# Analysis of BRCA1 large genomic rearrangements to identify individuals at risk of breast cancer

**Noemí García-Magallanes**
Universidad Politecnica de Sinaloa
**Alejandra Paola Martínez-Camberos**
Universidad Autonoma de Sinaloa Facultad de Ciencias Quimico Biologicas
**Emir Adolfo Leal-León**
Universidad Autonoma de Sinaloa Facultad de Ciencias Quimico Biologicas
**Verónica Judith Picos-Cárdenas**
Universidad Autonoma de Sinaloa Facultad de Medicina
**Enrique Johathan Romo-Martínez**
Universidad Politecnica de Sinaloa
**Fred Luque-Ortega**
Instituto Mexicano del Seguro Social
**Edith Eunice García-Alvarez**
Instituto Mexicano del Seguro Social
**María del Pilar Barbosa-Jasso**
Universidad Autonoma de Occidente
**José Alfredo Contreras-Gutierrez**
Hospital Civil de Culiacan
**Eliakym Arámbula Meraz** ( eliakymarambula@hotmail.com )
Universidad Autonoma de Sinaloa https://orcid.org/0000-0003-1026-7430

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# Abstract

Background. Breast cancer (BC) is a malignant neoplasm in which genetic and environmental aspects contribute to its development. In many cases, pathogenic mutations in BRCA1 are caused by large genomic rearrangements such as copy number variations, a mechanism of gene inactivation that increases the risk of BC. Therefore, identifying women at high risk of BC through genetic testing is of great importance. This study aimed to identify large BRCA1 rearrangements in patients with early-onset or family history of BC.

Methods. Peripheral blood from patients with (n= 38) and without (n= 30) BC was analyzed using the multiplex ligation-dependent probe amplification assay to detect large genomic rearrangements in BRCA1.

Results. One patient with sporadic BC showed an ambiguous deletion in exon 12 of the BRCA1 gene. To validate this result, full sequencing of this region was performed. The mutation detected by MLPA resulted in a false positive, showing that large genomic rearrangements in BRCA1 were absent in all subjects. Moreover, in this same patient, we detected the presence of c.4308T>C (rs1060915) polymorphism.

Conclusions. In our patients with BC, large genomic rearrangements in BRCA1 are held unaccountable for the development of the disease. We identified the presence of single nucleotide polymorphism, c.4308T>C (rs1060915), in a patient with sporadic BC.


# Background

Breast cancer (BC) is the most common malignant neoplasia in women worldwide. Environment and genetic background play important roles in the development of this disease and as a result, for the vast majority of women is not possible to identify a specific cause [1]. Approximately 5-10% of BC is identified of hereditary origin, with most mutations occurring in the tumor-suppressor gene BRCA1 (MIM#113705). Mutations in this gene also confer an increased risk in other types of cancer such as ovarian, fallopian tube, colon, prostate, male breast and pancreatic [2]; however, these alterations are mainly identified in individuals with family history of breast cancer, ovarian cancer, bilateral BC and early-onset BC [3, 4]; moreover, BC phenotypes such as medullary carcinoma and triple-negative (estrogen receptor, progesterone receptor and Her2/neu negative) have also been related with BRCA1 variations in 18% of patients under 50 years of age [3].

A selective group of these variations is known as large genomic rearrangements (LGRs). LGRs are responsible for as much as 23% of all identified BRCA1 mutations [5]. A type of LGR is copy number variation (CNV), occurs when a segment of DNA, 1 kb or longer, repeat at a different number in comparison with the reference genome [6]. Individuals that present pathogenic LGRs in BRCA1 (0-27%) have an estimated lifetime risk of 60% for BC, 59% for ovarian cancer and 83% for contralateral BC [7, 8].

It is well known that patients who receive treatment in an early stage of BC have higher survival rates. The identification of potentially pathogenic mutations in BRCA1 would allow taking clinical actions and preventive measurements such as prophylactic mastectomy, in the patient himself and his relatives [3]. Therefore, identifying women who are at high risk of BC through genetic testing has a very important implication. This study aimed to identify BRCA1 rearrangements in patients with early-onset or family history of breast cancer.

