Contribution of the -160C/A Polymorphism in the E-cadherin Promoter to Cancer Risk: A Meta-Analysis of 47 Case-Control Studies

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Abstract

Background: The -160C/A polymorphism (rs16260) of E-cadherin, a tumor repressor gene, has been shown to be a tumor susceptibility allele for various types of cancers. Because the significance of this polymorphism to cancer risk has been recognized, there are increasing studies investigating -160C/A in different types of cancers and ethnic populations. However, there is still uncertainty about the level of risk for a variety of cancers.

Methods: To resolve the controversial question raised by these studies as of March 2012 and provide more statistical power for detecting the significance of -160C/A, we performed a meta-analysis of 47 case-control studies in 16 types of cancers (18,194 cases and 20,207 controls). A meta-regression model and subgroup analysis were employed to identify the source of heterogeneity. Publication bias was evaluated, and sensitivity analysis and cumulative evidence assessment were also performed.

Results: Using fixed- and random-effects models, the -160AA homozygote was more susceptible to urothelial cancer compared with the -160CA heterozygote. Additionally, the -160A allele is an ethnicity-dependent risk factor for prostate and colorectal cancers. Carriers of the -160A allele in Asians and Europeans were more susceptible to prostate cancer, whereas their North American counterparts seemed tolerant. The -160AA homozygote plays a protective role for Europeans who develop colorectal cancer. The stability of these observations was confirmed by a one-way sensitivity analysis. However, the cumulative evidence for all cancer types was considered ‘weak’ using the Venice guidelines.

Conclusions: A meta-analysis indicated that the -160A allele of E-cadherin provides a higher risk for the development of prostate and urothelial cancers and a protective role for colorectal cancer in an ethnicity-dependent manner.

Introduction

E-cadherin, which has a widely acknowledged role in cell-cell adhesion, also functions as an invasion/tumor suppressor gene. Several immunohistochemical studies have reported a strong correlation between E-cadherin loss and the occurrence of tumors. The downregulation of E-cadherin is generally due to transcriptional repression [1]. The -160C/A polymorphism in the promoter region of the E-cadherin gene has been reported to have a direct effect on its transcriptional regulation and therefore may influence susceptibility to cancers [2]. To identify whether the -160C/A polymorphism of E-cadherin is involved in the pathogenesis of tumors in vivo, case-control studies concerning this allelic variation and cancer risk have been broadly performed. However, there is still uncertainty about the level of risk for a variety of cancers in a number of studies investigating the effect of -160C/A on different types of cancers and ethnic populations.

To resolve the controversial question raised by this evidence and provide more statistical power for detecting the significance of -160C/A to cancer risk, we performed a meta-analysis on the 160C/A polymorphism of E-cadherin and cancer risk with 47 case-control studies including 18,194 cases and 20,207 controls as of March 2012. The results indicated that the -160A allele of E-cadherin leads to a higher risk for the development of prostate and urothelial cancers and is an ethnicity-dependent risk factor for prostate and colorectal cancers. The significance of the -160C/A polymorphism in developing various types of cancer has received
increasing attention. However, further observation will be needed to improve the evaluation power of association.

Methods

Search Strategy

We conducted a systematic literature search using the databases MEDLINE (US National Library of Medicine, Bethesda, Maryland) and PubMed (National Center for Biotechnology, National Library of Medicine) as of March 2012 with the keywords “polymorphism of the E-cadherin gene,” “rs16260,” and “-160C/A,” in combination with “cancer,” “tumor,” “neoplasm,” or “carcinoma.” The full texts of the candidate articles were carefully examined for data extraction, and the reference lists were also reviewed to identify further relevant studies for our previous report [3].

Inclusion Criteria

Case-control studies with sufficient published data for estimating an odds ratio (OR) and corresponding 95 percent confidence interval (95% CI) were included in this meta-analysis. Published meta-analyses on the association of polymorphisms of E-cadherin with cancer risk were included in the assessment of evidence.

