Absence of the CHEK2 c.1100delC mutation in familial breast and ovarian cancer in Colombia: a case-control study [version 1; referees: awaiting peer review]

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Abstract

Background: BRCA1 and BRCA2 have been identified as high-penetrance breast cancer predisposition genes, but they only account for a small fraction of the inherited component of breast cancer. To explain the remaining cases, a polygenic model with a large number of low- to moderate-penetrance genes have been proposed; one of these, is the CHEK2 gene (Checkpoint Kinase 2). The objective of this study was to determine the role of the CHEK2 gene, specifically the c.1100delC mutation in familial breast cancer susceptibility in Colombian patients.

Methods: We screened 131 high-risk breast and/or ovarian cancer patients (negative for mutations in BRCA1 and BRCA2) and 131 controls for the germline mutation CHEK2 c.1100delC by allele-specific PCR.

Results: None of the cases or controls showed the CHEK2 c.1100delC mutation, neither as a homozygote nor as a heterozygote.

Conclusions: Our results suggest that the CHEK2 c.1100delC mutation is not a risk factor for genetic susceptibility to familial breast or ovarian cancer in the Colombian population. The absence of the CHEK2 c.1100delC mutation in our population show the importance of considering ethnic background before offering a genetic test.

Keywords

CHEK2, familial breast and ovarian cancer, Colombia, CHEK2 c.1100delC, moderate-penetrance
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Introduction

Breast cancer (BC) is the most common type of cancer among women, and in Colombia, it is the main cause of death by cancer in women. Of all cases of BC, approximately 5–10% have a strong inherited component, of which 25% is explained by germline mutations in the genes BRCA1 and BRCA2. To explain the BRCA1/2-negative cases, a polygenic model in which a large number of low- to moderate-penetrance genes as collectively responsible for the disease has been proposed.

CHEK2 has been proposed as a moderate penetrance BC susceptibility gene. This gene controls cell cycle and apoptosis and is activated in response to DNA double-strand breakage. Several mutations in the CHEK2 gene have been found, being the CHEK2 c.1100delC mutation the most studied; this is a truncating mutation in exon 10 that abolishes kinase activity of the protein. The role of this mutation in breast cancer was confirmed by Meijers-Heijboer et al., and in several other studies. The mutation CHEK2 c.1100delC was identified in approximately 5% of families with BC that did not have mutations in either BRCA1 or BRCA2 and was estimated to confer moderate risk (20–25%) of developing breast cancer for female mutation carriers.

Data on the contribution of moderate- or low-penetrance alleles to BC in South American populations are scarce. In this study, using a case-control design, we studied the CHEK2 c.1100delC mutation in order to investigate the potential influence of this variant on familial Breast and Ovarian Cancer (BOC) susceptibility in a Colombian cohort.

Materials and Methods

This was a case-control study conducted from 2009 to 2013 with 131 cases and 131 controls, which was carried out to determine the association between the CHEK2 c.1100delC mutation and increased risk of developing breast cancer. This study was approved by the Ethical Board of the School of Medicine of the University of Valle. Informed consent was obtained from all the participants.

Population Study

Cases

Our study cohort was composed of 131 Colombian familial breast/ovarian cancer (BOC) cases (BRCA1/BRCA2 negative). The patients were selected from the files of different health/Cancer centers after obtaining permission from each Center to participate in this study. These centers were located in the cities of Cali (Hospital Universitario del Valle, FUNCANCER, Clínica Rafael Uribe Uribe, Hematooncologos), Armenia (Oncólogos de Occidente), Cartagena (Fundación Hospital Infantil Napoléon Franco Pareja, Fundación mujeres por tus senos) and Bucaramanga (Insustny Oncologia e Investigación). The selected cases met, at least, one of the following criteria: at least three family members with breast or ovarian cancer at any age; two first degree family members affected, at least one, with breast cancer before 41 years of age or with ovarian cancer at any age; one breast cancer case diagnosed before 35 years or less; one ovarian cancer case diagnosed before age 31. For all index cases, breast and ovarian cancers were verified by the original pathology report. After the selection of the patients based in the inclusion criteria, they were asked to give a blood sample collected by a nurse or technician from each health/Cancer center and then sent to our laboratory.

Controls

The sample of healthy Colombian controls (n=131) consisted of unrelated individuals, with no personal or familial history of cancer, these individuals were interviewed and informed as to the aims of the study and who gave their informed written consent for anonymous testing. This control cohort was recruited in the same cities where the study cases were collected and also matched by age, sex and socioeconomic strata.

Genotyping

Genomic DNA was extracted from the blood samples of 131 the BC cases and the 131 healthy controls, for both groups the blood samples were collected specifically for this study. Samples were analyzed using allele-specific PCR (ASPCR) to detect the presence of CHEK2 c.1100delC, using the primers reported by Rashid et al. The products of the ASPCR were visualized by electrophoresis on an 8% polyacrylamide gel. The expected results were a band of 200 base pairs (bp) for the deletion of cytosine 1100 or a band 534 bp when the deletion is not present; if the sample showed both bands, it meant the presence of a heterozygote.