# Methods

*Study groups*

A cross-sectional, observational and comparative study was carried out to evaluate the association between copy number variations (CNV) in the BRCA1 gene and the risk of BC earlier in life, a total of 68 female patients were recruited from the Mexican Institute of Social Security (Instituto Mexicano del Seguro Social, IMSS) in Mazatlán and the Sinaloa Cancerology Institute (Instituto Sinaloense de Cancerología) in Culiacan, from August 2016 to September 2017. Venous blood was withdrawn from all participants who reviewed and signed the informed consent. Interviews were conducted to obtain information about their gynecological and obstetric parameters, personal and family history of cancer, and exposure to other risk factors associated with BC. Additional information was also obtained from the hospital databases. This research was approved by the Ethical Committees of the Mexican Institute of Social Security, the Sinaloa Institute of Cancerology and the Biomedical Science Program of the School of Chemistry and Biological Sciences at the Autonomous University of Sinaloa (Universidad Autónoma de Sinaloa).

The study population was divided into three groups, hereditary BC (HBC, n=18), sporadic BC (SBC, n=20) and healthy patients (HP, n=30). The groups included women from 18 to 50 years of age. In the case of BC patients (HBC and SBC), all patients were 50 years old or younger at the time of initial diagnosis, with histopathological confirmation, and for the specific case of HBC, meeting at least one of the criteria listed in the NOM-041- SSA2-2011 [9].

*Multiplex ligation-dependent probe amplification assay*

Genomic DNA was isolated from blood samples with the Gustincich method [10]. The DNA concentration was evaluated by the A260/A280 absorbance ratio with a Nanodrop-1000™ (NanoDrop Technologies) spectrophotometer. BRCA1 CNV was quantified by multiplex ligation-dependent probe amplification (MLPA) using the P002-D1 probe mix assay according to instructions provided by the manufacturer (MRC Holland). Amplified products were separated using an ABI PRISM 310 Genetic Analyzer™ (Applied Biosystems) and interpreted using the GeneMapper Software v4.0 (Applied-Biosystems) and the Coffalyser.net Software v14.0 (MRC Holland). Healthy patient data was used as a control.

*PCR and gene sequencing*

Specific primers for Exon 12 (forward 5´-AGGTGATTTCAATTCCTGTGCT-3´ and reverse 5´-GGCTCCATAATTACCCATGTGC-3´) were designed to confirm the predicted deletion detected from MLPA data. These primers can amplify the whole exon and MLPA probe hybridization site. To locate the deletion site as well as to confirm the data from MLPA analysis, resultant PCR products were subjected to direct sequencing.

*Statistical analyses*

To determine the statistical association between the variables and BC risk, we used analysis of variance. Chi-square, odds ratios (OR) and 95% confidence intervals (CI) were also estimated.

# Results

A total of 38 unrelated women were diagnosed with BC, including women with HBC and SBC, this represented a 55.88% of the total study population (Table 1). The onset age for tumor development in the HBC group was 45.6±8.4 and in the SBC group was 43.7±5.9. The status of estrogen receptor (ER), progesterone receptor (PR) and HER2 revealed that 18.42% of tumors were triple-negative (TN) and 42.86% of it were HBC.

The family history of BC among women suffering from this disease is very significant ($p < 0.001$) when compared with the HP group. 39.47% of women with BC (HBC and SBC) had a family history of BC whereas in healthy patients was inexistent. As shown in Table 1, most HBC patients presented family history of BC (77.78%), 27.78% of them had previous cancer [$p < 0.001$, OR 1.90 (CI 1.50-2.41)], 77.78% reported positive family history of BC [$p < 0.001$, OR 2.30 (CI 1.69-3.13)], having as many as 4 relatives with this disease, and 11.11% with 1 or 2 relatives with prostate cancer ($p = 0.190$).

We performed MLPA to analyze the presence of CNV in BRCA1. Results showed an ambiguous deletion (rate 0.49) in exon 12 that is distinguished when observing the different peak amplitudes of MLPA products (Figure 1). To further confirm this deletion, exon 12 was fully sequenced and compared with the reference sequence of BRCA1 (NG_005905) searching for coincidences in the basic local alignment search tool (BLAST) published by the National Centre for Biotechnology Information. Results showed no rearrangement in exon 12 and indicated that the MLPA probe hybridization site was intact. Furthermore, the sequencing of the amplified product revealed a single nucleotide polymorphism, c.4308T>C (rs1060915) (Figure 2).

# Discussion

In México, few studies have investigated the contribution of BRCA1 mutations to BC. To our knowledge, there are no published studies about the prevalence of CNV in BRCA1 in women from Sinaloa. With our research, we contributed to clarifying this situation.

In our study, only women diagnosed with BC presented a family history of cancer (breast, ovary, colon, pancreas and/or prostate cancer) related to mutations in BRCA1, providing further evidence about the importance of genetics as a risk factor for this pathology [11].

