Associations between Aromatase CYP19 rs10046 Polymorphism and Breast Cancer Risk: From a Case-Control to a Meta-Analysis of 20,098 Subjects

Begoña Pineda1, Miguel Ángel García-Pérez1,2, Antonio Cano1,3, Ana Lluch1,4, Pilar Eroles1*

1 Institute of Health Research INCLIVA, Valencia, Spain, 2 Department of Genetics, University of Valencia, Valencia, Spain, 3 Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Valencia, Spain, 4 Department of Haematology and Medical Oncology, University Hospital of Valencia, Valencia, Spain

Abstract

Lifetime exposure to estrogen is a factor that plays an important role in the pathogenesis and progression of breast cancer. Genetic variants in genes of the biosynthesis and metabolism of estrogen have been associated with breast cancer risk. Among them, the CYP19 gene encodes for aromatase, the enzyme that catalyzes the conversion of androgens to estrogens. The rs10046 polymorphism on the CYP19 gene has been related to levels of circulating estradiol and to the estradiol/testosterone ratio. To date, epidemiological studies of rs10046 have been performed in different populations with contradictory results. In the present study, we have conducted a case-control analysis (522 cases and 1221 controls) in the Spanish population. Furthermore, we have performed a meta-analysis including 20,998 subjects (7,998 cases and 12,100 controls) to summarize the data available for rs10046 and breast cancer risk. An odds ratio (OR) with a 95% confidence interval (CI) was applied to assess the association. The results of our case-control study show an association between the carriers of at least one C allele (dominant model) and breast cancer risk (OR = 1.29, 95% CI 1.01–1.66, p-value = 0.038). The meta-analysis shows no significant association with breast cancer risk in any of the genetic models tested. The analysis by ethnic subgroups also failed to produce associations. The evaluation of heterogeneity, influence analysis, and publication bias confirms the reliability of the analysis. We can conclude that the rs10046 polymorphism on CYP19 by itself does not constitute breast cancer risk. We cannot, however, reject the possibility that it could contribute (interact), together with other genetic variants, to modify the circulating levels of estradiol.

Introduction

In recent decades, breast cancer cases have increased worldwide [1] and today are the main cause of cancer mortality and morbidity in women [2]. Breast cancer is caused by environmental and genetic factors [3]. Two main genes, BRCA1 and BRCA2, are associated with hereditary breast cancer. Sporadic cases of breast cancer, however, may be related to variants in low-penetration genes such as polymorphisms [4,5].

Lifetime exposure to estrogen is another factor that plays an important role in breast cancer. It is known that this hormone is involved both in the development of the mammary gland, as well as in the pathogenesis and progression of breast cancer [6]. Based on this, the study of genes related to biosynthesis and the metabolism of estrogen is one way to identify possible candidate genes for breast cancer risk [7]. One of them is the CYP19 (P450arom) gene. The human CYP19 gene is located in the chromosome 15q21.2 region and is comprised of a 30 kb coding region [8]. This gene encodes for aromatase, an enzyme whose function is to catalyze the conversion of androgens into estrogens, a reaction known as aromatization. In premenopausal women, the main source of estrogens is the ovaries. Meanwhile, in postmenopausal women, aromatization takes place elsewhere such as in adipose tissue, skin, muscle, and liver cells.

There have been several epidemiological studies of polymorphisms on the CYP19 gene with the aim of finding associations between genetic variations and breast cancer risk. Some have found an association with an increased risk of breast cancer, such as that of a tetra-nucleotide repeat polymorphism in the intron 4 (TTTA)n [9]. Still, other polymorphisms studied have not shown a clear association with breast cancer risk, thus generating a situation of inconsistent results. This is the case of a C/T single nucleotide polymorphism (SNP) located in the 3′ untranslated region (3′-UTR) of the CYP19 gene (rs10046). Some studies have linked this polymorphism with breast cancer risk [10], however, others show different results [11,12,13]. This discrepancy in results led us to conduct a case-control study of this SNP in a population in Valencia (Spain). Additionally, we performed a meta-analysis of this polymorphism for the first time. It is a powerful tool for overcoming the problems of the small sample size and inadequate
statistical power of genetic studies. This approach gives more reliable results than a single case–control study can. The aim was to collect all results published to date about this polymorphism and to obtain conclusive results about their relevance in susceptibility to breast cancer.

