Polymorphisms of TP53 codon 72 with breast carcinoma risk: evidence from 12226 cases and 10782 controls

Wenlei Zhuo*1, Yunsong Zhang2, Zhaolan Xiang3, Lei Cai4 and Zhengtang Chen*1

Address: 1Institute of Cancer, Xinqiao Hospital, Third Military Medical University, Chongqing, PR China, 2Department of Thoracic Surgery, the 254th Hospital, Tianjin, PR China, 3Department of Otolaryngology, Southwest Hospital, Third Military Medical University, Chongqing, PR China and 4Institute of Hepatobiliary Surgery, Southwest Hospital, Third Military Medical University, Chongqing, PR China

Email: Wenlei Zhuo* - zhuowenlei@yahoo.com.cn; Yunsong Zhang - zhangyunsongtj@163.com; Zhaolan Xiang - gyriyu@126.com; Lei Cai - medliterature@163.com; Zhengtang Chen* - cztsyd@yahoo.com

* Corresponding authors

Abstract

Background: Previously, TP53 codon 72 polymorphisms have been implicated as risk factors for various cancers. A number of studies have conducted on the association of TP53 codon 72 polymorphisms with susceptibility to breast carcinoma and have yielded inconclusive results. The aim of the present study was to derive a more precise estimation of the relationship.

Methods: We conducted a search in the Medline, EMBASE, OVID, Sciedirect, and Chinese National Knowledge Infrastructure (CNKI) without a language limitation, covering all papers published up to Jan 2009. The associated literature was acquired through deliberate searching and selected based on the established inclusion criteria for publications.

Results: A total of seventeen case-control studies, including 12226 cases and 10782 controls, met the included criteria and thus were selected. Ultimately, the relevant data were extracted and further analyzed using systematic meta-analyses. Overall, no associations of TP53 codon 72 polymorphisms with breast carcinoma were observed (for Arg/Arg vs Pro/Pro: OR = 1.20; 95%CI = 0.96–1.50; for dominant model: OR = 1.12; 95%CI = 0.96–1.32; for recessive model: OR = 1.13; 95%CI = 0.98–1.31). In the subgroup analysis by ethnicity, statistically similar results were obtained when the data were stratified as Asians, Caucasians and Africans.

Conclusion: Collectively, the results of the present study suggest that TP53 codon 72 polymorphisms might not be a low-penetrant risk factor for developing breast carcinoma.

Backgrounds

Breast cancer is the second leading cause of cancer death in women, exceeded only by lung cancer in the world [1]. It is believed that some epidemic factors such as Oral contraceptive use [2]; obesity [3] and hyperinsulinemia [4] are probable factors increasing risks of developing breast carcinoma. Although many individuals exposed to these risk factors, breast cancer develops only in a small group of exposed people, implying that genetic factors might contribute to the carcinogenic mechanisms and complex interactions between many genetic and environmental factors might be the major cause of breast cancer.
Previously, a number of studies indicate that family history is a risk factor for breast cancer [5], indicating the possible roles for genetic variations on the increased susceptibility to breast cancer. Recent published meta-analyses suggest that polymorphisms of FokI [6], XRCC1 codon 399 [7] and methylenetetrahydrofolate reductase [8] might have a significant association with increased breast cancer risk. Nevertheless, conversely, some meta-analysis failed to suggest a marked association of increased susceptibility to breast cancer with polymorphisms of some genes, such as Estrogen receptor alpha [9], CYP1A1 [10] and base-excision repair pathway genes [11].

Recently, a growing body of research has conducted on the association of breast cancer risk with tumour suppressors. TP53, one of the most extensive studied genes as a tumor suppressor, has been thought to have a critical function in cell cycle regulation. In case of its mutation, this regulation could be lost, resulting in cell proliferation without control and development of cancer. Previously, TP53 mutation has been indicated to associate with risks of a number of cancers such as lung cancer [12], breast cancer [13] and colorectal cancer [14]. The loss of TP53 gene could damage its DNA-binding properties and transcription factor function, thus leading to aberrant cell proliferation. In human populations, the TP53 gene is polymorphic at amino acid 72 of the protein that it encodes.

Recently, much attention has been focused on possible associations of TP53 codon 72 polymorphisms and cancer risks. The most informative polymorphism in TP53 gene is located in exon 4 at codon 72, which encodes two distinct functional allelic forms arginine (Arg) and proline (Pro) because of a transversion G to C [15], resulting in different biochemical and biological protein features. Consequently, three distinct genotypes were created, namely, homozygous for arginine (Arg/Arg), homozygous for proline (Pro/Pro), and heterozygous (Arg/Pro). Previously, Arg variant has been thought to increase susceptibility to gastric cancer [16] and Arg homozygosity might contribute to cervical cancer [17]. Nevertheless, Pro homozygosity might have an association with lung [18] and hepatocellular cancer [19] risk. The heterozygous genotype Arg/Pro has been implicated as a risk factor for bladder cancer [20].