Large exon rearrangements have been investigated for more than a decade with unclear results; at the beginning, usage of conventional PCR yielded variable results due to the amplification of wild-type alleles in heterozygous cases [12]. Currently, new techniques such as MLPA are used to solve this problem, however, attention is required to recognize ambiguous data and exon deletions. Moreover, single nucleotide polymorphisms (SNPs) may also affect the correct amplification, leading to false apparent deletion in a single exon [13]. Thus, the results always need to be validated by another method.

Even though MLPA analysis did not show CNV in the BRCA1 gene, a larger study is necessary before its contribution in hereditary BC could be called null. Nowadays, more than 100 rearrangements have been described for BRCA1 [14]. In the case of the Hispanic population, the prevalence of CNV is over 10% and is mainly caused by a founder deletion of exons 9-12 [15]. When a pathogenic mutation is identified, preventive measures, such as prophylactic mastectomy, can be taken to reduce the risk of BC [4].

We detected an SNP (rs1060915), which is most prevalent in the South Asian population (alternative allele G=0.50), but also detected in lower frequency in American (G=0.36) [16]. This polymorphism acts as a benign mutation in women with BC, being a silent mutation that does not cause changes in the encoded amino acid (S1436S). The rs1060915 polymorphism is reported as a part of a common haplotype in BRCA1 [17, 18, 19], but its significance remains unclear.

# Conclusions

In this study, CNV in BRCA1 was absent in all studied subjects, we recommend a large study sample to reveal novel or reported genomic rearrangements among Mexican mestizo population. We identified the presence of rs1060915 polymorphism by direct sequencing a sample corresponding to a patient with sporadic BC.

BRCA1 mutations are also responsible for other malignant neoplasms (ovary, colon, pancreas, and prostate) among the young population, thus it is important to consider this hereditary background in the selection of risk patients.

# Declarations

- Ethics approval and consent to participate

This research is carried out under the agreements of the Declaration of Helsinki and was approved by the Ethical Committees of the Mexican Institute of Social Security, the Sinaloa Institute of Cancerology and the Biomedical Science Program of the School of Chemistry and Biological Sciences at the Autonomous University of Sinaloa (Universidad Autónoma de Sinaloa).

All participants reviewed and signed the informed consent.

- Consent for publication

Not applicable

- Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to the privacy policies of the health institutions involved but are available from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests

- Funding

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- Authors' contributions

NGM and APMC equal contribution in the Conceptualization, Investigation, Methodology, Formal analysis, Writing – Review & Editing the manuscript. EALL was a major contribution in Review and Editing the manuscript. VJPC, EJRM, FLO and MBJ supporting in Revision the manuscript. EEGA and JACG collaborate in provision of patients. EAM Supervision, ensuring that all listed authors have approved the manuscript before submission and that all authors receive the submission and all substantive correspondence with editors.

All authors read and approved the final manuscript.

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- Authors' information (optional)

Not applicable

# Abbreviations

BC. Breast cancer; CI. Confidence intervals; CNV. Copy number variation; ER. Estrogen receptor; HP. Healthy patients; LGRs. Large genomic rearrangements; MLPA. Multiplex ligation-dependent probe amplification; PR. Progesterone receptor; SBC. Sporadic breast cancer; SNPs. Single nucleotide polymorphisms; TN. Triple-negative