Materials and Methods

Case-control Study

Study population. Association analysis between the rs10046 polymorphism (CYP19) and breast cancer disease was performed in a case-control study. The study was performed in a Caucasian Spanish population composed of 322 breast cancer patients and 1221 controls recruited at the Clinic Hospital of Valencia (Spain) with a mean age at diagnosis of 51 years (range 21–89) and 51 years (range 18–86), respectively. The controls were women without malignant pathology recruited at the blood donor bank and women with non-malignant pathology from the menopause unit of the same hospital. The recruitment of the cases and controls was performed in the same interval of time ±0.5 years. The research protocols were approved by the ethics committee of the Institute of Health Research INCLIVA before the study began. All the participants in the study gave their written informed consent to participate in the study.

Genotyping. Genomic DNA was extracted from blood samples using the DNeasy tissue kit from Qiagen (Izasa, Madrid, Spain) or DNA Isolation Kit by MOBIO (Carlsbad, CA, USA) using minor modifications to the manufacturer protocol. A final elution volume of 100 µl was established. DNA quantity was measured by absorbance at 260 nm using a NanoDrop spectrophotometer, and DNA purity was evaluated by measurement of the 260/280 absorbance ratio. DNA samples were stored at −20°C. Genotyping analysis was performed by real-time PCR (5 ng/µl DNA), using the TaqMan SNP Genotyping Assays C_2834731_30 (Applied Biosystems) according to the manufacturer instructions. Thermal cycling and detection was performed on the ABI Prism 7900 using the Sequence Detection Software (Applied Biosystems). The results were analyzed using the allelic discrimination assay program of Sequence Detection Software version 2.4 (Applied Biosystems).

Statistical analysis. The SNPs genotype analysis of our study population was done with the SNPstats software [14] (allele and genotype distributions, association test, Hardy-Weinberg Equilibrium (HWE)). SNPstats association was based on binary logistic regression according to the response variable providing odds ratios (ORs), the confidence interval (CI), and the p-values for multiple inheritance models (dominant, recessive, over-dominant co-dominant, and log-additive). The lowest Akaike's Information Criterion and Bayesian Information Criterion value indicate the best inheritance genetic model for each specific polymorphism.

Meta-analysis

Literature search strategy for identification of the studies. We did a literature search in PubMed, Scopus and EBSCO data bases using the terms “breast cancer and rs10046”, along with additional terms such as “polymorphisms, SNPs and CYP19”, and all possible combinations. The studies for the meta-analysis were selected when they satisfied the following criteria: studies published by March 2012, case-control studies in humans, studies with genotype frequencies or OR data, information about HWE and information about procedure (adjusted or not, subgroups, etc.). In order to search more deeply, we reviewed the references of the selected articles to retrieve data that we could have ignored in the initial search.

Data extraction. Information was extracted from the articles by two of the authors following the criteria listed above (B.P. and P.E.). Disagreement was discussed and resolved between the two authors. In the event that a study presented subpopulations, these were taken to be different studies. The same was done for studies composed of a first set and a second independent validation set.

Statistical analysis. Raw data from comparable studies were analyzed jointly using likelihood methods. The estimate of association with breast cancer risk was evaluated using the fixed effect method [15] which calculates the ORs and the corresponding 95% CIs for individual studies and the global association. When the test was heterogeneous, the random-effects method [16] was applied. If heterogeneity was not corrected, we performed an influence analysis to determine the study responsible for that variability. Recessive, dominant, co-dominant, additive and over-dominant models were computed.

The analysis of heterogeneity between studies was performed by the Q statistic, with p-values <0.1 indicating significant heterogeneity [17]. We also used the I² statistic to quantify heterogeneity; values of 25% correspond to low heterogeneity, 50% to moderate heterogeneity and 75% to high heterogeneity [18]. The metaregression was performed with the SPSS package (Statistical Package for the Social Sciences, version 19.0), and a variable was considered a source of heterogeneity when the p-value was significant in the ANOVA analysis. Publication bias was assessed by funnel plots of effect sizes versus standard errors to identify significant asymmetry. Additionally, Egger's linear regression test [19] and Begg's test [20] were performed to evaluate the potential bias. The Gleser-Olkin method [21] was used to estimate the number of unpublished studies. Accumulative meta-analysis [22] was assessed by year of publication to evaluate the possible publication bias by time. Statistical analysis of association was done with SPSS, version 19.0.

Results

rs10046 Genotype in a Spanish Population

The distribution of the genotype frequencies in this polymorphism within the control group is in agreement with that expected under HWE with a p-value of <0.05. We also observed that the frequencies in this study were similar to those previously reported in the European population described by HapMap (http://hapmap.ncbi.nlm.nih.gov/).

