A Meta-Analysis of Interleukin-10 -592 Promoter Polymorphism Associated with Gastric Cancer Risk

Huiping Xue1*, Ying-Chao Wang1*, Bing Lin2*, Jianfu An3*, Lu Chen4*, Jinxian Chen4, Jing-Yuan Fang1*

1Division of Gastroenterology and Hepatology, Shanghai Jiao-Tong University School of Medicine Renji Hospital, Shanghai Institution of Digestive Disease and Key Laboratory of Gastroenterology & Hepatology, Ministry of Health Shanghai Jiao-Tong University, Shanghai, People’s Republic of China, 2Division of Nutrition, Zhongshan Hospital, Fudan University School of Medicine, Shanghai, People’s Republic of China, 3Bioinformatics Department, Shanghai Jiao Tong University School of Medicine, Shanghai, People’s Republic of China, 4Department of General Surgery, Renji Hospital, Shanghai, People’s Republic of China

Abstract

We aimed to explore the role of IL-10 -592 A/C SNP in the susceptibility to gastric cancer through a systematic review and meta-analysis. Each initially included article was scored for quality appraisal. 17 studies were eligible for the meta-analysis. We adopted the most probably appropriate genetic model (recessive model). Potential sources of heterogeneity were sought out via subgroup and sensitivity analyses, and publication biases were estimated. IL-10-592 AA genotype is associated with the reduced risk of developing gastric cancer among Asians and even apparently observed among Asians high quality subgroup, suggesting IL-10-592 AA genotype may seem to be more protective from overall gastric cancer in Asian populations. IL-10-592 AA genotype is also associated with the overall reduced gastric cancer susceptibility in persons with H. pylori infection compared with controls without H. pylori infection, suggesting IL-10-592 AA genotype may seem to be more protective from overall gastric cancer susceptibility in persons infected with H. pylori. IL-10-592 AA genotype is not associated with either pathologic subtypes (intestinal or diffuse) or anatomic subtypes (non-cardia or cardia) of gastric cancer susceptibility. Genotyping methods like direct sequencing should be highly advocated to be conducted in future well-designed high quality studies among different ethnicities or populations.

Introduction

Worldwide gastric cancer incidence has decreased but its mortality still ranks second [1–3]. In Asia [4], especially China [5], gastric cancer constitutes the peak lethal malignancy. As is widely known, infectious, dietary, environmental, and genetic factors are implicated in gastric carcinogenesis, but only a minority of persons exposed to risk factors such as Helicobacter pylori (H. pylori) infection ultimately develop gastric cancer [6], which implies that host genetic susceptibility plays an important role in developing gastric cancer [7–9]. Such various susceptibilities could be partially explained by single nucleotide polymorphisms (SNPs) of susceptible genes [7–9]. During the pathogenesis from chronic gastritis to gastric cancer spawned by H. pylori infection, host activated neutrophils and mononuclear cells can produce not only proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α but also anti-inflammatory cytokines like IL-10. Riveting, the level of IL-10 besides those of IL-1 and TNF-α could also be elevated in gastric mucosa infected with H. pylori.

IL-10, a potent pleiotropic cytokine, has the dual ability to immunosuppress or immunostimulate anti-cancer properties [10]. Interleukin-10 inhibits the production of pro-inflammatory cytokines by inhibition of T-helper 1 (Th1) lymphocytes and stimulation of B lymphocytes and Th2 lymphocytes and thus downregulates the inflammatory response [10–12]. The human IL-10 gene, located on chromosome 1q31–32, consists of five exons and four introns and one of polymorphisms is reported in its 5' -flanking region at position -592 A/C SNP [13].

In 2003, El-Omar EM et al. [14] and Wu MS et al. [15] almost simultaneously published their separate study on IL-10-592 A/C SNP. Since then, researchers have consecutively reported associations of IL-10-592 A/C SNP with the susceptibility to gastric cancer, but with mixed or conflicting results [16–30]. Up to now, there have been two relevant published meta-analysis articles focusing on IL-10-592 A/C SNP [31,32], but those two meta-analyses both failed to adopt the most likely appropriate genetic model, and thus the authentic values of statistical results could be compromised. Accordingly, the aim of our meta-analysis was to shed more light, using the most appropriate genetic model, on the role of IL-10-592 A/C SNP in the risk of developing gastric cancer and to identify possible sources of heterogeneity among the eligible studies.