In recent literature, inconclusive data regarding TP53 codon 72 were found in some cancers, such as gastric cancer in which controversial conclusions were obtained in Asians [21] and in individuals from Northern Brazil [22]. Similarly, up to date, published data on the possible association of TP53 codon 72 polymorphism with breast carcinoma have also generated controversial and inconclusive results. To the best of our knowledge, whether TP53 codon 72 polymorphism could increase breast cancer risk remains largely uncertain. To clarify this association may help us better understand the possible risk of breast cancer and therefore contribute to its prevention.

As a single study may have been underpowered in clarifying the relationship of TP53 codon 72 polymorphisms with breast carcinoma susceptibility, in the present study we performed evidence-based quantitative meta-analyses that can increase statistical power to address the association.

**Materials and methods**

**Literature search strategy for identification of the studies**

We carried out a search in the Medline, EMBASE, OVID, Sciencedirect, and Chinese National Knowledge Infrastructure (CNKI) without a language limitation, covering all papers published up to Jan 2009, with a combination of the following keywords: TP53, P53, codon 72, breast, carcinoma, neoplasm, tumor, cancer and polymorphism. The keywords were paired each time in order to get more relevant information. For example, the word "breast" was always kept and others were substituted in different moments.

We evaluated potentially associated publications by checking their titles and abstracts and then procured the most relevant publications for a closer examination. Moreover, the reference lists of the selected papers were also screened for other potential articles that possibly have been missed in the initial search. The following criteria were used for the literature selection for the further meta-analysis:

1. Studies concerning the association of TP53 codon 72 polymorphism with breast carcinoma;
2. Case–control or cohort studies;
3. Papers presenting the breast cancer diagnoses and the sources of cases and controls;
4. Articles offering the size of the sample, odds ratios (ORs) and their 95% confidence intervals (CIs) or the information that can help infer the results;
5. The number of individuals homozygous for arginine (Arg/Arg), proline (Pro/Pro) and heterozygous (Pro/Arg) in breast cancer cases and controls should be offered;
6. The methods of data collection and analysis should be statistically acceptable.

Accordingly, the following exclusion criteria were also used:

1. The design and the definition of the experiments were obviously different from those of the selected papers.

2. The source of cases and controls and other essential information were not offered;

3. The genetic distribution of the control group was inconsistent with Hardy-Weinberg equilibrium (HWE).

4. Reviews and duplicated publications.

After searching, we reviewed all papers in accordance with the criteria defined above for further analysis.

Data extraction
Data were carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria mentioned above. For conflicting evaluations, an agreement was reached following a discussion. If a consensus could not be reached, another author was consulted to resolve the dispute and then a final decision was made by the majority of the votes. The extracted information was entered into a database. For data not provided in the main text, the relevant information was obtained by contacting corresponding authors as possible as we could.

Statistical analysis
The odds ratio (OR) of TP53 codon 72 polymorphisms and breast cancer risk was estimated for each study. The pooled ORs were performed for additive model (Arg/Arg vs Pro/Pro), dominant model (Arg/Arg+Arg/Pro versus Pro/Pro) and recessive model (Arg/Arg versus Arg/Pro+Pro/Pro), respectively. For detection of any possible sample size biases, the OR and its 95% confidence interval (CI) to each study was plotted against the number of participants respectively. A Chi-square based Q statistic test was performed to assess heterogeneity. If the result of the heterogeneity test was $P > 0.05$, ORs were pooled according to the fixed-effect model (Mantel-Haenszel). Otherwise, the random-effect model (DerSimonian and Laird) was used. The significance of the pooled ORs was determined by Z-test. The HWE was assessed via Fisher’s exact test.

Publication bias was assessed by visual inspection of funnel plots[23], in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot indicates a possible publication bias. The symmetry of the funnel plot was further evaluated by Egger’s linear regression test[24]. In addition, fail-safe number for $P = 0.05$ ($N_{fs,0.05}$) [25] for the evaluation of the reliability of meta-analysis, defined as the number of negative results that could reverse the significant findings, was also used to estimate the robustness of the meta analysis. Statistical analysis was undertaken using the program Review Manager 4.2 and SAS 8.1 software.

Results

Study characteristics
A total of 131 studies regarding TP53 codon 72 with respect to breast cancer were searched and screened for retrieval, of which 97 irrelevant studies were excluded. Then, 9 studies [26-34] were excluded because each of them did not contain a control group. Next, of the remaining 25 studies, 2 studies[35,36] were excluded due to their insufficient data and 1 study [37] owing to its review characteristic. Afterwards, another 5 studies [38-42] were excluded because the genetic distributions of the control groups were not in agreement with HWE. Lastly, 17 case-control studies were selected (Figure 1). Of the all included 17 studies, 16 were written in English [43-58] and 1 [59] in Chinese.