Our results show an association between the rs10046 polymorphism on the CYP19 gene and breast cancer risk. The carriers of at least one C allele (dominant model) have 1.29 times increased risk of developing breast cancer (95% CI 1.01–1.66, p-value = 0.038) vs. non-carriers. The distribution of the rs10046 genotype among cases and controls and risk of breast cancer is listed in Table 1 (codominant and dominant models).

Meta-analysis Study Characteristics

A total of 14 studies were selected from the bibliography search regarding the association between the rs10046 polymorphism on the aromatase CYP19 gene and breast cancer. Two of these were excluded for not having been case-control studies [23,24]. Additionally, of the remaining 12 studies, 3 were excluded for not stating the genotype distribution [9,25,26]. Afterwards, the studies presenting different subpopulations according to ethnicity [27] or having a validation set [12] were divided into independent studies. Finally, 12 studies were accepted for the first association analysis using the genotype distribution in cases and controls.
Table 1. Genotypic and allelic frequencies of rs10046 and breast cancer risk.

| Polymorphism | Genotype | Cases (n = 522) | Controls (n = 1221) | OR (95%CI)* p-value |
|--------------|----------|----------------|-------------------|--------------------|
| rs10046      | TT       | 109 (20.9%)    | 311 (25.5%)       | Reference          |
| CYP19        | TC       | 278 (53.3%)    | 629 (51.5%)       | 1.26 (0.97–1.64)   | 0.094              |
|              | CC       | 135 (25.9%)    | 281 (23.0%)       | 1.37 (1.02–1.85)   | 0.038              |
|              | TC+CC    | 413 (79.1%)    | 910 (74.5%)       | 1.29 (1.01–1.66)   |                    |

(Figure 1). Table 2 lists the main characteristics of these studies: first author, publication year, original country, ethnicity, source of controls, genotype distribution of controls and cases, HWE p-value, mean age and menopausal status.

Meta-analysis Results

When the eligible studies were pooled in the meta-analysis, no significant association with breast cancer risk was found in any of the genetic models. The dominant model did not present homogeneity when either the fixed effect model or the random effect model were applied. We assessed an analysis of influence to discriminate if one of the studies was causing the heterogeneity. The Yoshimoto et al. [28] study shows the highest p-value for the heterogeneity test (p = 0.16), revealing it as the more discordant of all in the comparison. Consequently, we eliminated this study from further analysis. Additionally, the fixed effect model did not show homogeneity for two of the assayed models. To explore whether the sources of heterogeneity were mean age and menopausal status, we performed a meta-regression analysis. Neither of these variables explained the heterogeneity (F = 3.042; p = 0.247). The random effect model was chosen for all the analyses.

For the dominant model, the odds ratio obtained was 0.99 (95% CI: 0.91–1.08) (Figure 2). This result shows no association with the presence of the C allele and a predisposition to breast cancer.

Similarly, we proceeded to evaluate the possible association using the co-dominant (Figures 3 and 4), recessive (supplementary Figure 1), additive (supplementary Figure 2) and over-dominant models (supplementary Figure 3). In all cases, the analysis presents an OR, again, nearly 1, and the 95% CI crossed this limit. We can conclude that neither of the models presents an association with the risk to develop breast cancer for the rs10046.

Subgroup Analysis

Considering the possible impact of ethnic variations, we then performed two subgroup analyses in European, Asian and American populations, and in Caucasian, Asian and others ethnicities. The analyses of these groups failed to suggest an association between rs10046 and breast cancer risk in any of the models (Table 3).