Materials and Methods

Search Strategy

A systematic literature search was performed for articles regarding IL-10-592 A/C SNP associated with the risk of...
Table 1. Study Characteristics of genotypes in gastric cancer cases and controls in the analysis of Interleukin-10 -592 Promoter Genetic Polymorphism.

| First author                  | Year of publication | Quality assessment scores | Genotyping method | Total sample size | Number of controls | Number of cases | Study location | Ethnic group | P values for HWE Controls, genotypes(n) | All Cases, genotypes(n) |
|------------------------------|---------------------|---------------------------|-------------------|-------------------|-------------------|-----------------|---------------|-------------|----------------------------------------|------------------------|
| El-Omar EM et al.            | 2003                | 7.5                       | TaqMan            | 524               | 210               | 314             | USA           | Caucasians  | 0.427256638 127 70 13 178 101 35    |                        |
| Wu MS et al.                 | 2003                | 7                         | Direct sequencing | 450               | 230               | 220             | China         | Asians      | 0.231397685 20 83 127 27 105 88      |                        |
| Savage SA et al.             | 2004                | 5                         | ABI Genetic Analyzer | 470           | 386               | 84              | China         | Asians      | 0.38299498 171 166 49 36 39 9       |                        |
| Alpizar-Alpizar W et al.     | 2005                | 6                         | Pyrosequencing    | 88                | 44                | 44              | Costa Rica    | Caucasians  | 0.761073904 18 21 5 21 20 3         |                        |
| Zambon C F et al.            | 2005                | 5                         | TaqMan            | 773               | 644               | 129             | Italy         | Caucasians  | 0.696436614 353 245 46 70 42 17      |                        |
| Lee JY et al.                | 2005                | 5.5                       | RFLP              | 242               | 120               | 122             | South Korea   | Asians      | 0.059163504 7 60 53 8 62 52         |                        |
| Kamangar F et al.            | 2006                | 8                         | TaqMan            | 320               | 208               | 112             | Finland       | Caucasians  | 0.775545579 109 82 17 68 38 6        |                        |
| Sicinschi LA et al.          | 2006                | 5.5                       | Pyrosequencing    | 550               | 369               | 181             | Mexico        | Latinos     | 0.376818571 98 176 95 51 90 40        |                        |
| Sugimoto M et al.            | 2007                | 6.5                       | ASP               | 273               | 168               | 105             | Japan         | Asians      | 0.419149756 10 70 88 8 54 43         |                        |
| Garcia-González MA et al.    | 2007                | 7.5                       | TaqMan            | 808               | 404               | 404             | Spain         | Caucasians  | 0.075218023 245 131 28 237 143 24    |                        |
| Crusius JB et al.            | 2008                | 8.5                       | ABI real-time PCR | 1359             | 1122              | 237             | Europe        | Caucasians  | 0.049349054 642 397 83 148 78 11     |                        |
| Deng WY et al.               | 2008                | 4                         | Direct sequencing | 235               | 110               | 125             | China         | Asians      | 1.18833E-08 46 25 39 56 39 30         |                        |
| Kang JM et al.               | 2009                | 6.5                       | RFLP              | 665               | 332               | 333             | Korea         | Asians      | 0.591846755 41 145 146 34 157 142    |                        |
| Xiao H et al.                | 2009                | 6                         | RFLP              | 844               | 624               | 220             | China         | Asians      | 0.718880427 69 283 272 20 100 100     |                        |
| Ko KP et al.                 | 2009                | 7                         | Snapshot          | 408               | 325               | 83              | Korea         | Asians      | 0.040647499 37 121 167 11 33 39       |                        |
| Con SA et al.                | 2009                | 4.25                      | RFLP              | 243               | 191               | 52              | Costa Rica    | Latinos     | 0.015843753 103 65 23 16 26 10        |                        |
| Liu J et al.                 | 2011                | 6.5                       | RFLP              | 477               | 243               | 234             | China         | Asians      | 0.772829993 28 106 109 39 96 99       |                        |

