-1.81)	2.54 (0.39-16.68)

*Number of populations included.
†Score based on source of cases, source of controls, histologic confirmation of gastric cancer, blind genotyping, and association assessment (Addendum 1).
‡Age and sex matched.
§Controls, type 1: blood donors/healthy subjects/nongastroenterology patients; type 2: gastroenterology patients; and type 3: population-/neighbor-based sample.

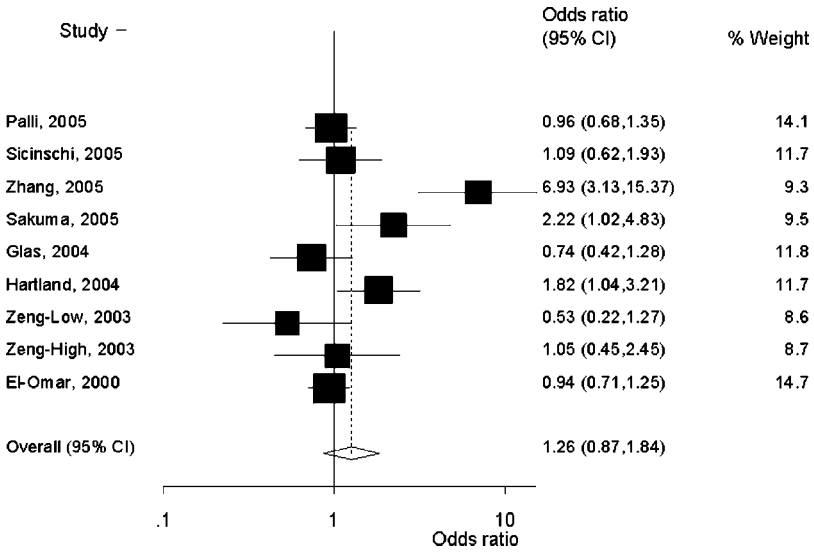

Figure 3. Random-effects ORs and 95% CIs of gastric cancer associated with the *IL1B+3954* polymorphism, based on a dominant model (T carriers versus C/C genotype). The studies are ordered by publication year. The *Q* statistic was 33.4, indicating the presence of heterogeneity ($P < 0.001$).

IL1B+3954 in Asians and Hispanics (14, 15, 31), a dominant genetic model was assumed.

Figure 3 presents the random-effects OR for gastric cancer risk for *IL1B+3954T* carriers compared with the C/C genotype. Under a random-effects model, individuals carrying the T allele had a nonsignificantly elevated gastric cancer risk compared with the C/C genotype. None of the available study characteristics (including genotyping technique) explained the heterogeneity. Based on the observed ethnic variations of the T-allele frequencies, a stratified analysis was done. There was marked heterogeneity across studies in Asians ($P < 0.001$). A moderate association was found between *IL1B+3954T* and gastric cancer risk in this ethnic group (fixed-effects OR, 1.84; 95% CI, 1.22-2.77; random-effects OR, 1.73; 95% CI, 0.59-5.05). In Caucasians, there was less heterogeneity ($P = 0.12$) across the studies. However, this SNP seemed unassociated with gastric cancer risk using either the random- or fixed-effects models. The effect of this polymorphism could not be evaluated in Hispanics due to limited data.

Subgroup analyses by histologic subtype and location were not done in any of the ethnic groups due to insufficient data for this SNP.

***IL1RN* VNTR Polymorphism and Gastric Cancer Risk.** Twenty-three studies in 25 populations tested this association (Table 4). The estimated OR_1, OR_2, and OR_3 were 1.67, 1.09, and 1.56, respectively. These estimates suggest a recessive effect of the allele 2; therefore, L/L and L/2 genotypes were combined and compared with 2/2 genotype. Homozygotes for allele 2 have a nonsignificantly elevated gastric cancer risk compared with carriers of allele L (Fig. 4). As mentioned, a dominant model was also considered; therefore carriers of allele 2 (L/2 and 2/2 genotypes) were compared with L/L genotype. Measures of heterogeneity across studies by both models are shown in Table 6.