Evaluation of Reliability

Heterogeneity is a potential problem when performing a meta-analysis. In the present study, we systematically performed an evaluation of the heterogeneity in each model using the Q test and I² statistic. The breach of the homogeneity condition led to the use of a less restrictive model (random effect model instead of fixed effect model). When the analysis was not homogeneous even using the random effect model we performed an influence analysis to discriminate study outliers and a meta-regression to explore whether the source of heterogeneity was the mean age and
| FIRST AUTHOR | YEAR | COUNTRY | ETHNICITY | SOURCE | GENOTYPE DISTRIBUTION (CONTROLS) | GENOTYPE DISTRIBUTION (CASES) | HWE | MEAN AGE | MENOPAUSAL STATUS |
|--------------|------|---------|-----------|--------|---------------------------------|--------------------------------|-----|----------|-------------------|
| Olson        | 2006 | US (Minnesota) | Caucasian | H      | NA                              | 167 (27%) 310 (51%) 134 (22%) 118 (26%) 240 (52%) 103 (22%) | –    | 94%      |                    |
| Haiman       | 2002 | US (Nurses’ Health Study) | Caucasian | H      | 167 (27%) 310 (51%) 134 (22%) 118 (26%) 240 (52%) 103 (22%) | –    | 94%      |                    |
| Raskin       | 2009 | Israel (Jews Ashkenazi) | Jews     | H      | NA                              | >0.05                          | –    | 94%      |                    |
| Ralph        | 2007 | USA* | Caucasian | H      | 883 (26.8%) 1650 (50.1%) 758 (23.1%) 461 (28.1%) 830 (50.6%) 349 (21%) | 0.05 | cases 9.8±9.3 |                    |
| Ralph (validation) | 2007 | USA* | Caucasian | H      | 883 (26.8%) 1650 (50.1%) 758 (23.1%) 461 (28.1%) 830 (50.6%) 349 (21%) | 0.05 | cases 9.8±9.3 |                    |
| Zhang        | 2008 | China | Asian     | H      | 120 (30.8%) 176 (45.1%) 94 (24.1%) 94 (31.3%) 151 (50.3%) 55 (18.3%) | 0.43 | cases 52.0±34.1 |                    |
| Kristensen   | 2000 | Norway | Caucasian | HP     | 53 (23%) 114 (48%) 69 (29%) 146 (31%) 240 (50%) 95 (19%) | >0.05 | –        |                    |
| Colomer      | 2008 | Spain | Caucasian | H      | 17 (26%) 29 (45%) 19 (29%) | –    | 94%      |                    |
| Fasching     | 2008 | Germany | Caucasian | H      | 362 (29%) 606 (48.6%) 279 (22.4%) | >0.05 | –        |                    |
| Dunning      | 2004 | Inglaterra | Caucasian | H      | 1049 (28.9%) 1773 (48.9%) 808 (22.2%) 739 (28%) 1286 (48.9%) 610 (23.1%) | 0.3 | cases 22–65 | 45–74 |
| Yoshimoto    | 2011 | Japan | Asian     | H      | 60 (21.7%) 120 (43.3%) 97 (35%) 160 (19.3%) 427 (51.7%) 239 (29%) | >0.05 | –        |                    |
| Garcia-Casado| 2010 | Spain (Valencia) | Caucasian | H      | 23 (24.2%) 57 (60%) 15 (15.8%) | >0.05 | range 30–64 |                    |
| Chen         | 2008 | China | Asian     | H      | 277 (31.6%) 436 (49.8%) 163 (18.6%) 178 (29.1%) 308 (50.4%) 125 (20.5%) | >0.05 | range 30–64 |                    |
| Iwasaki1     | 2009 | Japan | Asian     | H      | 69 (17.8%) 194 (50%) 125 (32.2%) 82 (21.1%) 188 (48.5%) 118 (30.4%) | >0.05 | range 30–64 |                    |
| Iwasaki2     | 2009 | Brasil (Japanese) | Asian    | H      | 13 (16.5%) 44 (55.7%) 22 (27.8%) 14 (17.7%) 41 (51.9%) 24 (30.4%) | >0.05 | range 30–64 |                    |
| Iwasaki3     | 2009 | Brasil | Caucasian | H      | 58 (15.3%) 100 (52.8%) 121 (61.9%) 67 (47.2%) 179 (97.2%) 133 (35.1%) | >0.05 | range 30–64 |                    |
| Pineda       | 2012 | Spain | Caucasian | H      | 311 (23.1%) 629 (51.5%) 281 (25.4%) 109 (20.9%) 278 (53.3%) 135 (25.9%) | 0.094 | –        |                    |

doi:10.1371/journal.pone.0053902.t002
menopausal status variables. An influential study was detected as the source of heterogeneity and was eliminated from further analysis. Another limitation of meta-analysis is publication bias. To check for potential problems, we performed two statistical analyses: Begg’s and Eggers. The Begg method checks the correlation between an effect and its variability. This analysis calculates the Kendall’s tau ($\tau$) coefficient. The absence of statistical significance suggests no publication bias. Of the three models assayed, only the recessive model showed significance (p-values = 0.14, 0.02 and 0.89 for dominant, recessive and over-dominant models, respectively). To confirm these results, we analyzed the data with the more sensitive Egger’s test which uses a linear regression between the reduced measure of the effect and the precision. The existence of bias was evaluated by the significance of the ordinate value in the origin for a value p $< 0.1$. This analysis confirmed there was no publication bias (p-values = 0.40, 0.52, 0.94 for dominant, recessive and over-dominant models, respectively).