*Data of cardia-subtype gastric cancer were accessible; † Data of noncardia-subtype gastric cancer were accessible; ‡ Data of sporadic diffuse-subtype gastric cancer were accessible; § Data of intestinal-subtype gastric cancer were accessible. Here the ancestry of predominant participants in this study is annotated as Spanish ethnicity, which should be treated as Latins rather than Caucasians [29]. RFLP: Restriction fragment length polymorphisms; TaqMan: 5′-nuclease polymerase chain reaction assays; Pyrosequencing: a method of DNA sequencing (determining the order of nucleotides in DNA) based on the “sequencing by synthesis” principle. It differs from Sanger sequencing, in that it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides. Direct sequencing: method of methylation analysis using bisulfite-treated DNA utilized PCR and standard dideoxynucleotide DNA sequencing to directly determine the nucleotides resistant to bisulfite conversion; ASP: the allele specific primer-polymerase chain reaction (ASP-PCR) method; Snapshot: the Snapshot assay which provides detection of certain SNPs.

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developing gastric cancer. The MEDLINE, EMBASE databases, Chinese National Knowledge Infrastructure (CNKI), Web of Science, and BIOSIS databases were used simultaneously with the combination of terms “Interleukin 10”, “IL-10”, “interleukin”, or “cytokine”; “gene”; “polymorphism”, “variant”, or “SNP”; and “gastric cancer”, “gastric carcinoma”, “diffuse gastric cancer” or “stomach cancer” from January 2000 to September 2011. The search was performed without any restriction on language. The scope of computerized literature search was expanded according to the reference lists of retrieved articles. The relevant original articles were also sought manually.

Study Selection
Studies concerning the association of IL-10-592 A/C SNP with the risk of developing gastric cancer were included if the following conditions were met: (i) any study described the association of IL-10-592 A/C SNP with gastric cancer; (ii) any study reported the numbers of both controls and gastric cancer cases; (iii) results were expressed as odds ratio (OR) with 95% confidence intervals (CI); and (iv) studies were case-control or nested case-control ones.

Methodological Quality Appraisal
To identify high-quality studies, we mainly adopted predefined criteria for Quality Appraisal [33,34,7–9]. The criteria cover credibility of controls, representativeness of cases, consolidation of gastric cancer, genotyping examination, and association assessment [7–9]. Methodological quality was independently assessed by two investigators (Y. Wang and B. Lin). Disagreements were resolved through discussion. Scores ranged from the lowest zero to the highest ten. Articles with the score lower than 6.5 were considered “low or moderate quality” ones, whereas those no lower than 6.5 were thought of as “high quality” ones.

Data Extraction
The following data from each article were extracted: authors, year of publication, country, ethnicity of participants (categorized as Caucasians, Asians, Latinos, etc.), study design, source of controls, number of controls and of cases, genotyping method, distribution of age and gender, Lauren’s classification (intestinal, diffuse, or mixed), and anatomical classification (cardia or non-cardia cancer).

The data were extracted and registered into two databases independently by two investigators (Y. Wang and B. Lin) who were blind to journal names, institutions or fund grants. Any discrepancy between these two investigators was resolved by the third investigator (H. Xue), who participated in the discussion with them and made an ultimate decision.

Statistical Analysis
All statistical analyses were performed using STATA statistical software (Version 10.1, STATA Corp, College Station, TX). Two-sided Ps < 0.05 were considered statistically significant. HWE in controls was calculated again in our meta-analysis. The chi-square goodness of fit was used to test deviation from HWE (significant at the 0.05 level). Odds ratios (OR) and 95% confidence intervals (95% CI) were employed to assess the strength of associations between IL-10-592 A/C SNP with gastric cancer risk. OR2, OR3, and OR5 regarding IL-10-592 A/C SNP were calculated for genotypes AA versus CC, CA versus CC, and AA versus CA, respectively.