Data Extraction

The following information was independently extracted from each study by two investigators: 1) publication date, first author, year of publication, and country of origin; 2) polymorphism of the E-cadherin gene and cancer types; 3) characteristics of cases and controls and genotyping method; and 4) number of cases and controls with heterozygous and homozygous genotypes. This information is summarized in Tables 1, S1 and S2.

Meta-analysis

Based on the inclusion criteria, 47 case-control studies were included. In total, 59 datasets were extracted based on the original data, which were divided by either region or cancer type. Relevant information on the studies is summarized in Table S1. The review process and outcomes of inclusion and exclusion are illustrated in Figure S1.

Hardy-Weinberg equilibrium was tested in control samples of each dataset by the chi-square method to assess the latent bias resulting from the deviation of genotype distribution. ORs were considered as estimates of relative risk and were combined across studies using fixed- or random-effects meta-analysis for low and high heterogeneity, respectively. Heterogeneity was assessed using the I2 statistic, which describes the degree of genuine differences across studies in a meta-analysis [4]. A meta-regression model was used to identify the source of heterogeneity [5], and subgroup analysis was also carried out. One-way sensitivity analysis was performed by removing one dataset at a time, was carried out to confirm the stability of the estimated OR (Figure 1). As shown in Table 5, when the Venice guidelines were applied, cumulative evidence for all cancer types was considered ‘weak.’ Detailed information on the assessment of each cancer type is summarized in Table S3.

Compared with our previous study [3], evidence on seven new types of cancer was reported, including pancreatic [11], nasopharyngeal [12], endometrial [13], cervical [13], ovarian [14], oral [15], liver [16], and thyroid [17] cancers and lymphoma [18]. There was no change concerning evidence on lung [19] and esophageal [20,21] cancers.

Breast Cancer

One additional study [13] was added to previous breast cancer studies [22,23], which led to a total of 1,142 cases and 1,063 controls. The -160A carriers were still not more susceptible to breast cancer (OR = 1.14, 95% CI = 0.96–1.36) with a fixed-effects model, and no heterogeneity (Q = 0.61, P = 0.89, I2 = 0%) was detected among these data sets.

Colorectal Cancer

Six new datasets from four studies [13,24–26] were added to previous data [21,27,28], which included 7,117 cases and 7,157 controls altogether. Using a random-effects model, the -160A carriers were not more susceptible to colorectal cancer compared
**Table 1.** Estimates of odds ratios and the corresponding 95% confidence intervals for AA and CA genotype and A allele carriers versus the CC genotype for 16 types of cancers analyzed by fixed- or random-effects models divided by cancer type and ethnicity as of March 2012.