Using the Gleser-Olkin method, we estimated the number of unpublished studies based on the number of known publications and their p-values. The low limit of the 95% CI was a negative value that was compatible with the hypothesis of absence of publication bias. Furthermore, we performed a cumulative meta-analysis for year of publication to verify the influence of time on the results observed by different groups. There were no tendencies related to time that might affect the data published (data not shown).

**Discussion**

The importance of breast cancer worldwide has led to a substantial increase in research in this field. The present efforts to fight the illness are focused in its better classification [29] and treatment, but without forgetting the relevance of prevention and early diagnosis. Among the factors to take into consideration in the early detection of breast cancer is the exposure to estrogens and to other hormones. Prospective studies have shown a direct association between circulating sex hormones with the risk of developing breast cancer in postmenopausal women [30]. Furthermore, the circulating levels of estradiol precursors and metabolites have also been related to increased risk of breast cancer. These circulating levels are largely under genetic control and, consequently, can be modified by polymorphisms on genes associated with estradiol. Therefore, it is logical to think that changes in genes that control the levels of estrogen, as in the case of rs10046 ($CYP19$), are potential candidates to predispose for this illness [30].

To date, several studies have been performed to evaluate this hypothesis in different populations, with contradictory results. Consequently, we have done a case-control study in a Spanish population with samples paired by age and menopausal status to assure that these variables have not affected the results. We obtained a significant association between carrying at least one C allele (dominant model) and the risk of breast cancer. This result was in agreement with previous works published, where the
frequency of the C allele is higher in cases vs. controls [11,30,31]. Especially relevant is the publication of Dunning et al. [30], where the study was carried out in more than 2000 cases and 3000 controls. Other authors however, have not detected a significant association or even found opposite results. Whereas Kristensen et al. found a predisposition role for the T allele [10], other authors have not found a clear association with breast cancer risk [12,13,27]. The reason for these conflicting results could be that the studies were developed in different populations, geographical areas and, additionally, with a variable number of samples. Though some of these case-control studies did not reach statistical significance, it is possible that the polymorphism contributed to levels of circulating sex hormones. At least some publications claim that this polymorphism is related to the levels of estradiol and the estradiol:testosterone ratio in normal postmenopausal women [30], a factor relevant in the development of breast cancer [6]. Unfortunately, in our case-control study, serum samples were not available for the determination of estradiol, which would have added important information. Published expression data show higher estrogen levels with higher repeats in [TTTA]n polymorphism on \( CYP19 \) found in linkage disequilibrium with rs10046 [32,33,34]. Additionally, a highly significant relationship between aromatase SNPs and circulating estrogen levels among postmenopausal women has been found by Haiman et al. [35]. Between the highly correlated tagging SNPs, there were polymorphisms in different haplotype blocks, including rs10046.

How this SNP can affect estrogen levels is not obvious. Different studies have shown SNPs that can affect phenotypic outcome by altering DNA binding sites [36,37,38], mRNA stabilization, folding, splicing [39,40,41] and modification of mechanisms involving the enhancement of transcription and the posttranslational regulation.

All the above highlights the importance of studying in depth the possible association of C>T rs10046 SNP with breast cancer risk. With that in mind, we have performed a meta-analysis, a reliable analytical tool for comparing the different data related to this polymorphism. The results of our meta-analysis showed no significant association with breast cancer risk in any of the genetic models tested. In all cases, the overall OR calculated is near the value of 1, indicating no existence of a trend or predilection for the rs10046 genetic variants between cases and controls. They were no significant differences in age and menopausal status across genotypes, as the meta-regression analysis revealed. The comparisons of subgroups based on population stratifications showed only slight discrepancies, data that in no case reached significance.