The above pairwise differences were used to determine the most appropriate genetic model. If OR1 = OR1 ≠ 1 and OR1 = 1, then a recessive model is suggested. If OR1 = OR1 ≠ 1 and OR1 = 1, then a dominant model is implied. If OR2 = 1/OR3 ≠ 1 and OR2 = 1, then a complete overdominant model is suggested. If OR3 > OR2 > 1 and OR3 > OR2 > 1, or OR3 < OR2 < 1 and OR3 < OR2 < 1, then a codominant model is indicated [35]. If a dominant model was indicated, the original grouping was collapsed and the new group of A carriers (AA+CA) was compared with CC genotype; if a recessive model was suggested, AA was compared to the group of CC plus CA; if a complete overdominant model was implied, the group of AA plus CC was compared with CA; or if a codominant model was insinuated, AA was compared with CA and with CC, respectively.

The Q statistic was used to test for heterogeneity among the studies included in the meta-analysis. A fixed-effects model, using Mantel–Haenszel (M-H) method, was used to calculate the pooled ORs when homogeneity existed on the basis of Q-test p value no less than 0.1. By contrast, a random-effects model, using DerSimonian and Laird method (D+L), was utilized if there was heterogeneity based on Q-test p value less than 0.1. The significance of pooled ORs was tested by Z test (P < 0.05 was considered significant).

Sensitivity analysis was performed, in which the meta-analysis estimates were computed after every one study being omitted in each turn. Finally, publication bias was assessed by performing funnel plots qualitatively, and estimated by Begg’s and Egger’s tests quantitatively.

Figure 1. The flow chart of literature search and study selection.
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Results

Literature Search and Study Selection

After comprehensive searching, a total of 236 articles in English and 6 in Chinese were retrieved. In our meta-analysis were initially included altogether 17 studies [14–30] which catered to the inclusion criteria. Those 17 studies were preliminarily appropriate to the meta-analysis of the associations with gastric cancer regarding IL-10-592 A/C SNP.

Four studies [24,25,28,29] were deviated from HWE. Generally speaking, any study that deviated from Hardy-Weinberg equilibrium through our calculation should have been removed; however, considering that the number of participants especially in the study [24] was large and given that sensitivity analyses would be conducted, we remained those four studies in our meta-analysis. Thus, 17 studies [14–30] with a total of 5730 controls and 2999 cases were ultimately eligible for the meta-analysis of IL-10-592 A/C SNP. The corresponding characteristics were seen in Table 1.

Overall Meta-analysis among Different Ethnicity Populations

\[ OR_1 (p \text{ value}), OR_2 (p \text{ value}), \text{ and } OR_3 (p \text{ value}) \] of IL-10-592 A/C SNP for overall ethnicities were 0.91 (p = 0.437), 1.00 (p = 0.950), and 0.87 (p = 0.030), respectively, potentially insinuating a recessive genetic model effect of putative protective A allele \((OR_1 = OR_3 < 1\text{ and } OR_2 = 1)\). Meanwhile, after ethnicity

![Flow chart of literature search and study selection](image-url)

Figure 2. Odds ratios (ORs) for associations between IL-10 -592 A/C SNP and gastric cancer risk (AA vs CA-plus-CC) among different ethnicity populations, in order of increasing publication year, 2003–2011. Studies were entered into the meta-analysis sequentially by year of publication. The sizes of the squares indicate the relative weight of each study. Weights were derived from random-effects analysis. Bars, 95% confidence interval (CI).

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Figure 3. Odds ratios (ORs) for associations between IL-10 -592 A/C SNP and gastric cancer risk (AA vs CA-plus-CC) among different ethnicities based on high quality and median-and-low quality subgroup analysis. The sizes of the squares indicate the relative weight of each study. Bars, 95% confidence interval (CI).