Different allele frequencies in ethnic groups indicated stratification by ethnicity. Under a dominant model, studies conducted in Asians were heterogeneous ($P = 0.01$), and *IL1RN*2* did not seem associated with gastric cancer risk. In Caucasians, comparison between *IL1RN*2* carriers and the L/L genotype showed a modest association (random-effects OR, 1.21; 95% CI, 0.99-1.47; 12 populations). Analyses stratified according to study characteristics showed that higher quality studies and those matched on sex and age reported larger ORs on average than studies of lower quality or unmatched ones.

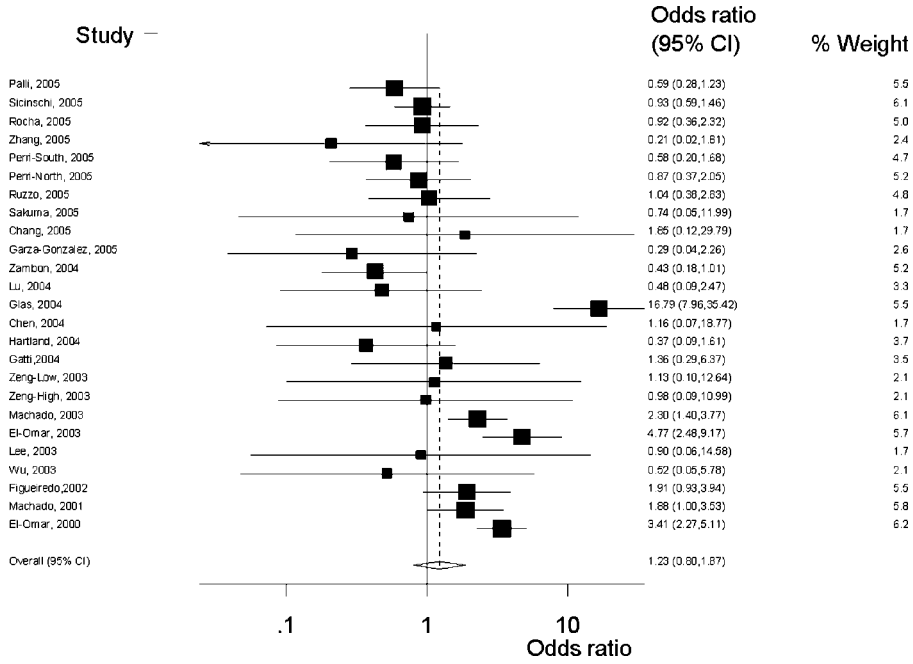

Figure 4. Random-effects ORs and 95% CIs of gastric cancer associated with the *IL1RN* VNTR based on the recessive genetic model (L carriers versus 2/2 genotype). The studies are ordered by publication year. The Q statistic was 103, indicating the presence of heterogeneity ($P < 0.001$). To include the 25 populations, 1 was added to the cells of five studies with no counts for the 2/2 genotype.

Table 6. ORs and 95% CIs of gastric cancer associated with *IL1RN* VNTR based on dominant and recessive genetic models