| Cancer type                  | No. of data set | No. of cases | No. of controls | AA (95% CI) | CA (95% CI) | (AA+CA) (95% CI) |
|------------------------------|-----------------|--------------|-----------------|-------------|-------------|-----------------|
| **Gastric**                  | 19              | 3,453        | 4,775           | 1.14        | 0.85, 1.52  | 1.01            |
| Asian                        | 11              | 2,164        | 2,558           | 0.96        | 0.63, 1.46  | 0.92            |
| European                     | 6               | 1,102        | 2,046           | 1.15        | 0.78, 1.69  | 1.18            |
| Others                       | 2               | 187          | 171             | 2.95        | 0.90, 9.69  | 1.36            |
| Healthy                      | 11              | 1,929        | 2,100           | 1.19        | 0.84, 1.70  | 0.93            |
| Healthy matched              | 3               | 356          | 367             | 1.14        | 0.14, 9.35  | 1.14            |
| CAG                          | 1               | 96           | 196             | 2.95        | 0.90, 9.69  | 1.36            |
| Free of cancer               | 4               | 1,072        | 2,112           | 1.10        | 0.80, 1.52  | 1.06            |
| **Colorectal**               | 9               | 7,117        | 7,157           | 0.85        | 0.71, 1.03  | 0.97            |
| Asian                        | 2               | 356          | 294             | 0.90        | 0.03, 25.97 | 1.22            |
| European                     | 7               | 6,761        | 6,863           | 0.85        | 0.74, 0.99  | 0.95            |
| Healthy                      | 5               | 6,325        | 5,877           | 0.82        | 0.63, 1.06  | 0.94            |
| Free of CRC                  | 3               | 686          | 1,034           | 0.85        | 0.58, 1.26  | 0.88            |
| Free of cancer               | 1               | 106          | 246             | 1.49        | 0.64, 3.43  | 1.32            |
| Esophageal                   | 2               | 407          | 490             | 1.03        | 0.27, 3.93  | 1.30            |
| **Prostate**                 | 10              | 3,570        | 3,304           | 1.36        | 0.93, 1.99  | 1.32            |
| Asian                        | 3               | 655          | 726             | 1.85        | 0.98, 3.50  | 1.51            |
| European                     | 5               | 2,251        | 2,106           | 1.31        | 0.83, 2.07  | 1.34            |
| Healthy                      | 2               | 664          | 472             | 1.12        | 0.21, 5.88  | 1.12            |
| Healthy matched              | 2               | 974          | 646             | 0.69        | 0.45, 1.06  | 1.12            |
| Healthy and BPH              | 2               | 1,895        | 1,765           | 1.65        | 0.90, 3.02  | 1.18            |
| BPH                          | 1               | 200          | 159             | 1.85        | 0.74, 52.37 | 2.10            |
| BPH and other                | 1               | 82           | 188             | 1.65        | 0.41, 6.62  | 3.83            |
| Urothelial                   | 5               | 1,064        | 1,124           | 2.58        | 1.40, 4.76  | 1.54            |
| Asian                        | 3               | 544          | 474             | 4.05        | 2.49, 6.60  | 1.82            |
| Others                       | 2               | 520          | 650             | 1.43        | 0.88, 2.34  | 1.17            |
| **Breast**                   | 4               | 1,142        | 1,063           | 1.14        | 0.83, 1.57  | 1.14            |
| Pancreatic                   | 1               | 254          | 101             | 1.25        | 1.21, 5.26  | 1.37            |
| Nasopharyngeal               | 1               | 162          | 140             | 3.84        | 1.04, 14.15 | 1.81            |
| Endometrial                  | 1               | 92           | 246             | 1.25        | 0.46, 3.38  | 2.07            |
| Cervical                     | 1               | 101          | 246             | 2.08        | 0.96, 4.48  | 1.05            |
| Ovarian                      | 1               | 207          | 256             | 0.69        | 0.20, 2.40  | 0.95            |
| Lung                         | 1               | 95           | 85              | 12.56       | 0.68, 231.61| 2.37            |
| Oral                         | 1               | 251          | 347             | 0.32        | 0.18, 0.57  | 0.66            |
| Liver                        | 1               | 131          | 347             | 0.77        | 0.42, 1.42  | 0.88            |
| Thyroid                      | 1               | 92           | 169             | 2.09        | 0.90, 4.87  | 2.42            |
| Lymphoma                     | 1               | 56           | 357             | 0.70        | 0.20, 2.47  | 0.94            |
| **Overall**                  | 59              | 18,194       | 20,207          | 1.21        | 1.03, 1.43  | 1.14            |

Statistically significant, with P<0.05 and a 95% confidence interval (CI) that does not include 1.0.

OR, odds ratio.

Stratified by ethnicity, including Asian, European, and others (North American and African).

Stratified by controls, including benign prostatic hyperplasia (BPH), BPH or visitors or requesting vasectomy (BPH and others), benign urological patients matched, chronic atrophic gastritis (CAG), free of colorectal cancer (free of CRC), free of cancer, healthy, healthy and BPH, healthy and free of cancer, healthy matched, and normal peritumoral tissues.