| Study ID | OR (95% CI) | Weight |
|---------|-------------|---------|
| High quality |            |         |
| El-Omar EM et al. (2003) | 1.90 (0.98, 3.69) | 4.53 |
| Kamangar F et al. (2006) | 0.64 (0.24, 1.66) | 2.59 |
| Garcia-Gonzalez MA et al. (2007) | 0.85 (0.48, 1.49) | 5.58 |
| Crusius JBA et al. (2008) | 0.61 (0.32, 1.16) | 4.89 |
| Subtotal (I-squared = 56.1%, p = 0.078) | 0.91 (0.54, 1.53) | 17.39 |
| Median and low quality |            |         |
| Asians |            |         |
| Wu MS et al. (2003) | 0.54 (0.37, 0.79) | 8.43 |
| Sugimoto M et al. (2007) | 0.63 (0.39, 1.03) | 6.50 |
| Kang JM et al. (2009) | 0.95 (0.70, 1.29) | 9.72 |
| Ko KP et al. (2009) | 0.84 (0.52, 1.36) | 6.65 |
| Liu J et al. (2011) | 0.90 (0.63, 1.29) | 8.65 |
| Subtotal (I-squared = 39.7%, p = 0.157) | 0.77 (0.61, 0.98) | 39.95 |
| Medi- and low quality subgroup analysis, OR1 (p value), OR2 (p value), and OR3 (p value) of IL-10 -592 A/C SNP among Asians were 0.82 (p = 0.080), 1.04 (p = 0.699), and 0.83 (p = 0.011), respectively, further suggesting a recessive genetic model effect of putative protective A allele (OR1 = OR3 < 1 and OR2 = 1). Thus, the genotype AA was compared with the combined genotype CA-plus-CC. As in figure 2, for overall gastric cancer no statistically significant finding could be observed among Caucasians and Latinos, respectively, whereas a statistically significant finding could be noted among Asians from the facts that the pooled ORs (95% CI, p value) were 1.03 (0.64–1.65, p = 0.913) and 1.10 (0.53–2.26, p = 0.802) for the former, respectively, but 0.81 (0.68–0.97, p = 0.019) for the latter. |         |

Further Subgroup Analysis

Specific data for IL-10-592 A/C SNP were classified in accordance with the quality appraisal scores, into high quality (scores no less than 6.5) and median-and-low quality (scores less than 6.5) subgroups among different ethnicities. A statistically significant finding was only witnessed in Asians high quality subgroup but not in Asians median-and-low quality subgroup, Caucasians high quality subgroup, Caucasians median-and-low quality subgroup, or Latinos median-and-low quality subgroup, given that the pooled ORs (95% CIs, p value) were 0.77 (0.61–0.96, p = 0.022), 0.90 (0.69–1.18, p = 0.437), 0.91 (0.54–1.53, p = 0.724), 1.29 (0.41–4.11, p = 0.664), or 1.10 (0.53–2.26, p = 0.802), respectively (Figure 3).

When gastric cancer was classified into non-cardia (or distal) and cardia subtypes, no statistically significant findings were found among non-cardia subtype or among cardia subtype on the grounds that the pooled ORs (95% CIs, p value) were 1.09 (0.57–2.11, p = 0.787) among non-cardia subtype and 0.78 (0.42–1.45, p = 0.432) among cardia subtype. In terms of pathology, gastric cancer could be classified into intestinal, diffuse, or mixed subtypes, and no statistically significant finding was observed in intestinal-subtype cancer or diffuse-subtype cancer, for the pooled ORs (95% CIs, p value) were 0.82 (0.64–1.06, p = 0.127) in the former and 0.89 (0.62–1.29, p = 0.546) in the latter.

In terms of H. pylori infection status, a statistically significant finding was found among H. pylori positive cancer patients in contrast as H. pylori negative controls, but no statistically significant
finding was found among *H. pylori* positive cancer patients in contrast as *H. pylori* positive controls, for pooled ORs (95% CIs, p value) were 0.67 (0.46–0.98, p = 0.041) in the former and 1.00 (0.75–1.32, p = 0.978) in the latter (Figure 4).

And when genotyping techniques were considered, a statistically significant finding was noted in direct sequencing subgroup but not in any other genotyping technique subgroup. In the direct sequencing, TaqMan, ABI Genetic Analyzer, Pyrosequencing, RFLP, ASP, ABI real-time PCR, and Snapshot genotyping technique subgroups, pooled ORs (95% CIs, p value) were 0.55 (0.40–0.75, p = 0.000), 1.25 (0.74–2.13, p = 0.406), 0.83 (0.39–1.75, p = 0.618), 0.80 (0.53–1.20, p = 0.273), 1.00 (0.84–1.19, p = 0.997), 0.63 (0.39–1.03, p = 0.067), 0.61 (0.32–1.16, p = 0.132), and 0.84 (0.52–1.36, p = 0.475), respectively (Figure 5).

