An Association of Caspase-10 (Ex 1228G>A) Polymorphism with Breast Cancer Risk: A Meta-Analysis of Case-Control Studies

Sanjib Chaudhary1,2*, Madhur Krishna B1, Sandip K Mishra1* and Dipti Ranjan Mishra2
1Cancer Biology Lab, Institute of Life Sciences, Nalco Square, Bhubaneswar, Odisha, India
2Gene Function and Regulation Group, Institute of Life Sciences, Nalco Square, Chandrasekharpur, Bhubaneswar, Odisha, India

Background: Breast Cancer (BC) is one of the primary causes of cancer related deaths in women worldwide. The incidence rate of BC has increased in recent times, especially in developing countries. Several polymorphisms have been linked with susceptibility of breast cancer risk. Cellular differentiation and homeostasis is maintained by a coordinated cascade of apoptotic genes, caspases and any alternation in this pathway can potentially lead to cancer. Transition mutation in the 9th exon of caspase-10 is associated with breast cancer susceptibility with few reservations. We performed meta-analysis to examine the association of caspase-10 on exon 9 1228G>A polymorphism with BC susceptibility.

Method: Three studies with a total of 31,300 BC cases and 34,331 healthy controls were included in the analysis. The present analysis was performed using comprehensive meta-analysis Version 3 (CMA Version 3) software. Either random or fixed models were used according to the degree of heterogeneity within the studies. Odds ratio with 95% CI was calculated and was considered to apprise the association of caspase-10 on exon 9 1228G>A polymorphism with BC susceptibility.

Results and Discussion: The present meta-analysis showed that caspase-10, exon 9 1228G>A polymorphism was not associated with breast cancer risk (OR=0.91, 95%CI=0.77-1.08, p=0.30). Homozygous mutant AA analysis also did not show any significant association with BC (OR=0.93, 95%CI=0.73-1.18, p=0.58). All the case-control studies included in the analysis were Caucasian origin, therefore a larger case-control studies in different ethnic groups is inevitable to better understand the association of caspase-10 1228G>A polymorphism with BC risk.

Conclusion: The current meta-analysis result suggests that caspase-10, exon 9 1228G>A polymorphism is not associated with breast cancer risk.

Keywords: Breast cancer; Caspase-10; Polymorphism; Meta-analysis
Materials and Methods

Literature search and study eligibility

An extensive search was done in Pubmed, Google scholar, Science-direct and cross-references were checked using keywords "caspase-10 and polymorphism", "caspase-10 polymorphism", "rs13010627" or "caspase-10 V410I" or "caspase-10 1228G>A" and "breast cancer". The articles that contained only available genotype frequencies, case-control studies, written in English and BC risk studies were included in the analysis. Those studies which included overlapping data, no case-control study for caspase-10 polymorphism and not associated with cancer risk were excluded from the study. Three studies with a total of 31,300 BC cases and 34,331 healthy controls were analysed.

Data extraction

The published data were independently collected by two investigators (SC and BMK) to extract the following information: first author, year of publication, country, ethnicity, number of case and controls, genotype and allele frequencies for each case-control study. The disagreement between the authors was resolved after group discussion amongst the investigators and final decision was taken by SKM.

Statistical analysis

The statistical analysis was performed using the comprehensive meta-analysis (CMA) V3 software (Biostat, USA). The CMA V3 has advantage over other software's for computing meta-analysis [13,14]. The odd ratio (OR) and confidence interval (CI) was measured to check the strength of the association of caspase-10 1228G>A with breast cancer. The heterogeneity and variation between the studies were estimated using I2-based Cochran’s Q statistic test. The inter-study variability that can attribute to heterogeneity can be represented by I2 value which ranges between 0 to 100%. The I2 values 0-25, 25-50, 50-75, 75-100% represent no, moderate, large and extreme heterogeneity [15]. When a significant p value (p<0.01) or I2 >50% indicated heterogeneity among the studies, the random effect model was used, or the fixed model was used [16,17]. The publication bias was checked by Begger funnel plot which was further re-evaluated using Egger's regression analysis, the p value of regression analysis less than 0.01 was considered to be significant. The sensitivity analysis was performed by sequential removal of the individual studies.