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with all genotypes (OR = 0.95, 95% CI = 0.85–1.05), and the heterogeneity among seven datasets was moderate \((Q = 12.09, P = 0.15, I^2 = 34\%)\). Then, we performed a subgroup analysis stratified by source of controls or ethnicity, and the source of heterogeneity was identified in the colorectal cancer-free control subgroup when it was divided by the source of controls \((Q = 4.82, P = 0.09, I^2 = 59\%)\) and in the European subgroup when it was divided by ethnicity \((Q = 9.31, P = 0.16, I^2 = 36\%)\). The heteroge-

### Table 2. Heterogeneity test for studies of each genotype in different cancer types (as of March 2012) with Cochrane’s Q-test and the quantity \(I^2\).

| Cancer type                  | AA   | CA   | (AA-CA) | No. of data sets |
|------------------------------|------|------|---------|------------------|
|                              | \(Q\) value | \(P\) value | \(I^2\) (%) | \(Q\) value | \(P\) value | \(I^2\) (%) |
| Gastric                      | 34.62 | 0.01 | 48      | 30.98 | 0.03 | 42 | 33.44 | 0.01 | 46 | 19 |
| Asian#                       | 19.74 | 0.03 | 49      | 14.67 | 0.14 | 32 | 13.89 | 0.18 | 28 | 11 |
| European#                    | 7.86  | 0.16 | 36      | 12.62 | 0.03 | 60 | 13.29 | 0.02 | 62 | 6 |
| Others#                      | 2.01  | 0.16 | 50      | 0.15  | 0.70 | 0  | 0.83  | 0.36 | 0  | 2  |
| Healthy#                     | 15.62 | 0.11 | 36      | 19.59 | 0.03 | 49 | 19.71 | 0.03 | 49 | 11 |
| Healthy matched\(\#\)       | 16.95 | 0.00 | 88      | 4.32  | 0.12 | 54 | 8.50  | 0.01 | 77 | 3  |
| CAG\(\#\)                   |      |      |         |       |      |     |       |     |    |    |
| Free of cancer               | 1.41  | 0.70 | 0       | 3.85  | 0.28 | 22 | 2.61  | 0.46 | 0  | 4  |
| Colorectal                   | 10.06 | 0.26 | 20      | 12.28 | 0.14 | 35 | 12.09 | 0.15 | 34 | 9  |
| Asian#                       | 3.53  | 0.06 | 72      | 0.05  | 0.83 | 0  | 0.52  | 0.47 | 0  | 2  |
| European#                    | 6.35  | 0.39 | 5       | 10.12 | 0.12 | 41 | 9.31  | 0.16 | 36 | 7  |
| Healthy#                     | 6.69  | 0.15 | 40      | 5.71  | 0.22 | 30 | 4.38  | 0.36 | 9  | 5  |
| Free of CRC                  | 1.65  | 0.44 | 0       | 4.35  | 0.11 | 54 | 4.82  | 0.09 | 59 | 3  |
| Free of cancer\(\#\)        |      |      |         |       |      |     |       |     |    |    |
| Esophageal                   | 3.20  | 0.07 | 69      | 0.03  | 0.86 | 0  | 0.46  | 0.50 | 0  | 2  |
| Prostate                     | 24.66 | 0.003| 63      | 22.57 | 0.007| 60 | 26.18 | 0.002| 66 | 10 |
| Asian#                       | 1.38  | 0.50 | 0       | 2.80  | 0.25 | 29 | 3.23  | 0.20 | 38 | 3  |
| European#                    | 12.75 | 0.01 | 69      | 16.83 | 0.002| 76 | 17.47 | 0.002| 77 | 5  |
| Others#                      | 7.88  | 0.005| 87      | 0.00  | 0.95 | 0  | 0.88  | 0.35 | 0  | 2  |
| Healthy#                     | 1.26  | 0.26 | 21      | 0.01  | 0.94 | 0  | 0.22  | 0.64 | 0  | 2  |
| Healthy matched\(\#\)       | 10.02 | 0.02 | 70      | 0.72  | 0.87 | 0  | 2.20  | 0.53 | 0  | 4  |
| Healthy and BPH\(\#\)       | 0.14  | 0.71 | 0       | 0.92  | 0.34 | 0  | 0.43  | 0.51 | 0  | 2  |
| BPH\(\#\)                   |      |      |         |       |      |     |       |     |    |    |
| BPH and others\(\#\)        |      |      |         |       |      |     |       |     |    |    |
| Urothelial                   | 14.28 | 0.006| 72      | 9.83  | 0.04 | 59 | 20.37 | 0.0004| 80 | 5  |
| Asian#                       | 2.30  | 0.32 | 13      | 8.94  | 0.01 | 78 | 16.37 | 0.0003| 88 | 3  |
| European#                    | 1.22  | 0.27 | 18      | 0.38  | 0.54 | 0  | 0.78  | 0.38 | 0  | 2  |
| Breast                       | 1.68  | 0.64 | 0       | 0.89  | 0.83 | 0  | 0.61  | 0.89 | 0  | 4  |
| Pancreatic                   |      |      |         |       |      |     |       |     |    |    |
| Nasopharyngeal               |      |      |         |       |      |     |       |     |    |    |
| Endometrial                  |      |      |         |       |      |     |       |     |    |    |
| Cervical                     |      |      |         |       |      |     |       |     |    |    |
| Ovarian                      |      |      |         |       |      |     |       |     |    |    |
| Lung                         |      |      |         |       |      |     |       |     |    |    |
| Oral                         |      |      |         |       |      |     |       |     |    |    |
| Liver                        |      |      |         |       |      |     |       |     |    |    |
| Thyroid                      |      |      |         |       |      |     |       |     |    |    |
| Lymphoma                     |      |      |         |       |      |     |       |     |    |    |
| Overall                      | 161.42| 0.0001| 64      | 138.89 | 0.0001| 58 | 177.76 | 0.0001| 67 | 59 |