Group of analysis	Dominant model (L/L genotype vs 2 carriers)				Recessive model (L carriers vs 2/2 genotype)			
	*n**	$P_{heterogeneity}$	Method of estimation		*n**	$P_{heterogeneity}$	Method of estimation	
			Fixed–effects	Random–effects			Fixed–effects	Random–effects
			OR (95% CI)	OR (95% CI)			OR (95% CI)	OR (95% CI)
Total	25	<0.01	1.20 (1.09-1.31)	1.17 (1.00-1.37)	20†	<0.01	1.73 (1.46-2.06)	1.29 (0.82-2.02)
Quality score‡ <5	8	0.01	0.91 (0.75-1.11)	0.93 (0.67-1.30)	6	<0.01	1.72 (1.14-2.59)	1.24 (0.30-5.09)
Quality score ≥5	17	0.02	1.31 (1.17-1.46)	1.29 (1.10-1.51)	14	<0.01	1.73 (1.43-2.10)	1.35 (0.88-2.07)
Asians	9	0.01	1.02 (0.82-1.26)	1.11 (0.77-1.61)	4	0.95	0.62 (0.21-1.85)	0.62 (0.21-1.85)
Quality score‡ <5	3	0.21	0.77 (0.54-1.10)	0.76 (0.48-1.20)	1	—	—	—
Quality score ≥5	6	0.01	1.20 (0.91-1.57)	1.40 (0.85-2.29)	3	0.86	0.65 (0.19-2.20)	0.65 (0.19-2.20)
Caucasians	12	<0.01	1.25 (1.11-1.40)	1.21 (0.99-1.47)	12	<0.01	2.11 (1.74-2.58)	1.59 (0.90-2.81)
Quality score‡ <5	4	0.01	0.92 (0.72-1.16)	0.94 (0.58-1.52)	4	<0.01	1.82 (1.18-2.80)	1.40 (0.23-8.60)
Quality score ≥5	8	0.17	1.38 (1.20-1.57)	1.36 (1.15-1.61)	8	<0.01	2.20 (1.76-2.75)	1.75 (1.07-2.88)
Matched§	3	0.96	1.66 (1.35-2.05)	1.66 (1.35-2.05)	3	0.04	3.27 (2.36-4.52)	2.88 (1.47-5.65)
Unmatched	9	0.01	1.10 (0.95-1.26)	1.08 (0.86-1.35)	9	<0.01	1.64 (1.28-2.10)	1.30 (0.62-2.73)
<200 subjects	0	—	—	—	0	—	—	—
200-400 subjects	8	0.01	1.18 (0.99-1.39)	1.16 (0.87-1.53)	8	<0.01	2.34 (1.76-3.12)	1.78 (0.80-3.98)
>400 subjects	4	0.01	1.32 (1.12-1.55)	1.27 (0.92-1.76)	4	<0.01	1.93 (1.47-2.53)	1.26 (0.50-3.16)
Controls type‖ 1	7	0.03	1.07 (0.90-1.28)	1.08 (0.82-1.42)	7	<0.01	2.01 (1.57-2.79)	1.58 (0.67-3.71)
Controls type 2	2	0.29	0.95 (0.71-1.26)	0.95 (0.70-1.29)	2	<0.01	1.03 (0.59-1.79)	0.92 (0.21-4.00)
Controls type 3	3	0.99	1.66 (1.39-2.03)	1.66 (1.39-2.03)	3	<0.01	2.69 (1.97-3.67)	2.18 (0.73-6.48)
Hispanics	4	0.24	1.24 (0.98-1.57)	1.23 (0.91-1.67)	4	0.70	0.91 (0.62-1.34)	0.91 (0.62-1.34)
Quality score‡ <5	1	—	—	—	1	—	—	—
Quality score ≥5	3	0.25	1.18 (0.92-1.51)	1.16 (0.85-1.57)	3	0.56	0.89 (0.59-1.32)	0.89 (0.59-1.32)

*Number of populations included.
†Only studies that have counts on 2/2 genotype were included.
‡Score based on source of cases, source of controls, histologic confirmation of gastric cancer, blind genotyping, and association assessment (Addendum 1).
§Age and sex matched.
‖Controls, type 1: blood donors/healthy subjects/nongastroenterology patients; type 2: gastroenterology patients; and type 3: population-/neighbor-based sample.

The four studies conducted in Hispanics appeared homogeneous. Using the L/L genotype as the reference, a weak association was found between *IL1RN**2* and gastric cancer risk. Under a recessive model, larger ORs were observed for the majority of the analytic approaches; heterogeneity across studies also increased.

Stratification by histologic subtype was done by both genetic models. Under a recessive model, six studies conducted in Caucasians were considered (5, 6, 13, 30, 35, 39). Seven studies were analyzed considering a dominant model (four studies in Caucasians, one in Asians, and two in Hispanics; refs. 6, 13, 17, 29, 30, 35, 36). Two reports were excluded (5, 39) because their comparison could not be incorporated into our analysis (L carriers versus 2/2 genotype). Using random-effects models, elevated intestinal-subtype gastric cancer risks were observed in both models (OR, 2.26; 95% CI, 1.08-4.74 and OR, 1.22; 95% CI, 0.69-2.13 based on a recessive and dominant model, respectively). Heterogeneity was noted for both groups of studies in Caucasians. Studies conducted in Hispanics appeared