### Sensitivity Analysis

Meta-analyses were conducted repeatedly when each particular study had been removed. The results indicated that fixed-effects estimates and/or random-effects estimates before and after the deletion of each study were similar at large, suggesting high stability of the meta-analysis results. As shown in Figure 6, the most influencing single study on the overall pooled estimates seemed to be the study conducted by Wu et al.[15], the sensitivity analysis, however, indicated high stability of the results from the facts that the ORs (95% CI, p value) were 0.88 (0.74–1.05, p = 0.152) before the removal of that study and 0.92 (0.79–1.08, p = 0.332) after the removal of that study. In view of the study [24] conducted by Crusius JB et al. which is deviated from HWE, the ORs (95% CI, p value) were 0.86 (0.74–0.99, p = 0.037) before the removal of that study and 0.87 (0.75–1.01, p = 0.063) after the removal of that study for the all ethnicity, indicating moderate to high stability of the results. Similarly, for the other three studies with deviation from HWE [25,28,29], removal of the three studies one by one altered ORs (95% CI, p value) from 0.86 (0.74–1.00, p = 0.050), 0.86 (0.74–1.00, p = 0.050), and 0.86 (0.74–1.00, p = 0.050) to 0.86 (0.74–1.00, p = 0.050), 0.86 (0.74–1.00, p = 0.050), and 0.86 (0.74–1.00, p = 0.050), respectively, indicating high stability of the results. (The illustrating figures were omitted due to the length of paper).

### Cumulative Meta-analysis

Cumulative meta-analyses of IL-10 -592 A/C SNP association were also conducted among Asians (Figure 7 part A) and among Caucasians (Figure 7 part B) via the assortment of total number of sample size. As shown in Figure 7 part A, the inclination toward significant reverse associations with overall gastric cancer, though...
somewhat undulated, was obviously seen among Asians, whereas in Figure 7 part B, the opposite tendency was observed among Caucasians.

Publication Bias Analysis

Publication bias was preliminarily examined by funnel plots qualitatively and estimated by Begg’s and Egger’s tests quantitatively. Its funnel plot (Figure 8) showed that dots nearly symmetrically distributed, predominantly within pseudo 95% confidence limits. P values were 0.902 in Begg’s test and 0.914 in Egger’s test, separately, also suggesting no publication bias.

Discussion

In our meta-analysis, a statistically significant finding could be noted with the overall reduced risk of gastric cancer among Asians but not among Caucasians or Latinos (AA vs CA-plus-CC); the opposite tendency toward the risk of gastric cancer could also be observed between Caucasians and Asians via cumulative meta-analysis sorted by publication time and the number of total samples. Thus, IL-10-592 AA genotype may seem to be more protective from overall gastric cancer susceptibility among Asians. To be sure, the different or even conflicting risk associations, if so, among different ethnicities should be further meticulously investigated and reconfirmed in the future.

Our further subgroup analyses also indicate that a statistically significant finding was only witnessed in Asians high quality subgroup but not in Asians median and low quality subgroup, Caucasians high quality subgroup, Caucasians median and low quality subgroup, or Latinos median and low quality subgroup (AA vs CA-plus-CC). It is natural that high-quality studies should be designed in the future so as to accurately explore the real associations between IL-10-592 AA genotype and gastric cancer susceptibility among different ethnicities.