\(\#\) Stratified by ethnicity, including Asian, European, and others (North American and African).

\(\#\) Stratified by controls, including benign prostatic hyperplasia (BPH), BPH or visitors or requesting vasectomy (BPH and others), benign urological patients matched, chronic atrophic gastritis (CAG), free of colorectal cancer (free of CRC), free of cancer, healthy, healthy and BPH, healthy and free of cancer, healthy matched, kindreds, and normal peritumoral tissues.

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neity could be attributed to one dataset from Grunhage et al. [24], in which the association was investigated between the -160C/A polymorphism and familial colorectal cancer. After exclusion of this dataset, the heterogeneity was effectively decreased to 'low' ($Q = 8.64, P = 0.28, I^2 = 19\%$), and the pooled OR estimated in the fixed-effects model was 0.93 (95% CI = 0.87–0.99, $P = 0.03$). The estimated OR of the -160A allele in Europeans was 0.85 (95% CI = 0.74–0.99, $P = 0.03$), with low heterogeneity ($Q = 6.35, P = 0.39, I^2 = 5\%$), indicating that it played protective roles in colorectal cancer.

Prostate Cancer

Two additional studies [29,30] were added to previous prostate cancer studies [31–38], resulting in a total of 3,570 cases and 3,304 controls. The genotype distribution in controls from two studies [31,32] was significantly deviated from Hardy-Weinberg equilibrium ($P < 0.05$). After excluding these datasets, the pooled OR estimated in -160A carriers was 1.33 (95% CI = 1.18–1.50), indicating the same predisposition to prostate cancer as before excluding these datasets (OR = 1.24, 95% CI = 1.13–1.37). To clarify the possible sources of the significant heterogeneity among these datasets ($Q = 26.18, P = 0.002, I^2 = 66\%$), we performed a subgroup analysis according to the source of controls and ethnicity, respectively. Stratification by source of controls effectively decreased the heterogeneity ($I^2_{healthy} = 0\%, I^2_{healthy-matched} = 0\%, I^2_{healthy and benign prostatic hyperplasia} = 0\%$); however, this decrease may also be due to a reduction in power for the $Q$-test. When stratified by ethnicity, the -160A allele was revealed to be an ethnicity-dependent risk factor for prostate cancer. ORs estimated using the random-effects model were greater than 1.0 for both Asians (OR = 1.56, 95% CI = 1.16–2.08) and Europeans (OR = 1.25, 95% CI = 1.02–1.55), while no relationship was found between the -160A allele and the progression of prostate cancer in North Americans (OR = 1.10, 95% CI = 0.86–1.41).