Addendum 1. Scale for Quality Assessment

Criteria	Score
Representativeness of cases	
Selected from any population cancer registry	2
Selected from any gastroenterology/surgery service	1
Selected without clearly defined sampling frame or with extensive inclusion/exclusion criteria	0
Source of controls	
Population- or neighbor-based	3
Blood donors	2
Hospital-based (nongastroenterology patients)	1
Healthy volunteers, but without total description	0.5
Gastroenterology patients	0.25
Not described	0
Ascertainment of gastric cancer	
Histopathologic confirmation	2
Diagnosis of gastric cancer by patient medical record	1
Not described	0
Genotyping examination	
Genotyping done under ''blinded'' condition	1
Unblinded or not mentioned	0
Association assessment	
Assessed association between genotypes and gastric cancer with appropriate statistics and examining confounders and effect modifiers (including *H. pylori* infection)	2
Assessed association between genotypes and gastric cancer with appropriate statistics and examining confounders and effect modifiers, but without including *H. pylori* infection)	1
Assessed association between genotypes and gastric cancer with appropriate statistics without examining confounders and effect modifiers	0

Addendum 2. Study Characteristics of the 25 Revised Reports. The Studies Are Presented in Decreasing Order Based on the Date of Publication

First author, year of publication (reference)	Country, region	Controls/cases*	Gastric cancer cases	
			Anatomic site [†]	Histologic type [†,‡]
Palli, 2005 (28)	Italy, central	546/185	Antrum: 56.6%, body: 20.2% and other, including cardia	All (I: 59.5%, D: 32.5%, and M: 8%)
Sicinschi, 2005 (29)	Mexico, south	377/183	Cardia and noncardia	All (I: 36%, D: 53%, and M: 11%)
Rocha, 2005 (26)	Brazil, south	541/168	Noncardia	ND
Zhang, 2005 (14)	China, northwestern (high gastric cancer risk region)	166/154	ND	ND
Perri, 2005 (30)	Italy, south and north (low and high gastric cancer prevalence)	South (S): 146/86; north (N): 216/98	S = proximal: 26%, distal: 74%; N = proximal: 14%, distal: 86%	All (S= I: 79%, D or M: 21% N= I: 92%, D or M: 8%)
Ruzzo, 2005 (6)	Italy, central	100/138	ND	Intestinal (55%) and diffuse (45%)
Sakuma, 2005 (31)	Japan	103/140[¶]	Corpus (68%) and antrum	Intestinal and diffuse (50%)
Chang, 2005 (32)	South Korea, Seoul	434/234	Cardia (8.5%) and noncardia (91.5%)	All (I: 50%, D: 49.6%, and M: 0.4%)
Garza-Gonzalez, 2005 (24)	Mexico, north	215/63	Distal	Intestinal (46%) and diffuse (54%)
Zambon, 2004 (33)	Italy	644/129	Noncardia	All (I: 52%, D: 30%, and M: 18%)
Lu, 2004 (34)	China, northern	300/250	Corpus (36%), antrum (46%), cardia, and unclassified (18%)	All (I: 42%, D:40% and M: 18%)
Glas, 2004 (35)	Germany	145/88	Noncardia	All (I: 52%, D: 26%, and M: 22%)
Yang, 2004 (19)	China	258/280	Cardia (~43%) and others [††]	ND
Chen, 2004 (36)	Taiwan	164/142	ND	All (I: 58%, D:30% and M: 12%)
Lee, 2004 (37)	South Korea, Seoul	433/331	ND	All (I: 40%, D: 57%, and M: 3%)
Gatti, 2004 (17) [‡‡]	Brazil, north	56/56	ND	Intestinal (43%) and diffuse (57%)
Hartland, 2004 (38)	United Kingdom, Newcastle upon Tyne	286/59	Gastric and gastroesophageal cancer	ND
Zeng, 2003 (25)	China, low and high risk regions	Low risk:192/84 High risk: 169/86	Noncardia	ND
Machado, 2003 (39)[§§]	Portugal, Northern	306/287	Cardia (15.5%) and noncardia (84.5%)	All (I: 45%, D:31% and M: 24%)
El-Omar, 2003 (18)	United States (New Jersey, Connecticut, and Washington State)[‖‖]	210/188	Noncardia[¶¶]	Intestinal (34%) and diffuse (47%)
Wu, 2003 (40)	Taiwan-China	230/220	Cardia (14%) and noncardia (86%)	Intestinal (51%) and diffuse (49%)
Lee, 2003 (16)	South Korea, Seoul	172/190	Antrum (34.7%) and body (46.8%)	Intestinal (15%), diffuse (80%) and unclassified (5%)
Figueiredo, 2002 (5) [†††]	Portugal, Porto	136/222	Cardia (17.6%) and noncardia	All (I: 53%, D:23% and M: 24%)
Machado, 2001 (13)	Portugal, Northern	220/152	ND	All (I: 50%, D:24% and M: 26%)
El-Omar, 2000 (4)	Poland, Warsaw	429/366	Cardia, noncardia and unknown [‡‡‡]	All, including indeterminate and unclassified [‡‡‡]