The results of our work show that the case-control studies restricted to a limited population provide different results than do those with a wide representation of the population. To extrapolate results and to come to relevant conclusions of the possible influence of a factor in a disease seems essential to realize global studies. The meta-analysis approach allows us to obtain relevant conclusions and simultaneously to summarize and to unify the studies in the field. On the basis of our results, polymorphism
Table 3. Association between rs10046 and breast cancer risk stratified by subgroups.

| GENETIC MODEL | Subgroup A | n | RECESSIVE | OR | 95% CI | DOMINANT | OR | 95% CI | ADDITIVE | OR | 95% CI | CC vs. TT | OR | 95% CI | CT vs. TT | OR | 95% CI | OVERDOMINANT | OR | 95% CI |
|---------------|------------|---|-----------|-----|---------|----------|-----|---------|----------|-----|---------|-----------|-----|---------|-----------|-----|---------|-------------|-----|---------|
|               |            | 0 |           |  1.03 | 0.89–1.18 | 0.95 | 0.86–1.06 | 0.95 | 0.86–1.05 | 0.95 | 0.84–1.08 | 0.85–1.06 | 0.95 | 0.84–1.07 |
|               |            | 1 |           |  0.94 | 0.70–1.26 | 1.00 | 0.76–1.32 | 0.98 | 0.72–1.34 | 0.94 | 0.61–1.44 | 1.03 | 0.84–1.27 | 1.02 | 0.93–1.11 |
|               |            | 2 |           |  1.08 | 0.79–1.48 | 1.01 | 0.86–1.18 | 0.99 | 0.84–1.17 | 0.94 | 0.72–1.21 | 1.02 | 0.86–1.21 | 1.15 | 0.91–1.44 |

| GENETIC MODEL | Subgroup B | n | RECESSIVE | OR | 95% CI | DOMINANT | OR | 95% CI | ADDITIVE | OR | 95% CI | CC vs. TT | OR | 95% CI | CT vs. TT | OR | 95% CI | OVERDOMINANT | OR | 95% CI |
|---------------|------------|---|-----------|-----|---------|----------|-----|---------|----------|-----|---------|-----------|-----|---------|-----------|-----|---------|-------------|-----|---------|
|               |            | 0 |           |  0.99 | 0.86–1.14 | 1.00 | 0.89–1.13 | 1.00 | 0.87–1.14 | 0.99 | 0.82–1.20 | 1.01 | 0.91–1.11 | 1.00 | 0.94–1.07 |
|               |            | 1 |           |  1.08 | 0.79–1.48 | 1.01 | 0.86–1.18 | 0.99 | 0.84–1.17 | 0.94 | 0.72–1.21 | 1.02 | 0.86–1.21 | 1.15 | 0.91–1.44 |
|               |            | 2 |           |  1.15 | 0.85–1.56 | 0.84 | 0.57–1.24 | 0.87 | 0.60–1.27 | 0.95 | 0.62–1.46 | 0.77 | 0.52–1.16 | 0.80 | 0.60–1.07 |

Subgroup A corresponds to American (0: Haiman, Ralph, Ralph validation, Iwasaki3), European (1: Kristensen, Dunning, Pineda) and Asian (2: Chen, Iwasaki1, Iwasaki2, Zhang) populations. Subgroup B corresponds to Caucasian (0: Haiman, Ralph, Ralph validation, Iwasaki1, Iwasaki2, Zhang) and other ethnicities (2: Iwasaki3). OR: odds ratio, CI: confidence interval. The Random effect model was used to perform the analysis.

doi:10.1371/journal.pone.0053902.t003

Figure 4. Meta-analysis of OR for rs10046 polymorphism associated with breast cancer (CC vs. TT).
doi:10.1371/journal.pone.0053902.g004
rs10046 is not capable of modifying the risk to develop breast cancer. Nonetheless, several studies associated this polymorphism to circulating hormonal levels [30]. Additionally, rs10046 has been related to altered disease-free survival in the subgroup of premenopausal breast cancer patients [9]. Polymorphisms in linkage disequilibrium with rs10046, as in the case of rs4664, rs700518 and rs700519, has been associated with variable efficacy of treatments [23,24,42] and breast cancer survival [43,44].

The data available is not sufficient to affirm that it is an activating polymorphism, but published data suggest that it could be related to an advantage in the protein structure that makes it more active [23]. In the absence of a mechanistic explanation, however, strong linkage disequilibrium with other polymorphisms remains possible. There are some studies about polymorphisms in linkage disequilibrium with rs10046 which describe a significant association with efficacy of the aromatase inhibitor letrozole in patients with breast cancer, as is the case for rs700518 [42] and rs4664 [23,24]. The latter SNP has also been reported to be associated with HER2 status of tumors [9], circulating steroid hormones [35] and histological grade and tumor size in postmenopausal women [35,43]. Haiman et al. reported a significant relationship between this SNP and circulating estrogen levels among postmenopausal women. A haplotype analysis has also been performed on the CYP19 gene in most of these studies. Some specific haplotypes, including rs10046, were associated with an increased risk of breast cancer with concurrent proliferative fibrocystic conditions [31] and with clinical efficacy of letrozole [42]. Moreover, the haplotype studies conducted by Raskin et al. showed a trend to association with breast cancer risk in BRCA1 carriers aged <50 years [26].