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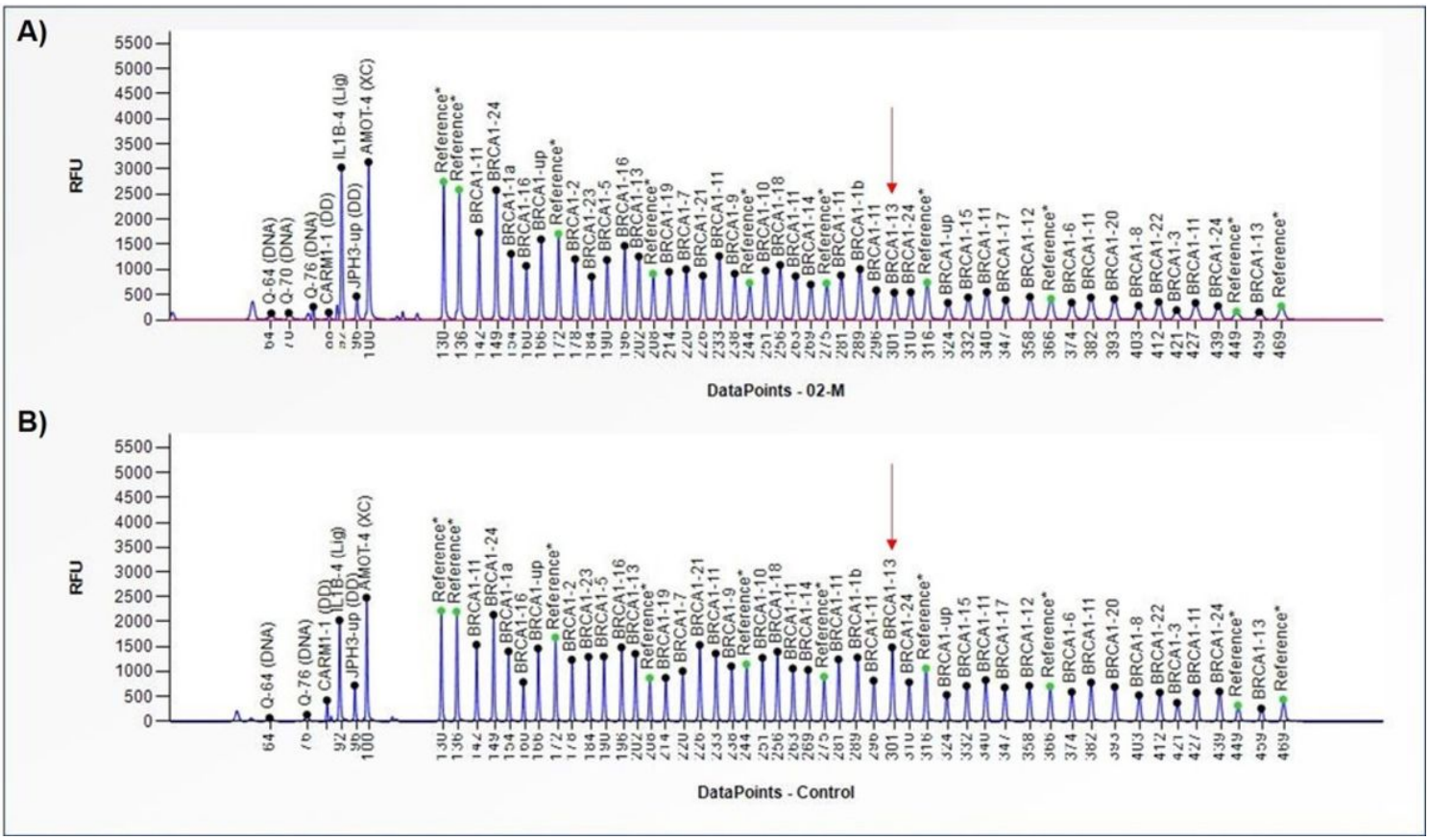
# Table

Table 1. Characteristics of the study population

| Characteristic | HBC n (%) | SBC n (%) | NS n (%) | Total n (%) | *P value* |
|---|---|---|---|---|---|
| Number | 18 (26.47) | 20 (29.41) | 30 (44.11) | 68 (100) | |
| Age at diagnosis (mean ± SD) | 45.6 ± 8.4 | 43.7 ± 5.9 | 42.4 ± 5.7 | 42.0 ± 6.2 | 0.323 |
| **High risk features** | | | | | |
| Previous cancer | 5 (27.78) | 0 (0) | - | 5 (7.35) | 0.001* |
| Bilateral BC | 2 (11.11) | 2 (10.00) | - | 4 (10.53) | |
| Family history BC: 1st-3rd degree (%) | 14 (77.78) | 1 (5.00) | 0 (0) | 15 (22.06) | 0.001* |
| Family history OC: 1st-3rd degree (%) | 1 (5.56) | 0 (0) | 0 (0) | 1 (1.47) | - |
| Family history PC: 1st-3rd degree (%) | 2 (11.11) | 1 (5.00) | 0 (0) | 3 (4,41) | 0.190 |
| **Tumor type (%)** | | | | | |
| DC | 10 (55.56) | 15 (75.00) | - | 25 (65.79) | 1.000 |
| LC | 1 (5.56) | 1 (5.00) | - | 2 (5.26) | 1.000 |
| DC-LC | 2 (11.11) | 2 (10.00) | - | 4 (10.53) | 0.075 |
| Other | 5 (27.78) | 2 (10.00) | - | 7 (18.42) | 0.034* |
| **Molecular subtype** | | | | | |
| Luminal | 10 (55.56) | 15 (75.00 | - | 25 (65.79) | 0.420 |
| Her-2 | 1 (5.56) | 1 (5.00) | - | 2 (5.26) | 0.863 |
| Triple negative | 3 (16.67) | 4 (20.00) | - | 7 (18.42) | 0.436 |

BC, Breast cancer; HBC, Hereditary BC; SBC, Sporadic BC; NS, Normal subject; BC, Breast cancer; OC, Ovarian cancer; PC, Prostate cancer; DC, Ductal carcinoma; LC, Lobulillar carcinoma. *Statistical significance by ANOVA Test.