We established a database according to the extracted information from each article. The relevant information was listed in Table 1. According to the lists, the first author and the number and characteristics of cases and controls for each study as well as other necessary information were presented. As shown in Table 2, the distributions of TP53 codon 72 genotype of the included studies were also presented. The controls of the included studies were all in accordance with HWE.

Test of heterogeneity
We analyzed the heterogeneity of Arg/Arg versus Pro/Pro and dominant model (Arg/Arg+Arg/Pro versus Pro/Pro) as well as recessive model (Arg/Arg versus Arg/Pro+Pro/Pro). As shown in Table 3, the heterogeneity for the overall data was significant in each of the above three models respectively because the $P$ values were less than 0.05 for Q-tests. Thus, random-effect models were utilized for the meta-analyses.

Meta-analysis results
Table 3 lists the main results of the meta-analysis. No evidence showed that individuals who carry Arg allele have an increased or decreased risk of breast carcinoma compared with those who carry Pro allele.

In the present study, a total of 17 studies were included. Nevertheless, the study conducted by Weston et al. [44] concerned both Caucasians and Africans. Thus, the data...
were extracted respectively and further assessed by Revman 4.2 software. Consequently, the following results reported 18 studies.

As shown in Table 3, for Arg/Arg vs Pro/Pro, the data available for our meta-analysis were obtained from 18 case-control studies of 7377 cases and 6450 controls, of which 6288 cases and 5112 controls had the Arg/Arg genotype and 1089 cases and 1338 controls had the Pro/Pro genotype of the TP53 codon 72. The overall OR was 1.20 (95% CI = 0.96–1.50) and the test for overall effect Z value was 1.58 (P > 0.05). For dominant model (Arg/Arg+Arg/Pro versus Pro/Pro), the data available for our meta-analysis were obtained from 18 case-control studies containing 12226 cases and 10782 controls, of which 11137 cases and 9444 controls had the combined genotypes of Arg/Arg and Arg/Pro, while 1089 cases and 1338 controls had the homozygote Pro/Pro genotype. The overall OR was 1.12 (95% CI = 0.96–1.32) and the test for overall effect Z value was 1.47 (P > 0.05). For recessive model (Arg/Arg versus Arg/Pro+Pro/Pro), the data were extracted from the 18 case-control studies concerning 12226 cases and 10782 controls, of which 6288 cases and 5112 controls had the wild-type homozygote Arg/Arg genotype while 5938 cases and 5670 controls had the combined variant genotypes (Arg/Pro and Pro/Pro) of the TP53 codon 72. The overall OR was 1.13 (95% CI = 0.98–1.31) and the test for overall effect Z value was 1.65 (P > 0.05).

Considering the possible impact of ethnic variation on the results, we conducted subgroup analysis concerning Asians, Caucasians and Africans, respectively. Likewise, the subgroup analyses failed to suggest marked association between TP53 codon 72 polymorphisms and breast cancer risk in Asians, Caucasians and Africans.

**Sensitivity analysis**

In order to compare the difference and evaluate the sensitivity of the meta-analyses, we also presented the results of the fixed-effect models as listed in Table 3. In all, the results were not significantly different between the two models, suggesting the robustness of the meta-analyses. Moreover, we also conducted one-way sensitivity analysis[60] to evaluate the stability of the meta-analysis. The statistical significance of the results was not altered when any single study was omitted (data not shown), confirming the stability of the results. Hence, results of the sensitivity analysis suggest that the data in this meta-analysis are relatively stable and credible.

**Bias diagnostics**

Funnel plots were created for assessment of possible publication biases. Then, Egger's linear regression tests were used to assess the symmetric of the plots. As shown in Table 4, for the dominant model, the data suggest that the funnel plot is symmetrical. However, for the additive and recessive model, the results indicate possible asymmetric of the funnel plots. Therefore, we further calculated the Nfs 0.05 for evaluation of the stability of the results. Consequently, the Nfs 0.05 were 237, 143 and 271 for additive, dominant model and recessive model respectively, which were more than five times of the number of the included studies, suggesting that the results of these meta-analyses are relatively stable and the publication biases might not have an evident influence on the results of the meta-analyses.

**Discussion**

In the present study, the results of meta-analyses showed that individuals with TP53 codon 72 polymorphism might not have significant associations with increased or decreased susceptibility to breast carcinoma.