Results

Characteristics of the studies

According to the inclusion criteria, three studies were included for the analysis [10-12]. The characteristics of the study are represented in Table 1. All the studies included in the analysis were of Caucasian origin. A total of 31,300 BC cases and 34,331 healthy controls were examined for the association of caspase-10 1228G>A polymorphism with breast cancer susceptibility.

Table 1: Caspase-10 1228G>A polymorphism characteristics of studies included in meta-analysis.

<table>
<thead>
<tr>
<th>Study (ref)</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Cancer type</th>
<th>Case</th>
<th>Control</th>
<th>Confirming method</th>
<th>P value HWE</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
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<td>GG</td>
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<td>Engel</td>
<td>2010</td>
<td>Multicenter</td>
<td>European</td>
<td>BC</td>
<td>3368</td>
<td>486</td>
<td>18</td>
<td>2903</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>433</td>
<td>19</td>
<td>RFLP-PCR</td>
<td>NA</td>
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<tr>
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<td>2009</td>
<td>Multicenter</td>
<td>European</td>
<td>BC</td>
<td>23456</td>
<td>3352</td>
<td>109</td>
<td>26597</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3706</td>
<td>126</td>
<td>Taqman/Sequenom iPLEX</td>
<td>NA</td>
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<tr>
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<td>Germany</td>
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<td></td>
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<td></td>
<td></td>
<td>85</td>
<td>6</td>
<td>TaqMan/ Sequencing</td>
<td>0.0076</td>
</tr>
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</table>

Publication bias

Begger's funnel plot and Egger's regression analysis was performed to check the publication bias. Egger's regression analysis was used to measure asymmetry of the funnel plot. The funnel plots were symmetrical and also the Egger's linear regression analysis showed no statistical significance for publication bias (p>0.05). The funnel plots are depicted in Figures 1a-1e.

Begger's funnel plot of publication bias has been categorised and plotted as Overall A vs G, Homozygous mutant AA vs GG, Heterozygous mutant GA vs GG, Dominant Genetic model AA+GA vs GG, Recessive Genetic model AA vs GG+GA. Publication bias was assessed in studies assaying odds ratio of cancer associated with CASP10 1228G>A polymorphism. Begger's funnel plot showed no publication bias within the studies.

Figure 1(a): Begger’s funnel plot of publication bias: Overall A vs G.
Heterogeneity test

The heterogeneity among the studies was checked by using Q-test and I² statistics. The overall allele (A vs. G), heterozygous mutant (GA vs. GG) and dominant genotype model (AA+GA vs. GG) showed heterogeneity; therefore the random model was used (A vs. G: Q=9.48, Ph=0.009, I²=78.90; GA vs. GG: Q=6.44, Ph=0.04, I²=68.97; AA+GA vs. GG: Q=8.13, Ph=0.01, I²=75.40). Since homozygous mutant (AA vs. GG) and recessive genotype model (AA vs. GG+GA) showed no heterogeneity the fixed model was used (AA vs. GG: Q=2.83, Ph=0.24, I²=29.46; AA vs. GG+GA: Q=2.64, Ph=0.26, I²=24.39). All details are mentioned in Table 2.

Table 2: Statistics to test publication bias and heterogeneity.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Egger’s regression analysis</th>
<th>Heterogeneity analysis</th>
<th>Model used for meta-analysis</th>
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<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>95% Confidence Interval</td>
<td>P value</td>
</tr>
<tr>
<td>A vs. G</td>
<td>-2.85</td>
<td>-24.41</td>
<td>0.1</td>
</tr>
<tr>
<td>AA vs. GG</td>
<td>-1.59</td>
<td>-11.28</td>
<td>0.08</td>
</tr>
<tr>
<td>GA vs. GG</td>
<td>-2.35</td>
<td>-19.73</td>
<td>0.1</td>
</tr>
<tr>
<td>AA+GA vs. GG</td>
<td>-2.64</td>
<td>-22.43</td>
<td>0.1</td>
</tr>
<tr>
<td>AA vs. GG+GA</td>
<td>-1.54</td>
<td>-10.96</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Meta-analysis results