Results

A total of 262 samples (131 study cases and 131 matched-controls) were analyzed for the presence of the CHEK2 c.1100delC mutation. The clinical characteristics of the families included in this study are listed in Table 1 (Also see Dataset 1). After allele-specific PCR analysis, none of the cases or controls showed the CHEK2 c.1100delC mutation. All the samples showed only the heavier band (534bp) (Supplementary Figure 1). This indicates that the all the samples were homozygotes for the normal allele.

Discussion

This is the first study conducted in the Colombian population for CHEK2 c.1100delC mutation. Our results have shown that none of the 262 analyzed samples carried the CHEK2 c.1100delC mutation, suggesting that the frequency of this mutation is extremely low (or not present) in the Colombian population.

Similar results have been reported for populations in other South American countries. Although studies are scarce, in the few populations that have been evaluated for the presence of this variant, it has not been found in Chile and Mexico, or has been found at very low frequencies in Brazilian population.

Worldwide, CHEK2 c.1100delC is absent in Spain and all Asian populations studied to date, including those in India, Japan, China, Korea, Singapore, the Philippines, Pakistan, and Malaysia. The mutation is present in populations of Galicia.
Table 1. Distribution of breast and/or ovarian cancer cases included in this study according to inclusion criteria.

<table>
<thead>
<tr>
<th>Selection Criteria</th>
<th>Family n (%)</th>
</tr>
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<tbody>
<tr>
<td>$\geq 3$ family members with breast cancer, at least one with ovarian cancer</td>
<td>13 (9.9%)</td>
</tr>
<tr>
<td>$\geq 3$ family members with breast cancer</td>
<td>37 (28.2%)</td>
</tr>
<tr>
<td>2 family members with breast cancer, at least one with ovarian cancer</td>
<td>9 (6.9%)</td>
</tr>
<tr>
<td>2 family members with breast cancer</td>
<td>36 (27.5%)</td>
</tr>
<tr>
<td>1 family member with breast cancer and at least one with ovarian cancer</td>
<td>12 (9.2%)</td>
</tr>
<tr>
<td>Single affected individual with breast cancer $\leq$ age 35</td>
<td>21 (16.0%)</td>
</tr>
<tr>
<td>Single affected individual with Ovarian cancer $\leq$ age 31</td>
<td>3 (2.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>131 (100%)</strong></td>
</tr>
</tbody>
</table>

(northwest Spain)$^{16-41}$, 1.4% of the Northern European countries including Finland, the UK and the Netherlands$^{8,11,42}$ and the United States$^{43-45}$.

An explanation for this variability, proposes CHEK2 c.1100delC as an allele whose frequency is distributed along a population-gradient, which would have originated in populations of Northern Europe (with high frequencies) and decreasing towards the regions of Southern Europe (Basque Country, Spain and Italy)$^{39}$. This may explain the absence of the allele in the Colombian population, which is a mixture, in different proportions, of European-Spanish, African and Native American ancestry$^{46}$. Hence, the probability that the allele would have reached our population is low. As reported in several studies, it is evident that the contribution of CHEK2 c.1100delC mutation to the burden of cancer varies according to the ethnic group, and from country to country$^{47}$.

We found that the CHEK2 c.1100delC mutation is not present or is present at an extremely low frequency in familial BOC cases and controls in our Colombian cohort.

Based on our findings, we suggest that genotyping of the CHEK2 c.1100delC mutation in genetic testing for breast cancer susceptibility in the Colombian population should not be recommended. However, further studies are required to confirm the contribution of this variant in the Colombian population.

The study of CHEK2 mutations in the Latin American population has been focused mainly in the c.1100delC mutation. However, databases like ExAC (Exome Aggregation Consortium) showed the presence of other germline mutations in the CHEK2 gene in Latin American samples that could generate cancer susceptibility$^{48}$. Accordingly, it would be important to examine other mutations in the Colombian population and its association with the development of familial BOC.

Data availability
Dataset 1: Raw data for “Genotype and type of cancer present in the studied patients and their families” 10.5256/f1000research.13368.d207084

Competing interests
The authors declare no conflicts of interest.

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Supplementary Material
Supplementary Figure 1: Allele specific PCR products for the CHEK2 c.1100delC mutation.

Polyacrylamide gel electrophoresis at 8% (29:1 acrylamide:bisacrylamide) of allele specific PCR products for the CHEK2 c.1100delC variant for cases and controls. Lanes 1 - 5 show the amplified product of Colombian patients with breast and/or familial ovarian cancer; lanes 7-11 show the amplified product of healthy controls; Lane 6: molecular weight marker of 100 base pairs. all individuals show an amplified product of 537 bp which corresponds to the normal genotype. The letters “p.b.” in the picture are the spanish initials for base pairs (“pares de bases”).

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