A previous meta-analysis conducted by Koushik et al. [61] regarding cervical cancer suggests that homozygote Arg/Arg genotype increases susceptibility to both squamous cell carcinoma and adenocarcinoma. While another meta-analysis [62] indicates that Arg/Arg genotype only associates with increased risk of cervical adenocarcinoma but not squamous cell carcinoma. Then, Sousa et al. [63] failed to demonstrate Arg/Arg genotype as a risk marker for the development of cervical lesions in most of European countries. Conversely, nonassociations of TP53 codon 72 polymorphism with lung carcinoma [64] and gastric cancer [65] risk were found by meta-analysis. Nevertheless, An updated meta-analysis concerning lung cancer implied that Pro allele is a low-penetrant risk factor for
developing lung cancer [66]. Thus, whether TP53 codon 72 polymorphism contributes to susceptibility to cancers varies in different types of cancer. In the present study, no evidence showed TP53 codon 72 polymorphism as a risk factor for breast cancer.

The underlying mechanisms by which TP53 polymorphism influences cancer risk are not fully understood. TP53 is the most frequently investigated gene that is often mutated in a variety of cancers. Nevertheless, several single-nucleotide polymorphisms have been studied and reported in TP53 gene [67]. The polymorphism of TP53 codon 72 occurs in a proline-rich region that is thought to play a critical role in the growth suppression and apoptotic functions of TP53 protein [68]. The two polymorphic variants differ in their capability of binding the transcriptional protein, activating transcription and suppressing the transformation of some primary cells [69]. For example, Arg variant might induce cell apoptosis and suppress transformation more efficiently than Pro variant do, which may be due to the ability of the Arg variant to localize in mitochondria that regulates the release of cytochrome c into cytosol. However, the present meta-analysis indicates that neither Arg nor Pro carriers may have a significant association with breast cancer risk. It is likely that TP53 codon 72 polymorphisms rarely affect the tumorigenesis and progression of breast carcinoma. Considering that the same polymorphism may play different roles in cancer susceptibility among different ethnic populations and the frequencies of single nucleotide polymorphisms may be different ethnicity, we stratified the data by race into three groups concerning Asians, Caucasians or Afri-

Table 1: Characteristics of studies included in the meta-analysis

| First Author     | Publication Year | Number of Cases | Number of Controls | Types of Cases | Type of controls | Method             | Country      | Ref. No. |
|------------------|------------------|-----------------|--------------------|----------------|------------------|--------------------|--------------|---------|
| Själander        | 1996             | 212             | 689                | Ductal carcinoma (>80% of the total cases) | Pooled individuals from several controls | PCR-RFLP | Sweden | 43       |
| Weston, Li       | 1997             | 81              | 147                | NS             | 50 healthy people (age-matched) | AS-PCR | USA | 44       |
| Wang-Gohrke      | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Buyru, Huang, Katiyar | 2003, 2003, 2003 | 115, 200, 77    | 76, 282, 41       | 76 healthy people; 282 healthy people; 41 normal healthy women (age-matched) | NS               | PCR-RFLP | Turkey | 47       |
| Li               | 2002             | 28              | 50                 | NS             | 50 healthy people (age-matched) | PCR-RFLP | China | 45       |
| Wang-Gohrke      | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |
| Li               | 2002             | 577             | 579                | NS             | NS               | PCR-RFLP | Germany | 46       |

AS-PCR: Allele Specific PCR; RFLP: restriction fragment length polymorphism; CTPP: confronting two-pair primers; NS: not specified
cans, respectively. Ultimately, statistically similar results were obtained, confirming nonassociation of TP53 codon 72 polymorphism with breast cancer risk.

A well-known risk factor, HPV infection, is thought to have an association with increased susceptibility to some cancers such as cervical [70] and oral cancer [71]. Evidence suggests that P53Arg72 protein may be more susceptible than P53Pro72 protein to HPV mediated degradation, thus increasing risk of HPV associated cancers [17]. Growing body of literature indicates HPV infection as a possible risk factor for breast cancer [72]. However, we did not further investigate the possible association of HPV infection with TP53 codon 72 polymorphism due to the insufficient data in the primary included studies.

Table 2: Distribution of TP53 codon 72 genotype among breast cancer cases and controls included in the meta-analysis