**Table 3.** Adjusted $R^2$ and corresponding $I^2$ from the meta-regression models.

| Covariate                          | AA        | CA        | (AA-CA)   | No. of datasets |
|------------------------------------|-----------|-----------|-----------|----------------|
|                                    | $I^2$ (%) | Adjusted $R^2$ | $P$ value | $I^2$ (%) | Adjusted $R^2$ | $P$ value |
| Ethnicity$^o$                      | 61        | 12        | 0.05      | 58        | −6         | 0.67      | 66        | −1         | 0.44      | 59        |
| Cancer type$^i$                    | 50        | 46        | 0.04      | 46        | 55         | 0.08      | 54        | 49         | 0.03      | 59        |
| Control$^i$                        | 58        | 25        | 0.12      | 46        | 47         | 0.01      | 54        | 53         | 0.002     | 59        |
| Ethnicity and cancer type          | 46        | 59        | 0.02      | 48        | 41         | 0.17      | 56        | 41         | 0.07      | 59        |
| Ethnicity and control              | 55        | 36        | 0.04      | 46        | 41         | 0.01      | 53        | 54         | 0.001     | 59        |
| Cancer type and control            | 50        | 51        | 0.15      | 28        | 100        | 0.003     | 33        | 100        | 0.0003    | 59        |
| Ethnicity, cancer type and control | 45        | 63        | 0.07      | 30        | 80         | 0.01      | 33        | 88         | 0.001     | 59        |

$^o$Ethnicity, including Asian, European, and others (North American and African);

$^i$Cancer type, including breast, colorectal, esophageal, gastric, gynecological, lung, nasopharyngeal, pancreatic, prostate, urothelial, oral, liver, thyroid and lymphoma;

$^j$Controls, including benign prostatic hyperplasia (BPH), BPH or visitors or requesting vasectomy (BPH and others), benign urological patients matched, chronic atrophic gastritis (CAG), free of colorectal cancer (free of CRC), free of cancer, healthy and BPH, healthy and free of cancer, healthy matched, and normal peritumoral tissues.

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**Table 4.** Harbord test of each genotype in different cancer types (as of March 2012) with coefficient and standard error.

| Cancer type | AA       | CA       | (AA-CA)   | No. of datasets |
|-------------|----------|----------|-----------|----------------|
|             | Coef.    | Std. err. | $P$ value | Coef.          | Std. err. | $P$ value | Coef. | Std. err. | $P$ value |
| Breast      | 0.12     | 1.60     | 0.95      | −0.15          | 1.23      | 0.91      | 0.08  | 1.02      | 0.94      | 4         |
| Colorectal  | −0.17    | 0.66     | 0.81      | 0.94          | 0.65      | 0.19      | 0.80  | 0.67      | 0.27      | 9         |
| Gastric     | 1.01     | 0.92     | 0.29      | 1.10          | 0.90      | 0.24      | 1.44  | 0.91      | 0.13      | 19        |
| Prostate    | 2.12     | 1.09     | 0.09      | 3.12          | 1.42      | 0.06      | 3.54  | 1.43      | 0.04      | 10        |
| Urothelial  | 3.65     | 5.63     | 0.56      | 4.06          | 0.77      | 0.01      | 6.37  | 1.46      | 0.02      | 5         |
| Overall     | 1.23     | 0.41     | 0.004     | 1.55          | 0.37      | 0.000     | 1.86  | 0.41      | 0.000     | 59        |

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Contribution of the -160C/A in CDH1 to Cancer Risk
Figure 1. One-way sensitivity analysis for the stability of observations in the meta-analysis. The pooled odds ratios (ORs) and 95% confidence intervals (CIs) of the -160A allele carriers are evaluated by comparing to the CC genotype, omitting each dataset in each type of cancer (as of March 2012). The pooled ORs are calculated with a random-effects model. The numbers on the x-axis refer to the studies extracted. 22a, Sweden; 22b, Czech Republic; 24a, Familial; 24b, Sporadic; 26a, Phase 1; 26b, Phase 2; 41a, Beijing; 41b, Linqu; 51a, Canada; 51b, Germany; 51c, Portugal; total, no dataset omitted.