homogeneous, and a moderate association was observed (OR, 1.60; 95% CI, 0.90-2.82 based on a dominant model). *IL1RN**2 did not seem associated with diffuse-subtype gastric cancer risk in either model.

No associations for noncardia gastric cancer were observed when data from nine studies (5, 16, 18, 24-26, 33, 35, 39) were analyzed considering a recessive model. Under a dominant model, a meta-analysis of noncardia gastric cancer in nine populations (three in Caucasians, four in Asians, and two in Hispanics, 16, 18, 24-26, 31, 33, 35, excluding 5 and 39, due to incompatible presentation of data) showed nonsignificant associations in all ethnic groups.

Discussion

The wide variation in gastric cancer incidence across populations worldwide may be influenced by differences in genetic susceptibility. It was our goal in this meta-analysis to examine the strength of the association of *IL1B* and *IL1RN*

Addendum 2. Study Characteristics of the 25 Revised Reports. The Studies Are Presented in Decreasing Order Based on the Date of Publication (Cont'd)

Source of controls	Population based	Matched variables in recruitment	*H. pylori* status adjustment	Quality score
Randomly selected from the municipality list of two regions	Yes	Region	Yes	8.0
Clinical services in the same hospitals as cases	No	Age, sex and region	Yes	6.0
Blood donors	No	None	Yes	5.0
Checking-up examinees in the same hospital as cases	No	Age, sex and ethnicity	No	4.0
Blood donors	No	None	No§	4.0
Blood donors	No	Age and sex	Yes‖	7.0
Healthy volunteers	No	None	Yes	3.5
Gastroenterology patients from the same hospital as cases	No	None	Yes	5.25
Gastroenterology patients (gastritis, intestinal metaplasia or peptic ulcer)	No	None	Yes	5.25
Patients with gastroduodenal diseases**	No	None	No§	3.25
Random sample from a cancer screening program	Yes	Age and sex	Yes	8.0
Blood donors	No	Ethnicity	No	4.0
Randomly selected from neighbors	Yes	Age, sex and region	Yes	10.0
Mild gastritis or normal at endoscopy	No	Ethnicity	Yes	5.25
Healthy volunteers	No	None	No	3.5
Blood donors	No	None	No§	4.0
Healthy volunteers	No	Region and ethnicity	Yes	5.5
Volunteers from two universities	No	None	Yes	5.5
Blood donors	No	None	No§	6.0
Random-digit dialing or health care financing administration records	Yes	Age and sex	Yes***	9.0
Minimal gastritis or normal at endoscopy	No	Age, sex, ethnicity and blood collection date	No§	4.25
Health Promotion center in the same hospital as cases	No	Age and sex	No	5.0
Shipyard workers with nonatrophic gastritis	No	None	Yes	5.25
Blood donors	No	None	No	5.0
Random sample of Warsaw residents‡‡‡	Yes	Age and sex	No	8.0