| First author | Cases | Controls | HWE (control) |
|--------------|-------|----------|---------------|
|              | Arg/Arg | Arg/Pro | Pro/Pro | Arg/Arg | Arg/Pro | Pro/Pro | Chi-square | P |
| Själander    | 24     | 93       | 95      | 61      | 253     | 375     | 3.681      | 0.055 |
| Weston (Caucasian) | 6     | 27       | 32      | 3       | 42      | 72      | 1.189      | 0.276 |
| Weston (African)  | 6     | 9        | 12      | 14      | 4       | 0001    | 0.979      |
| Li           | 11     | 11       | 6       | 10      | 26      | 14      | 0.109      | 0.741 |
| Wang-Gohrke  | 282    | 221      | 49      | 300     | 203     | 40      | 0.485      | 0.486 |
| Buyru        | 64     | 39       | 12      | 21      | 43      | 12      | 1.657      | 0.198 |
| Huang        | 64     | 100      | 36      | 114     | 138     | 30      | 1.545      | 0.214 |
| Katsar       | 20     | 51       | 6       | 9       | 24      | 8       | 1.205      | 0.272 |
| Mabrouk      | 18     | 9        | 3       | 19      | 26      | 4       | 1.432      | 0.231 |
| Kalem        | 26     | 13       | 3       | 10      | 32      | 9       | 3.326      | 0.068 |
| Tommiska     | 825    | 617      | 109     | 403     | 278     | 52      | 0.183      | 0.669 |
| Baynes       | 1107   | 768      | 148     | 1177    | 854     | 166     | 0.414      | 0.520 |
| Gochhaisn    | 86     | 109      | 48      | 76      | 160     | 97      | 0.413      | 0.521 |
| Khadang      | 83     | 109      | 29      | 75      | 90      | 40      | 1.873      | 0.171 |
| Schmidt      | 2797   | 2008     | 386     | 2024    | 1523    | 287     | 0.001      | 0.983 |
| Sprague      | 823    | 570      | 89      | 705     | 490     | 83      | 0.03       | 0.862 |
| Zhang        | 21     | 45       | 17      | 47      | 87      | 33      | 0.406      | 0.524 |
| Akkiprik     | 25     | 50       | 20      | 46      | 49      | 12      | 0.038      | 0.846 |

Table 3: Main results of the pooled data in the meta-analysis

| No. of cases/controls | Arg/Arg vs Pro/Pro | (Arg/Arg+Arg/Pro) vs Pro/Pro | Arg/Arg vs (Arg/Pro+Pro/Pro) |
|-----------------------|--------------------|------------------------------|-----------------------------|
|                       | OR (95%CI)         | P (Q-test)                   | OR (95%CI)                  | P (Q-test)                   | OR (95%CI)                  | P (Q-test) |
| **Random-effect model** | | | | | | |
| Total                 | 12226/10782        | 1.20 (0.96–1.50)             | 0.11 0.000                  | 1.12 (0.96–1.32)             | 0.14 0.01                  | 1.13 (0.98–1.31)             | 0.10 0.000 |
| Caucasian             | 11549/9830         | 1.15 (0.91–1.44)             | 0.24 0.001                  | 1.11 (0.95–1.30)             | 0.17 0.06                  | 1.09 (0.93–1.27)             | 0.28 0.000 |
| Asian                 | 631/873            | 1.36 (0.61–3.03)             | 0.45 0.000                  | 1.19 (0.67–2.10)             | 0.55 0.006                 | 1.22 (0.72–2.05)             | 0.46 0.002 |
| African               | 46/79              | 1.46 (0.38–5.62)             | 0.58 0.76                   | 1.12 (0.31–4.10)             | 0.86 0.45                  | 1.60 (0.63–4.06)             | 0.32 0.22 |
| **Fixed-effect model** | | | | | | |
| Total                 | 12226/10782        | 1.09 (0.99–1.20)             | 0.10 0.000                  | 1.09 (0.99–1.19)             | 0.06 0.01                  | 1.04 (0.99–1.10)             | 0.13 0.000 |
| Caucasian             | 11549/9830         | 1.07 (0.96–1.18)             | 0.24 0.001                  | 1.08 (0.98–1.19)             | 0.12 0.06                  | 1.03 (0.98–1.09)             | 0.25 0.000 |
| Asian                 | 631/873            | 1.27 (0.94–1.71)             | 0.12 0.000                  | 1.16 (0.89–1.51)             | 0.26 0.006                 | 1.15 (0.92–1.44)             | 0.22 0.002 |
| African               | 46/79              | 1.47 (0.39–5.62)             | 0.57 0.76                   | 1.17 (0.33–4.14)             | 0.80 0.45                  | 1.67 (0.80–3.48)             | 0.17 0.22 |

Heterogeneity is a potential problem when interpreting the results of meta-analysis [73]. In the present study, significant between-study heterogeneity existed in overall comparisons. Nevertheless, when the data were stratified by race, the heterogeneity was decreased or removed, suggesting that differences of genetic backgrounds and the environment existed among different ethnicities. In the present meta-analysis, we excluded the studies in which the control groups were deviate from HWE. Thus, the between-study heterogeneity might be reduced. Moreover, random-effect models were used for combination of the data. Accordingly, the results may be credible and stable although the heterogeneity seemed evident.

Some limitations might be included in this study. First, in this meta-analysis, most published studies and papers
written in English or Chinese were searched. Moreover, articles published in other languages, cited by PubMed, were also searched, it is possible that some relevant studies might be missed. Hence, some inevitable publication biases might exist in the results, though the NF0.05 showed no remarkable publication biases in the meta-analyses. Second, in the subgroup analysis, the number of studies regarding Africans was relatively limited. It may be underpowered to explore the real association. Thus, the results may be interpreted with caution. Third, whether the experimental and control groups were from the same socio-economic status or the same geographic area have not been clearly presented in some of the included original papers, leading to possible biases. Furthermore, the sample sizes of some included studies are rather small, which might be one of the reasons contributing to the between-study heterogeneity. Therefore, a number of further studies with large sample sizes with well-matched controls are required. Besides, gene-gene and gene-environment interactions should also be considered in the further studies.