In our study, we have not done any analysis of polymorphism in linkage disequilibrium with rs10046. This could be a limitation, as it could have provided more information on the role of CYP19 in breast cancer. Nevertheless, it should be noted that these studies were taken into account when we performed the meta-analysis even though some of them were excluded from the final statistical analysis due to lack of necessary data.

In conclusion, despite the limitations, the results of the present meta-analysis suggest that rs10046, by itself, does not directly affect the risk to suffer breast cancer. Further extensive studies to clarify the influence of CYP19 polymorphisms on estradiol circulation levels are necessary.

Supporting Information

Figure S1 Meta-analysis of OR for rs10046 polymorphism associated with breast cancer (recessive model). (TIF)

Figure S2 Meta-analysis of OR for rs10046 polymorphism associated with breast cancer (additive model). (TIF)

Figure S3 Meta-analysis of OR for rs10046 polymorphism associated with breast cancer (over-dominant model). (TIF)

Acknowledgments

The authors thank sample donors and all the field workers who supported the data collection.

Author Contributions

Reviewed the manuscript: MAG AC AL. Conceived and designed the experiments: MAG PE. Performed the experiments: BP PE. Analyzed the data: PE AL. Contributed reagents/materials/analysis tools: MAG PE AL AC. Wrote the paper: BP PE.

References

1. Pedraza AM, Pollan M, Pastor-Barrionuevo R, Cabanes A (2012) Disparities in breast cancer mortality trends in a middle income country. Breast cancer research and treatment.
2. Hortobagyi GN, de la Garza Salazar J, Pritchard K, Amadori D, Haidinger R, et al. (2005) The global breast cancer burden: variations in epidemiology and clinicopathologic profile. Cancer 109: 1940-1948.
3. Zhang L, Gu L, Qian B, Hao X, Zhang W, et al. (2009) Association of genetic polymorphisms of ER-alpha and the estradiol-synthesizing enzyme genes CYP19 and CYP19 with breast cancer risk in Chinese women. Breast cancer research and treatment 114: 327-338.
4. Sole X, Guino E, Valis J, Iuema R, Moreno V (2006) SNPssets: a web tool for the analysis of association studies. Bioinformatics 22: 1929-1929.
5. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. Journal of the National Cancer Institute 22: 719-748.
6. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Controlled clinical trials 7: 177-188.
7. Loo J, Ioannidis JP, Schmid CH (1997) Quantitative synthesis in systematic reviews. Annals of internal medicine 127: 820-826.
8. Huggins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 537-540.
9. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629-634.
10. Plummer MD, Schildkraut J, Negri E, Torre L, Autier P, et al. (2006) Risk of breast cancer according to postmenopausal hormone replacement therapy use: an update of a pooled analysis of 53 epidemiological studies. Cancer Epidemiol Biomarkers Prev 15: 1085-1101.
11. Glenter LJ, Olkin I (1996) Models for estimating the number of unpublished studies. Statistics in medicine 15: 2493-2507.
the aromatase gene defines a subgroup of postmenopausal breast cancer patients with poor response to neoadjuvant letrozole. BMC cancer 10: 36.

25. Olson JE, Ingle JN, Ma CX, Pellemounter LL, Schaid DJ, et al. (2007) A comprehensive examination of CYP19 variation and risk of breast cancer using two haplotype-tagging approaches. Breast cancer research and treatment 102: 237–247.

26. Raskin L, Lejbkowicz F, Barnett-Greenough O, Dishon S, Almog R, et al. (2009) BRCA1 breast cancer risk is modified by CYP19 polymorphisms in Ashkenazi Jews. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 18: 1617–1623.

27. Iwasaki M, Hamada GS, Nishimoto IN, Netto MM, Motola J, Jr., et al. (2010) Dietary isoflavone intake, polymorphisms in the CYP17, CYP19, 17beta-HSD1, and SHBG genes, and risk of breast cancer in case-control studies in Japanese, Japanese Brazilians, and non-Japanese Brazilians. Nutrition and cancer 62: 466–475.