*Data are mainly based on the information described in Materials and Methods in the respective reports.
†ND, not described.
‡All = intestinal (I), diffuse (D), and mixed (M) types.
§Although *H. pylori* status was assessed, the statistical models for *IL1B* and *IL1RN* gene polymorphisms were not adjusted or stratified by this variable.
‖Cases and controls were *H. pylori* negative.
¶Only information from the category "Total gastric cancer" was analyzed.
**The control group includes patients with gastritis, duodenitis, esophagitis, and subjects without endoscopic lesions.
†† Information taken from Shen et al. (22).
‡‡ Total number of cases is based on the information presented in Table 2.
§§Information from subjects with chronic gastritis was not considered in our analysis.
‖‖Information taken from Gammon et al. (21).
¶¶Only information for noncardia gastric cancer cases was analyzed from this article.
***The *H. pylori* serologic analysis did not include the total sample.
††† Control group included only subjects with nonatrophic gastritis.
‡‡‡ Information taken from Chow, et al. (20).

gene polymorphisms with gastric cancer risk and to identify sources of heterogeneity in the studies. To our knowledge, this is the first meta-analysis that addresses this subject.

IL1B and *IL1RN* are part of a gene family cluster located on chromosome 2q21. Although it would be desirable to examine the risk of gastric cancer associated with a limited number of haplotypes for these polymorphisms, unfortunately such data are not yet available. Consequently, we have examined associations of each polymorphism independently to learn which associations are the strongest in the current literature. It is important to note that these associations do not identify a causal allele, but may reflect the effect of one or more other polymorphisms in linkage disequilibrium with the associated allele. Nevertheless, identification of marker alleles can be useful, especially in combination with other factors associated with risk.

Our results are consistent with studies that show *IL1B-511T* to be associated with gastric cancer risk in Caucasians, following a dominant genetic model. This association is particularly strong when intestinal-subtype and noncardia gastric cancer cases were examined. Our results are also consistent with reports that show this allele to be unassociated with gastric cancer risk in Asians. The reason for this ethnicity-specific effect may be the high prevalence of the putative risk allele *IL1B-511T* in cancer-free Asian subjects (50% in Asians versus 33% in Caucasians). When the measure of association is the relative risk, it is more difficult to detect an increased risk if the frequency of the risk allele is high in the population. It may also be that other gastric cancer risk factors, such as prevalence of *H. pylori* infection, prevalent *H. pylori* virulent factors, lifestyle, diet, or other environmental risk factors for which Asians and Europeans vary, may be responsible for the different associations observed in these ethnic groups.

Despite the well-known linkage disequilibrium between *IL1B-511T* and *IL1B-31C* (4, 53), we did not find a significant association with gastric cancer in Caucasians for *IL1B-31*C SNP. Only a few studies evaluated both *IL1B-511* and *IL1B-31* polymorphisms in the same subjects. It is likely that the smaller sample size used in the *IL1B-31* meta-analysis in Caucasians influenced the results.

The meta-analysis of *IL1B+3954* had the smallest number of subjects. Although an interesting trend was seen, particularly in Asians, no clear associations were observed. Additional large studies are necessary to clarify these relationships.

Analysis of studies of *IL1RN* VNTR had many similarities with those of *IL1B-511*: again, ethnicity and study quality were sources of heterogeneity. Under a dominant model, significant associations of gastric cancer and allele 2 of *IL1RN* were observed in Caucasians, in high-quality studies, in those with matched controls, and in those with population-based controls. No significant associations were observed in Asians. This finding and the fact that the allele 2 is rare in Asians suggest that this genetic marker may be of less importance and effect for that ethnic group. The small sample size used in the meta-analyses of Hispanics restricts interpretation. Similar results were observed under a recessive model. Among the polymorphisms we examined, *IL1RN* VNTR may be most vulnerable to genotyping error, due to more efficient amplification of the short allele 2 by PCR (54). It is known that nondifferential misclassification errors tend to underestimate associations. In the same way, differences in the results of the genetic models, particularly the increase of heterogeneity in the recessive model, may be related to misclassification errors. The recessive model is likely to have a stronger misclassification effect than the dominant one, due to the unpredictable proportion (across the studies) of subjects with the genotype L/2 misclassified as 2/2, due to artifactual disappearance of the harder-to-amplify longer allele in heterozygotes. This situation contrasts to that of the dominant model, in which the misclassification effect is masked by combining of L/2 and 2/2 genotypes as the exposure group. Studies of the *IL1RN* VNTR may benefit from use of the methodology of detection of fluor-tagged PCR products, a more sensitive protocol than standard analysis by ethidium bromide staining of agarose gels.