In summary, despite the limitations, the results of the present meta-analysis suggest that genetic variations of TP53 codon 72 may not have a marked association with breast cancer risk.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
WZ and YZ conceived of the study, and carried out the collection of the literatures. LC helped with the statistical analysis and manuscript drafting. ZC and WZ conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

References
1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ: Cancer statistics. CA Cancer J Clin 2007, 57:43-66.
2. Kahlenborn C, Modugno F, Potter DM, Severn WB: Oral contraceptives as a risk factor for premenopausal breast cancer: a meta-analysis. Mayo Clin Proc 2006, 81:1290-1302.
3. Carmichael AR: Obesity and prognosis of breast cancer. Obes Rev 2006, 7:333-340.
4. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Rohan TE, Manson JE, Li J, Ho GY, Xue X, Anderson GL, Kaplan RC, Harris TG, Howard BV, Wylie-Rosett J, Burk RD, Schrickler HD: Insulin-like growth factor-I, breast cancer risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2009, 101:48-60.
5. Pharoah PD, Day NE, Duffy S, Easton DF, Ponder BA: Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer 1997, 71:800-809.
6. Tang C, Chen H, Wu M, Yuan H, Du Y: Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat 2009.
7. Saadat M, Ansari-Lari M: Polymorphism of XRC1 (at codon 399) and susceptibility to breast cancer, a meta-analysis of the literatures. Breast Cancer Res Treat 2008. doi: 10.1007/s10549-008-0051-0.
8. Zintzaras E: Methylene tetrahydrofolate reductase gene and susceptibility to breast cancer: a meta-analysis. Clin Genet 2006, 69:327-36.
9. Gonzalez-Zueto L, M. Vásquez AA, Rivadeneira F, Siemes C, Hofman A, Stricker BH, Pols HA, Utterlinden AG, van Duijn CM: Estrogen receptor alpha polymorphisms and postmenopausal breast cancer risk. Breast Cancer Res Treat 2008, 107:415-419.
10. Masson LF, Sharp L, Cotton SC, Little J: Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. Am J Epidemiol 2005, 161:901-915.
11. Zhang Y, Newcomb PA, Egan KM, Titus-Ernstoff L, Chanoek S, Welch R, Brinton LA, Lissowksa J, Bardin-Pikolajczak A, Pelponska B, Szeszenia-Dabrowa N, Zatonski W, Garcia-Closs M: Genetic polymorphisms in base-excision repair pathway genes and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2006, 15:353-358.
12. Bumroongkit K, Rannala B, Traisathit P, Srikuumool M, Wongchai Y, Kangwanpong D: TP53 gene mutation of lung cancer patients in upper northern Thailand and environmental risk factors. Cancer Genet Cyogenet 2008, 185:20-27.
13. Assumpció J, Seidinger AL, Ribeiro RC, Zambetti GP, Ganti R, Sriravastava K, Shurtleff S, Pei D, Zelener LC, DuRoth RM, Brandalise SR, Youdeh M, Ansari R, Semeraro D, Hormazdi M, Fakheri H, Rakhsahi N, De Levis L, Currie M, Cama A, Malekzadeh R, Iacobelli S, Mariani-Costantini R: P53 mutations in colorectal cancer from northern Iran: Relationships with site of tumor origin, microsatellite instability and K-ras mutations. J Cell Physiol 2008, 216:543-550.
14. Aragona A, Lee PS, Ransberg MF, Saya H: Codon 72 polymorphism of the TP53 gene. Nucleic Acids Res 1990, 18:4961.
15. Shen H, Solari A, Wang X, Zhang Z, Xu Y, Wang L, Hu X, Guo J, Wei Q: P53 codon 72 polymorphism and risk of gastric cancer in a Chinese population. Oncol Rep 2004, 11:1115-1120.
16. Storey A, Thomas M, Kaila A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L: Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998, 393:229-234.
17. Wang YC, Lee HS, Chen SK, Chang YY, Chen CY: prognostic significance of p53 codon 72 polymorphism in lung carcinomas. Eur J Cancer 1999, 35:226-230.
18. Yu MW, Yang SY, Chiu YH, Chang YC, Liaw YF, Chen CJ: A p53 genetic polymorphism as a modulator of hepatocellular carcinoma risk in relation to chronic liver disease, familial tendency, and cigarette smoking in hepatitis B carriers. Hepatology 1999, 29:697-702.
19. Mabrouk I, Baccouche S, El-Abed R, Mokdad-Gargouri R, Mosbah A, Said S, Daoud J, Frieh X, Mlidi R, Gargouri A: No evidence of correlation between p53 codon 72 polymorphism and risk of