The meta-analysis did not show any association between caspase-10 1228G>A polymorphism with BC. The overall variant allele A was not associated with BC (OR=0.91, 95%CI=0.77-1.08, p=0.30). In addition, heterozygous mutant AG (OR=0.94, 95%CI=0.81-1.09, p=0.46), homozygous mutant AA (OR=0.93, 95%CI=0.73-1.18, p=0.58), dominant (OR=0.92, 95%CI=0.78-1.09, p=0.36) and recessive models
(OR=0.93, 95%CI=0.73-1.18, p=0.57) also indicated no significant association of CASP10 1228G>A polymorphism with breast cancer (Figure 2). Sensitivity analysis performed after omitting each single study showed no statistically significant influence (Figure 3).

**Figure 2**: Forest plot of CASP10 1228G>A polymorphism in association with breast cancer risk: Meta-analysis was performed using previously published literatures using Comprehensive meta-analysis V3 software (CMA V3). Random or fixed model used for the analysis for calculating the combined effect of the studies.

**Discussion**

In the pooled study of 31,300 Breast Cancer (BC) cases and 34,331 healthy controls, we demonstrated that minor allele A of caspase-10 (1228G>A) was not associated with BC (OR=0.91, 95%CI=0.77-1.08). There was heterogeneity between the studies, but the source was unknown. Sensitivity analysis was performed by omitting any single study, but no significant influence was found.

Caspases are highly specific proteolytic proteins that cleave substrates in a caspase-cascade manner in apoptotic cells and during development processes. The apoptotic pathway involving caspases is highly conserved in eukaryotes. Caspase activation in apoptosis-committed cells leads to enzymatic caspase cascade which degrade important structural proteins, proteins responsible for DNA-repair. However, alternation in this process results into diverse disease conditions [7]. Caspase-10 one of the key mediators in apoptosis contained several functional polymorphisms which may cause cancer risk [9,18,19]. The polymorphism caspase-10 1228G>A is found five amino acid upstream of the cleavage site and seven amino acids downstream of the conserved active-site motif (QACXG penta peptide) which contains the catalytic cysteine residue [20]. The variation for G to A at 1228 position affects the caspase cleavage and activation. The G to A variation is shown to cause BC risk; however, there were inconsistencies in the study.

Our study could not find any association of caspase-10 (1228G>A) with BC specifically in Caucasian. There were limitations regarding this meta-analysis should be addressed. Firstly, the study included for the analysis was few even though the sample size was large. Secondly, all the studies included were only of European origin, therefore we could not conclude the role of A minor allele in other ethnic population. Thirdly, the source of heterogeneity among studies could not be address. Fourthly, the case and control were included based on well-defined inclusion criteria; however, other factors not included may have influenced our findings.
Figure 3: Sensitivity analysis (leave-one-out) of meta-analysis of risk of breast cancer women with CASP10 1228G>A polymorphism: Sensitivity analysis was performed by removing one study each time using Comprehensive meta-analysis V3 software (CMA V3). Either random or fixed model was used for the analysis for calculating the combined effect of the studies. Sensitive analysis results showed no effect of single study on odds ratio.

Conclusion

In conclusion, this meta-analysis of 31,300 cases and 34,331 controls of BC suggested that caspase-10 (1228G>A) polymorphism was not associated with the disease. As there was very little study on this polymorphism, the evidence remains limited.

Therefore, there is a need of larger case-control studies of different ethnic groups along with other confounding factors in order to have conclusive result.

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Author Contributions

SC, BMK performed the inclusion of database searches, reviewed and selected eligible literature. SC, BMK and DRM analysed the data and performed the statistical analysis. SKM and SC are responsible for the concept/reagents/materials/analysis tools. SKM, SC wrote the manuscript.
Funding

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Availability of Data and Materials

All the authors confirmed the availability of data and materials.

Conflict of Interest

Authors have declared no conflict of interest.

Consent for Publication

Not applicable.

Ethics Approval

Not applicable.

References