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stratification showed that heterogeneity in the European subgroup was significant \((Q=13.29, P=0.02, I^2=62\%)\). This finding might be mainly attributed to the dataset from Humar et al. [50], in which diffuse gastric cancer was investigated. As a special histological form of gastric cancer, diffuse gastric cancer was more prevalent in younger age groups. The heterogeneity was effectively removed after exclusion of this dataset \((Q=6.49, P=0.17, I^2=38\%)\), as expected. The OR estimated for the \(-160A\) carriers was 1.05 (95% CI = 0.90–1.18) in pooled datasets, 0.93 (95% CI = 0.80–1.08) in Asians, 1.20 (95% CI = 0.91–1.58) in Europeans, and 1.53 (95% CI = 0.99–2.36) in others. The association of \(-160A\) allele carriers with the progression of gastric cancer in Europeans disappeared, whereas susceptibility was still evident in Asians and pooled datasets \((P=0.05)\). The exact number of cases and controls across datasets for each cancer type is shown in Table 1. The estimated OR indicated that the \(-160A\) allele of the \(E\)-cadherin gene provided a higher risk for the development of lung, nasopharyngeal, thyroid, endometrial and oral cancer, but the credibility of these associations was considered ‘weak’ after application of the Venice interim guidelines [9]. Because of the significance of the \(-160C/A\) polymorphism in human cancers, much more data will be provided in the future to enhance the statistical power in these cancer types.

### Discussion

The meta-analysis performed in this paper indicated that the \(-160A\) homozygote predisposed its carriers to urothelial cancer. Carriers of the \(-160A\) allele had an increased risk of prostate cancer. The ethnicity-dependent susceptibility of \(-160A\) carriers to gastric cancer [3] disappeared with the inclusion of updated evidence, whereas susceptibility was demonstrated in prostate cancer. The credibility of single studies that investigated the association of the \(-160A\) allele with lung, nasopharyngeal, pancreatic, thyroid, endometrial and oral cancer was considered ‘weak,’ which requires further verification. No evidence was found that the \(-160A\) allele predisposed its carriers to breast, colorectal, esophageal, gynecological, gastric, or liver cancer or lymphoma.

The meta-analysis, which is not maintained, may become out of date or misleading. Bias and greater heterogeneity arose because of the further inclusion of new evidence, which suggests the requirement for more studies concerning the \(-160C/A\) polymor-

| Cancer type       | \(AA\) scheme | evidence | \(CA\) scheme | evidence | \((AA+CA)\) scheme | evidence |
|-------------------|---------------|----------|---------------|----------|-------------------|----------|
| Colorectal        | ACB           | weak     | ACC           | weak     | ACC               | weak     |
| Gastric           | BCC           | weak     | BCC           | weak     | BCC               | weak     |
| Prostate          | BCC           | weak     | BCC           | weak     | BCC               | weak     |
| Urothelial        | BCA           | weak     | BCC           | weak     | BCC               | weak     |
| Breast            | BCC           | weak     | BCC           | weak     | BCC               | weak     |
| Esophageal        | CCC           | weak     | CCB           | weak     | CCB               | weak     |
| Pancreatic        | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Nasopharyngeal    | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Endometrial       | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Cervical          | CCB           | weak     | CCC           | weak     | CCC               | weak     |
| Ovarian           | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Lung              | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Oral              | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Liver             | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Thyroid           | CCB           | weak     | CCB           | weak     | CCB               | weak     |
| Lymphoma          | CCB           | weak     | CCB           | weak     | CCB               | weak     |