Table 4: Publication bias tests (Egger’s linear regression test and Nfs0.05) for TP53 codon 72 polymorphisms

| Genetic type          | Coefficient | Standard Error | t     | P value | 95% CI of intercept | Nfs0.05 |
|-----------------------|-------------|----------------|-------|---------|---------------------|---------|
| Arg/Arg vs Pro/Pro    | 2.757       | 1.043          | 2.641 | 0.018   | (0.544, 4.970)      | 237     |
| Arg/Arg+Arg/Pro vs Pro/Pro | 1.172   | 0.659          | 1.778 | 0.094   | (-0.225, 2.570)     | 143     |
| Arg/Arg vs Arg/Pro+Pro/Pro | 2.726   | 1.183          | 2.305 | 0.034   | (0.219, 5.234)      | 271     |
bladder or breast carcinoma in Tunisian patients. Ann Y Acad Sci 2003, 1010:764-770.

21. Li Y, Li N, Zheng W, Li Q, Wu TX, Yao X, Du L, Wei ML, Wu XT. p53 codon 72 polymorphism and gastric cancer: a meta-analysis of the literature. Int J Cancer 2007, 121:1481-1486.

22. Khayat AS, Lobo Gatti L, Moura Lima E, de Assumpção PP, Nascimento Motta FJ, Harada ML, Casartelli C, Marques Payão SL, Cardoso Smith MA, Buscio RR. Polymorphisms of the TP53 codon 72 and WRN codon 1367 in individuals from Northern Brazil with gastric adenocarcinoma. Clin Exp Med 2005, 5:161-168.

23. Munafò MR, Clark TG, Flint J: Assessing publication bias in genetic association studies: evidence from a recent meta-analysis. Psychol Res 2005, 129:39-44.

24. Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. BMJ 1997, 315:629-634.

25. Rosenthal R: The "file drawer problem" and tolerance for null results. Bull Psychol Bull 1979, 86:638-641.

26. Vieira JO, da Silva ID, Higo PE, Nogueira-de-Souza NC. Gebrim LH: Study of p53 codon 72 polymorphism in patients with breast cancer. Eur J Gynaecol Oncol 2008, 29:364-367.

27. Bonafe M, Ceccarelli C, Farabegoli F, Santini D, Taffurelli M, Barbis C, Nanni E, Trn M, Ceccarelli G, Oliveri F, Franceschi C: Retention of the p53 codon 72 arginine allele is associated with a reduction of disease-free and overall survival in arginine/proline heterozygous breast cancer patients. Clin Cancer Res 2003, 9:4860-4864.

28. Xu Y, Yao L, Ouyang T, Li J, Wang T, Fan Z, Lin B, Lu Y, Xie Y: p53 Codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res 2005, 11:7328-7333.

29. Siddique MM, Balram C, Fiser-Malyszewska L, Aggarwal A, Tan A, Tan P, Soo KC, Sabapathy K: Evidence for selective expression of the p53 codon 72 polymorphisms: implications in cancer development. Cancer Epidemiol Biomarkers Prev 2005, 14:2245-2252.

30. Toyama T, Zhang Z, Nishio M, Hamaguchi M, Kondo N, Iwase H, Iwata H, Takahashi S, Yamashita H, Fuji Y: Association of TP53 codon 72 polymorphism and the outcome of adjuvant therapy in breast cancer patients. Breast Cancer Res 2007, 9:R53.

31. Hamaguchi M, Nishio M, Toyama T, Sugihara H, Kondo N, Fuji Y, Yamashita H: Possible difference in frequencies of genetic polymorphisms of estrogen receptor alpha, estrogen metabolism and p53 genes between estrogen receptor-positive and -negative breast cancers. Jpn J Clin Oncol 2008, 38:734-742.

32. Vannini I, Zoli W, Tesei A, Rossetti M, Sansone P, Storci G, Passardi A, Masa I, Ricci M, Gussofino F, Fabbrini F, Ulielli P, Brigliadori G, Amadori D, Bonafe M, Ceccarelli G, Oliveri F, Franceschi C: Codon 72 arginine allele in cell survival in vitro and in the clinical outcome of patients with advanced breast cancer. Tumour Biol 2008, 29:145-151.

33. Lunn SS, Chua HW, Li H, Li WF, Rao N, Wei J, Shao Z, Sabapathy K: MDM2 SNPs contribute to breast cancer risk, with increased risk for women with breast cancer in Northern Greece. Cancer Epidemiol Biomarkers Prev 2007, 16:348-356.

34. Khadang B, Fattahi MJ, Talei A, Dehaghani AS, Ghaderi A: Association of the p53 codon 72 polymorphism with colorectal cancer in Iranian women. Ann Y Acad Sci 2003, 1010:764-770.