28. Yoshimoto N, Nishiyama T, Toyama T, Takahashi S, Shiraki N, et al. (2011) Genetic and environmental predictors, endogenous hormones and growth factors, and risk of estrogen receptor-positive breast cancer in Japanese women. Cancer science 102: 2065–2072.

29. Eroles P, Bosch A, Alejandro Perez-Fidalgo J, Lluch A (2012) Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways. Cancer treatment reviews 38: 698–707.

30. Dunning AM, Dowsett M, Healey CS, Tee L, Lahen RN, et al. (2004) Polymorphisms associated with circulating sex hormone levels in postmenopausal women. Journal of the National Cancer Institute 96: 936–945.

31. Chen C, Sakoda LC, Doherty JA, Loomis MM, Fish S, et al. (2008) Genetic variation in CYP19A1 and risk of breast cancer and fibrocystic breast conditions among women in Shanghai, China. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 17: 3457–3466.

32. Gemelli L, Mapi L, Merletti D, Picariello L, Falchetti A, et al. (2004) A polymorphic CYP19 TTTA repeat influences aromatase activity and estrogen levels in elderly men: effects on bone metabolism. The Journal of clinical endocrinology and metabolism 89: 2803–2810.

33. Skorupski P, Krol J, Starega J, Adamiak A, Jankiewicz K, et al. (2006) An alpha-1 chain of type I collagen Sp1-binding site polymorphism is associated with reduced risk of hip osteoarthritis defined by severe joint space narrowing in elderly women. Arthritis and rheumatism 52: 1431–1436.

34. Chen X, Truong TT, Weaver J, Bove BA, Cattie K, et al. (2006) Intronic alterations in BRCA1 and BRCA2: effect on mRNA splicing fidelity and expression. Human mutation 27: 427–433.

35. Haiman CA, Dossus L, Setiawan VW, Stram DO, Dunning AM, et al. (2007) Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. Cancer research 67: 1893–1897.

36. Bond GL, Hu W, Bond EE, Robbins H, Lutzker SG, et al. (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591–602.

37. Kimm J, Zinula JM, Nevitt MC, Lui L, Hochberg MC, et al. (2005) Type I collagen alpha1 Sp1 transcription factor binding site polymorphism is associated with reduced risk of hip osteoarthritis defined by severe joint space narrowing in elderly women. Arthritis and rheumatism 52: 1431–1436.

38. Skorupski P, Krol J, Starega J, Adamiak A, Jankiewicz K, et al. (2006) An alpha-1 chain of type I collagen Sp1-binding site polymorphism in women suffering from stress urinary incontinence. American journal of obstetrics and gynecology 194: 346–350.

39. Jin Y, Dietz HC, Montgomery RA, Bell WR, McIntosh I, et al. (1996) Glanmann thrombasthenia. Cooperation between sequence variants in cis during splice site selection. The Journal of clinical investigation 98: 1745–1754.

40. Park IH, Lee YS, Lee KS, Kim SY, Hong SH, et al. (2011) Single nucleotide polymorphisms of CYP19A1 predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer. Cancer chemotherapy and pharmacology 68: 1263–1271.

41. Darabi H, Czene K, Vedern S, Li Y, Liu J, et al. (2011) Genetic variation in the androgen estrogen conversion pathway in relation to breast cancer prognosticators. Breast cancer research and treatment 127: 505–509.

42. Bond GL, Hu W, Bond EE, Robbins H, Lutzker SG, et al. (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591–602.

43. Duan J, Wainwright MS, Comerón JM, Saitou N, Sanders AR, et al. (2003) Synonymous mutations in the human dopaminergic receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Human molecular genetics 12: 205–216.

44. Jin Y, Dietz HC, Montgomery RA, Bell WR, McIntosh I, et al. (1996) Glanmann thrombasthenia. Cooperation between sequence variants in cis during splice site selection. The Journal of clinical investigation 98: 1745–1754.

45. Park IH, Lee YS, Lee KS, Kim SY, Hong SH, et al. (2011) Single nucleotide polymorphisms of CYP19A1 predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer. Cancer chemotherapy and pharmacology 68: 1263–1271.

46. Darabi H, Czene K, Vedern S, Li Y, Liu J, et al. (2011) Genetic variation in the androgen estrogen conversion pathway in relation to breast cancer prognosticators. Breast cancer research and treatment 127: 505–509.

47. Long JR, Kataoka N, Shu XO, Wen W, Gao YT, et al. (2006) Genetic polymorphisms of the CYP19A1 gene and breast cancer survival. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 15: 2115–2122.