Additionally, 5[14,18,20,23,24] out of 17 eligible studies were dealt with noncardia-subtype gastric cancer and 3 [14,23,24] with
cardia-subtype gastric cancer. No statistically significant findings could be noted with either subtype (AA vs CA-plus-CC). 5 studies [20-22,23,26] in our meta-analysis were dealt with pathologically intestinal-subtype gastric cancer and 4 [21-23,26] out of 17 studies were dealt with pathologically diffuse-subtype gastric cancer. No statistically significant finding could be noted in either intestinal-subtype or diffuse-subtype cancer (AA vs CA-plus-CC). As is known, cardia-subtype gastric cancer differs from noncardia-subtype gastric cancer in etiology, pathology, carcinogenesis, and/or prognosis [36–38], so is intestinal-subtype cancer versus diffuse-subtype cancer. It could be said that the indiscriminate combination of cardia-subtype and noncardia-subtype cases or of intestinal-subtype and diffuse-subtype cases in the majority of eligible studies may mask or at least underestimate the strength of the real associations [7–9].

Furthermore, it was reported that gastric cancer develops in those with \textit{H. pylori} infection rather than in uninfected ones [39]. In our meta-analysis, a statistically significant reverse association with gastric cancer susceptibility was noted in direct sequencing genotyping technique subgroup but not in any other subgroup. We have previously mentioned that the most statistically significant result witnessed in direct sequencing technology in meta-analysis does not demonstrate that other technologies cannot be used. Nevertheless, the genotyping results by means of a novel genotyping technique should better be confirmed using direct sequencing. Under this circumstance, the novel genotyping technology can be seen as valid as direct sequencing [40]. Indeed, the sensitivity and specificity of those genotyping techniques need to be further explored so as to seek out the optimal approaches which could minimize the genotyping errors [7–9]. We advocate that direct sequencing should be further conducted in future studies.

Finally, the strength of our meta-analysis could be summarized as follows. We sought to find as many publications as we could by means of various searching approaches. We laid more emphasis on assessing biases across studies and pinpointing the potential sources of heterogeneity via subgroup analyses, and sensitivity analyses. We assessed the publication biases by means of Begg’s and Egger’s tests as well as funnel plot tests. Thus, we convince that the results of our meta-analysis, in essence, are sound and reliable.

Certainly, inevitable limitations could still be found in our meta-analysis. Firstly, the information extracted from the included studies is predominantly about genotypes associated with overall gastric cancer susceptibility, while less accessible is more important information regarding pathologic subtypes or anatomic subtypes of gastric cancer or regarding \textit{H. pylori} infection status. Thus, the results of subgroup analyses in line with specific subtypes or \textit{H. pylori} infection status may mask or at least underestimate the strength of the real associations [7–9].

With the advent of new genotyping technologies like seminested polymerase chain reaction, TaqMan allelic discrimination test, direct sequencing, the allele specific primer-polymerase chain reaction, pyrosequencing, Snapshot, or real-time PCR, we can anticipate an explosion of genetic association studies in the future.
Figure 7. Cumulative meta-analysis of associations between the IL-10 -592AA genotype, as compared with the combined CA-plus-CC genotype, and gastric cancer risk among different ethnicity populations sorted by publication year and the total number of sample size. Horizontal line, the accumulation of estimates as each study was added rather than the estimate of a single study. A) among Asians; B) among Caucasians.
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Secondly, given that merely published studies are included in our meta-analysis, publication bias could potentially occur, though no statistically significant publication bias is noted in our meta-analysis. Thirdly, moderate to severe heterogeneity could be witnessed across the included studies. Nonetheless, in an attempt to minimize the potential bias, we designed a rigorous protocol before conducting meta-analysis, and utilized explicit methods for literature search, study selection, data extraction, statistical analysis, genetic model adoption and sensitivity analysis [40,41].

In conclusion, IL-10-592 AA genotype may seem to be more protective from overall gastric cancer susceptibility among Asians and may also seem to be more protective from overall gastric cancer susceptibility in persons infected with *H. pylori*. IL-10-592 AA genotype is not associated with either pathologic subtypes (intestinal or diffuse) or anatomic subtypes (non-cardia or cardia) of gastric cancer susceptibility in our meta-analysis. Such genotyping methods as direct sequencing should be highly advocated to be conducted in future well-designed high quality studies among different ethnicities or populations.

### Author Contributions

Conceived and designed the experiments: HX JF JC. Performed the experiments: YW BL HX JA LC. Analyzed the data: YW JA BL HX JC. Contributed reagents/materials/analysis tools: HX BL YW JA LC. Wrote the paper: HX JF.

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