\(P=0.0005\), nasopharyngeal [12] \(OR=2.02, 95\% CI =1.20–3.41, P=0.0005\), thyroid [17] \(OR=2.33, 95\% CI =1.39–3.99, P=0.0001\), endometrial [13] \(OR=1.93, 95\% CI =1.19–3.14, P=0.0008\), oral [15] \(OR=0.57, 95\% CI =0.41–0.80, P=0.0001\), pancreatic [11] \(OR=1.62, 95\% CI =2.63, P=0.05\), liver [16] \(OR=0.85, 95\% CI =0.56–1.29, P=0.44\), cervical [13] \(OR=1.22, 95\% CI =0.77–1.95, P=0.39\), and ovarian [14] \(OR=0.93, 95\% CI =0.63–1.37, P=0.71\) cancer and lymphoma [18] \(OR=0.91, 95\% CI =0.51–1.60, P=0.74\).
phism and cancer risk, especially those with rigorous selection of case and control samples and the reporting of more studies with a large sample size and negative results. In addition to publication bias, which is popular in meta-analyses, different mechanisms can lead to asymmetry in funnel plots, including true heterogeneity resulting from improper study design [35].

The authors combined case-control studies, which are relatively more practical and inexpensive than prospective cohort studies in the investigation of relationships between suspected risk factors and diseases, especially those with low incidence, such as cancers. However, the crucial concern in the design of case-control studies is choosing case and control samples, especially a proper control population, given the explicit diagnostic criteria for cancers. Ideal controls should be a general group of persons without the disease of interest, from which qualified cases arise once diagnosed. This general group does not exclude those with other kinds of disease, whereas no relationship should be expected between the healthy status of the control and the investigated 'risk factor' because the correlation may exaggerate or understate the overall estimated OR [59].

Controls selected in studies investigating the association between the -160C/A polymorphism and prostate cancer risk could be divided into healthy [30,32], healthy matched [31,33,35,38], benign prostatic hyperplasia [29], healthy and benign prostatic hyperplasia [34,37] and benign prostatic hyperplasia or others [36]. Subsequent subgroup analysis stratified by controls in data sets of prostate cancer indicated homogeneity in each strata, indicating that the between-study variance in the prostate subgroup resulted from different controls. However, it should also be noted that the reduced heterogeneity may also result from a reduction in power for the Q-test because of the small sample size in some subgroups.

Furthermore, a question arose because of the low expression level of E-cadherin in benign prostatic hyperplasia [60,61] and urothelial diseases [62,63], which could also have resulted from the -160C/A polymorphism in the promoter region of E-cadherin. If the relationship between the -160C/A polymorphism of E-cadherin and benign prostatic hyperplasia and other urothelial diseases could not be excluded, the selection of patients with these diseases as controls may not be suitable. We tested Hardy-Weinberg equilibrium at the polymorphism site in the control samples, and deviation could be a symptom of disease association [64]. However, there was no guarantee that following Hardy-Weinberg equilibrium excluded a relationship between allele distribution and susceptible diseases [65].

Deviation from Hardy-Weinberg equilibrium in a random sample could be due to inbreeding, population stratification, or selection, and may be indicative of problematic assays [64,65]. Heterogeneity in evidence concerning urothelial cancer was successfully reduced to zero after the exclusion of studies that significantly deviated from Hardy-Weinberg equilibrium, what may indicate an inappropriate choice of control samples in those studies. We observed that the estimated OR qualitatively changed when the AA homozygote carriers are at a higher risk for the development of prostate and urothelial cancers. The association between the -160A allele and lung, nasopharyngeal, thyroid, endometrial and oral cancer indicated by single studies needs further validation.

Supporting Information

Figure S1 The flow diagram for the review process and outcomes of inclusion and exclusion. (TIF)

Figure S2 Meta-analysis of -160A association with fourteen types of cancers (as of March 2012). Odds ratios (ORs) and 95% confidence intervals (CIs) are displayed at a logarithmic scale. Events and total represent the number of -160A allele carriers and all the genotypes respectively. (TIF)

Table S1 Characteristics of the 47 case-control studies included in this meta-analysis. (DOC)

Table S2 Distribution of three genotypes at the E-cadherin -160C/A polymorphic site among case and control samples from 47 case-control studies in this meta-analysis. (DOC)

Table S3 Detailed information on the assessment of evidence in each cancer type. (DOC)

Author Contributions

Conceived and designed the experiments: LW GW. Performed the experiments: LW GW. Analyzed the data: LW GW CL. Contributed reagents/materials/analysis tools: CL BF JK. Wrote the paper: LW GW CL JK. Financial support: JK.
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