None of the polymorphisms (for which sufficient data were available) appeared associated with diffuse-subtype gastric cancer risk. Diffuse cancers, although they may be associated with *H. pylori* infection, have a different pathway of development from that of intestinal-subtype gastric cancer (55, 56). Epidemiologic and histopathologic evidence have shown that the intestinal-subtype is related to gastric atrophy and intestinal metaplasia, whereas the diffuse subtype is not usually preceded by a histologically identifiable precursor lesion (57).

Although few studies presented results stratified by anatomic site, we found a significant association between *IL1B-511*T and noncardia gastric cancer risk. Diverging patterns of gastric cancer incidence by location suggest that tumors in the proximal (cardia) and distal (noncardia) stomach may represent two different nosologic entities (58). Differences in findings across studies may reflect interstudy variation in the proportion of tumors of different sites and histologic patterns. The proportion of diffuse-subtype gastric cancer varied from 8% to 80% across studies, and the proportion of cardia gastric cancer varied from 8.5% to 26% across studies. Combining intestinal and diffuse subtypes, as well as cardia and noncardia cases, may cause underestimation of the magnitude of the associations.

Mechanisms by which the *IL1B* and/or *IL1RN* gene polymorphisms may confer gastric cancer susceptibility have been proposed, mainly related to the predisposition for increased IL-1β production or IL1Ra reduction. Carriers of *IL1B-511T*, *IL1RN**2, and *IL1B+3954T* alleles are high producers of IL-1β (9, 59-61). Acute and chronic inflammation in the gastric mucosa induced by *H. pylori* infection is accompanied by IL-1β production, which enhances the immune response and inhibits gastric acid secretion. Resultant hypochlorhydria permits pH-sensitive bacteria to colonize the stomach, potentially converting ingested nitrates into carcinogenic *N*-nitroso-compounds. It has been hypothesized that prolonged hypochlorhydria may lead to gastric atrophy and subsequently to gastric cancer (7). However, hypochlorhydria may also lead to reactive increased production of gastrin, a potent cell growth factor implicated in many processes, including neoplastic transformation (62). In parallel, prolonged inflammation causes excessive free radical production, which, in the presence of insufficient antioxidant defenses, may lead to lipid peroxidation and DNA damage (63, 64).

The associations found gain credence by *in vivo* studies in animal models demonstrating the important role of IL-1β in gastric carcinogenesis. In a Mongolian gerbil model, Takashima et al. (65) reported that acid secretion is decreased by *H. pylori* infection, which is accompanied by increase in mRNA expression of IL-1β in gastric mucosa. More recently, Tu et al. (66), using a transgenic mouse model, showed that overexpression of IL-1β can directly induce gastric atrophy and dysplasia in the absence of *H. pylori* infection.

Recent *in vitro* evidence supports the idea that both *IL1B-511* and *IL1B-31* are functional SNPs affecting the promoter activity of the *IL1B* gene (67). The presence of the minor allele in both SNPs produced greater promoter activity in a monocyte cell line than was observed by either minor allele alone. Furthermore, two other SNPs at −3737 and −1464 modulated the promoter activity, when both −31 and −511 carried minor alleles. Therefore these two SNPs, *IL1B-3737* and *IL1B-1464*, are likely candidates to test for association with gastric cancer risk. The same study noted that these four SNPs were present in Caucasians and African-Americans in four common haplotypes, two of which contain minor alleles at both −31 and −511. It follows that gene association studies examining only −511 and/or −31 are combining the effects of two haplotypes. Separation of these two haplotypes may provide a clearer understanding of genetic risk factors for gastric cancer.