# Figures




## Figure 1

CNV results of BRCA1 exon 13. Electropherogram of a test sample (A) is compared to that of a reference sample (B) showing a decrease in one probe of the test sample (red arrows). The numbering of exons used is the traditional exon numbering (exons 1a, 2, 3 and 5-24) in which exon 4 is not present, which differs from NCG NG_005905.2.

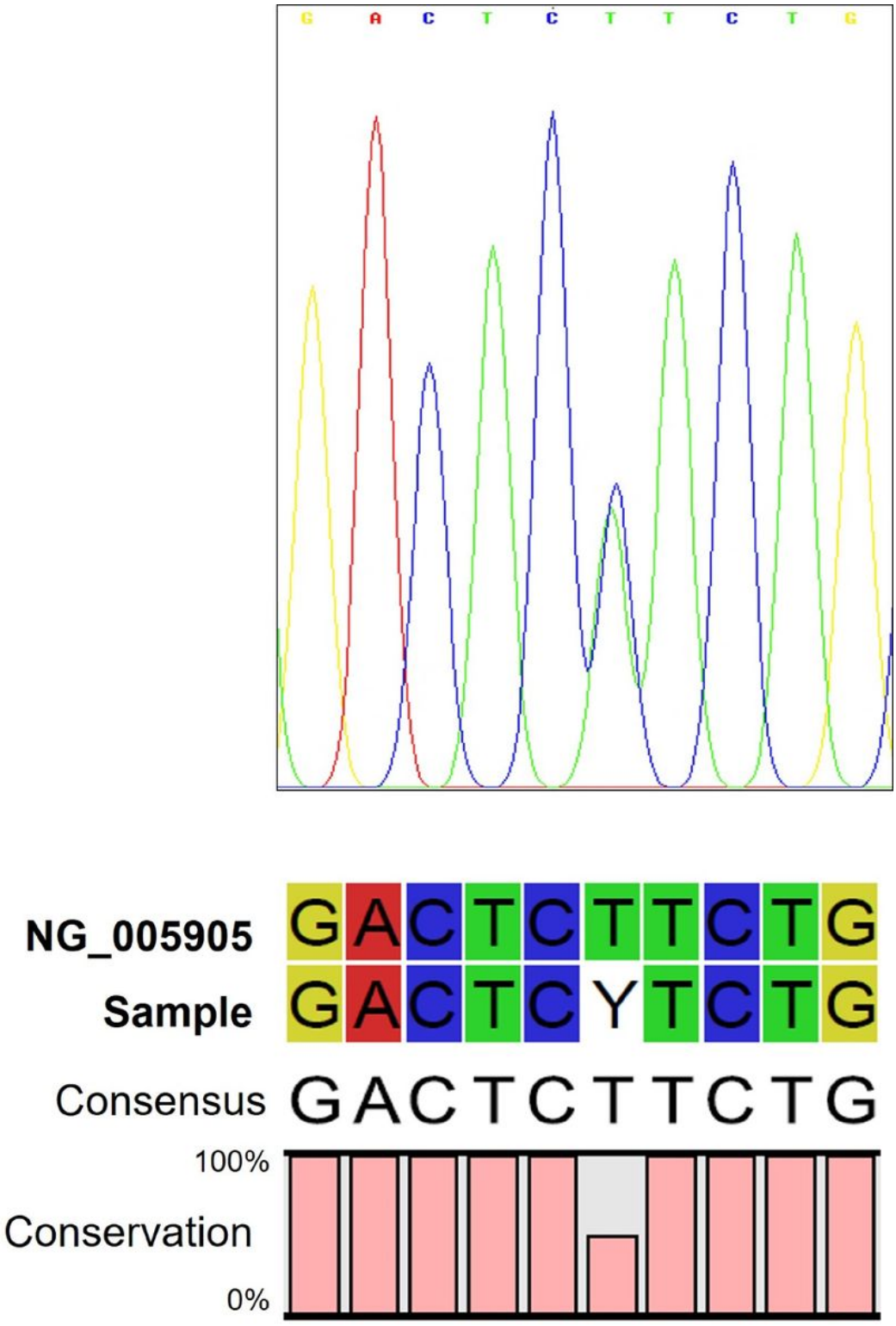



**Figure 2**

Single nucleotide polymorphism (SNP) c.4308T>C in exon 12 of BRCA1. SNP (Y) detected in a patient with sporadic breast cancer.