35. Kaledi TG, Lambropoulos AF, Gueorguiev M, Chrisafi S, Papazisis KT, Kotsis A: The association of p53 mutations and p53 codon 72 polymorphism with breast cancer in Northern Greece. Breast Cancer Res 2007, 22:57-65.

36. Tommiska J, Eerola H, Heinonen M, Salonen L, Kotsis A: MDM2 SNP309 G allele increases risk but the T allele is associated with familial breast cancer. Breast Cancer Res 2007, 9:R27.

37. Gohchait S, Bakhrair S, Barwai N, Vadhana S, Darvishi K, Raish M, Gupta P, Husain SA, Bameiez RN: Implication of BRCA2 -26G>A 5' untranslated region polymorphism in susceptibility to sporadic breast cancer and its modulation by p53 codon 72 Arg>Pro polymorphism. Breast Cancer Res 2007, 9:R71.

38. Baynes C, Healey CS, Pooley KA, Scollen S, Luben RN, Thompson DJ, Pharoah PD, Easton DF, Ponder BA, Dunning AM, SEARCH breast cancer association consortium: Do MDM2 SNPs and TP53 R72P interact in breast cancer susceptibility? A large pooled series from the breast cancer association consortium. Cancer Res 2007, 67(19):9584-9590.

39. Sprague BL, Trentham-Dietz A, Garcia-Closas M, Newcomb PA, Titus-Ernstoff L, Hampton JM, Chanocek SJ, Haines JL, Egan KM:
Genetic variation in TP53 and risk of breast cancer in a population-based case control study. *Carcinogenesis* 2007, 28:1680-1686.

58. Akkiprik M, Sonmez O, Gulluoglu BM, Caglar HB, Kaya H, Demirkalem P, Abacioglu U, Sengozer M, Sav A, Ozer A: *Analysis of p53 Gene Polymorphisms and Protein Over-expression in Patients with Breast Cancer.* *Pathol Oncol Res* 2008. DOI:10.1007/s12253-008-9129-6.

59. Zhang W, Jin MJ, Chen K: *Association of p53 polymorphisms and its haplotypes with susceptibility of breast cancer.* *Zhong Jiao Da Xue Xue Bao Yi Xue Ban* 2007, 36:561-566.

60. Tobias A: *Assessing the influence of a single study in the meta-analysis estimate.* *Statistical Methods in Medical Research* 1999, 8:15-17.

61. Koushik A, Platt RW, Franco EL: *p53 codon 72 polymorphism and cervical neoplasia: a meta-analysis review.* *Cancer Epidemiol Biomarkers Prev* 2004, 13:11-22.

62. Jee SH, Won SY, Yun JE, Lee JE, Park JS, Ji SS: *Polymorphism p53 codon-72 and invasive cervical cancer: a meta-analysis.* *Int J Gynecol Obstet* 2004, 85:301-308.

63. Sousa H, Santos AM, Pinto D, Medeiros R: *Is the p53 codon 72 polymorphism a key biomarker for cervical cancer development? A meta-analysis review within European populations.* *Int J Mol Med* 2007, 20:731-741.

64. Matalidou A, Eitem T, Houlston RS: *TP53 polymorphisms and lung cancer risk: a systematic review and meta-analysis.* *Mutagenesis* 2003, 18:377-85.

65. Zhou Y, Li N, Zhuang W, Liu GJ, Wu TX, Yao X, Du L, Wei ML, Wu XT: *P53 codon 72 polymorphism and gastric cancer: a meta-analysis of the literature.* *Int J Cancer* 2007, 121:1481-1486.

66. Li Y, Qiu LX, Shen XK, Lv X, Qian XF, Song Y: *A meta-analysis of TP53 codon 72 polymorphism and lung cancer risk: Evidence from 15,857 subjects.* *Lung Cancer* 2009.

67. Pietsch EC, Humbey O, Murphy ME: *Polymorphisms in the p53 pathway.* *Oncogene* 2006, 25:1602-1611.

68. Dumont P, Leu JL, Della Pietra AC 3rd, George DL, Murphy MF: *The codon 72 polymorphic variants of p53 have markedly different apoptotic potential.* *Nat Genet* 2003, 33:357-365.

69. Chang CC, Hsieh YY, Tsai FJ, Tsai CH, Tsai HD, Lin CC: *The proline form of p53 codon 72 polymorphism is associated with endometriosis.* *Fertil Steril* 2002, 77:43-45.

70. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS: *Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis.* *Am J Epidemiol* 2008, 168:123-137.

71. Miller CS, Johnstone BM: *Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982–1997.* *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001, 91:622-635.

72. Amarante MK, Watanabe MA: *The possible involvement of virus in breast cancer.* *J Cancer Res Clin Oncol* 2009, 135:329-337.

73. Munafò MR, Flint J: *Meta-analysis of genetic association studies.* *Trends Genet* 2004, 20(9):439-444.