Although gastric cancer incidence rates in some populations of Central and South America are among the highest in the world (68), currently available data regarding Hispanic populations are limited. Studies in these groups should be done and interpreted with caution due to the natural genetic admixture of varied ethnic groups (Amerindian, Caucasian, and African). We found no data regarding subjects of African descent, although African-Americans have a higher gastric cancer incidence than Caucasians (69).

We limited our meta-analysis to studies of associations of *IL1B* and *IL1RN* gene polymorphisms and gastric cancer risk. It was not our intention to address associations between these polymorphisms and gastric precancerous lesions. Nevertheless, it is interesting to note that although our meta-analysis did not find association between *IL1B-511T* and gastric cancer risk in Asians, this polymorphism has been associated with hypochlorhydria, atrophic gastritis, and intestinal metaplasia in this ethnic group in *H. pylori*–infected subjects (70, 71). Also, in Asians, Furuta et al. (72) reported that in *H. pylori*–infected patients, *IL1B-511* T/T and C/T genotypes were associated with increased inhibition of gastric acid secretion, and more widespread and more severe *H. pylori* infection, when compared with C/C genotype. This apparent incongruence deserves further investigation. It is not clear if the final stages of the gastric carcinogenic process are determined by a set of forces that differ from those involved in earlier stages.

Inverse associations between *IL1B−511T/−31C* and gastroesophageal reflux disease risk have been recently reported (73, 74). It is reasonable that alleles associated with gastric cancer and gastric atrophy would also be associated with a decrease in risk of an acid-related condition, such as gastroesophageal reflux disease.

The interpretation of this report should be made within the context of its limitations. The studies contributing to the summary estimates are vulnerable to various sources of bias. Tumor misclassification, by histology or location, may be substantial in some studies, particularly those lacking details about methods aimed at reducing misclassification. Genotyping inaccuracy may also be common; quality control measures for genotyping were not well documented in most reports. In some studies, controls were younger on average than cases; such control groups may include individuals with genotypes of interest who later develop cancer within the age range of the case group. The resulting case-control contrast would underestimate the relative risk associated with the genotype. The observation of stronger effects in studies that matched by age suggests that studies that failed to do so were vulnerable to this bias. In addition, the *IL1B* and *IL1RN* gene polymorphisms may be associated with population characteristics related to gastric cancer risk factors, such as *H. pylori* infection and smoking. Failure to control for those factors is another potential source of bias.

Another type of limitation, especially for *IL1B-31* and *IL1B+3954* analyses, was the small number of studies and, consequently, limited statistical power. The small sample size also limited the ability to conduct more meaningful subgroup analyses. Unavailability of raw data from the original studies limited the evaluation of gene-environment interactions. Another limitation is a potential English language bias. It is possible that the non-English literature contains studies that differ from those included here. Finally, population stratification may have affected the results of the constituent studies in the meta-analyses, as we documented that the frequency of the risk alleles varied considerably across ethnic groups.

Our analysis suggests recommendations for future genetic association studies of gastric cancer. Potential confounding factors and effect modifiers, such as age, sex, ethnicity, *H. pylori* status, *cagA* and *vacA* status, and smoking should be examined. Controls should be selected to represent the genotype distribution of the source population for the cases; at the very least, the age distribution should match that of the case series. Cases should be classified by location and histologic subtype with subtype-specific results presented. Future studies should ensure an adequate sample size taking into account the genotype frequencies, the effect size of interest, and the possibility of interactions. Strict quality control should be implemented in the DNA extraction and genotyping (including blind procedures), and deviation from HWE among controls should be noted. Use of more sensitive genotyping techniques is highly recommended to evaluate the *IL1RN* VNTR. Because gastric cancer is a multifactorial disease, more studies should focus on testing haplotypes and gene-environment interactions, as this might elucidate further the genetics of this complex disease.

In conclusion, although residual heterogeneity beyond factors addressed in this analysis was observed, our findings provide evidence that there are ethnic-specific associations between *IL1B* and *IL1RN* gene polymorphisms and gastric cancer risk. Conflicting results of previous reports may be explained by variation in the allele frequencies across ethnic groups, interstudy variation of the histologic subtype, and anatomic location of gastric cancer cases, as well as study quality.

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