

## ORIGINAL RESEARCH ARTICLE

# Examining *BRCA1* and *BRCA2* Mutations in Gastric Cancer Using Next Generation Sequencing: Relevance for Diagnosis and Treatment Strategies

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**Abstract:** Gastric cancer (GC) presents a significant global health dilemma, being the fifth most widespread cancer on a global scale and the third major contributor to cancer-related deaths. Given the intricacies of this malignancy, there is an urgent need for a thorough investigation into its molecular basis. In this study, we investigated the role of *BRCA1* and *BRCA2* mutations in Pakistani GC patients and their functional implications. Using Next-Generation Sequencing (NGS), we analyzed *BRCA1/2* genes in a cohort of 31 Pakistani GC patients, revealing pathogenic mutations not previously documented in this context. Expression analyses of *BRCA1* and *BRCA2* genes at both mRNA and protein levels uncovered a consistent down-regulation in GC samples with pathogenic mutations. This down-regulation extends beyond the canonical role of *BRCA1/2* in DNA repair, suggesting a broader impact on cellular processes. The clinical significance of these findings lies in the potential of these pathogenic mutations as biomarkers for GC diagnosis and treatment. Furthermore, our study explored potential drugs from the DrugBank database capable of up-regulating *BRCA1/2* gene expression in GC treatment. Pathway analysis revealed the involvement of *BRCA1/2* genes in diverse pathways, emphasizing their relevance in GC progression. Despite limitations, including a relatively small sample size, this study sheds light on the molecular intricacies of GC and offers promising avenues for further research and personalized therapeutic approaches. In conclusion, our study unveils the presence of pathogenic *BRCA1* and *BRCA2* mutations in Pakistani GC patients, shedding light on their potential as biomarkers and therapeutic targets. These findings offer promise for enhancing the management and treatment outcomes of this malignancy.

**Keywords:** Gastric cancer; *BRCA1*; *BRCA2*; Mutations; Next Generation Sequencing

## 1. Introduction

Gastric cancer (GC), also known as stomach cancer, is a formidable global health concern with a significant impact on morbidity and mortality<sup>[1,2]</sup>. It ranks as the fifth most common cancer and the third leading cause of cancer-related deaths worldwide, making it imperative to delve into the molecular underpinnings of this malignancy<sup>[3,4]</sup>. Recent advances in genomic research have revolutionized our understanding of cancer, including GC, by shedding light on the genetic alterations

that drive tumorigenesis and influence therapeutic responses<sup>[5,6]</sup>. One such avenue of investigation focuses on mutations in the *BRCA1* and *BRCA2* genes, traditionally associated with hereditary breast and ovarian cancer but increasingly recognized for their relevance in various cancer types, including GC<sup>[7,8]</sup>.

GC is a heterogeneous disease with various histological subtypes, including intestinal, diffuse, and mixed types<sup>[9,10]</sup>. The etiology of GC is multifactorial, involving an intricate interplay between genetic and environmental factors. While environmental factors such as diet, smoking, and *Helicobacter pylori* infection have been extensively studied in the context of gastric carcinogenesis, genetic factors are increasingly recognized as crucial contributors to the development of this malignancy<sup>[11,12]</sup>. While somatic mutations in key driver genes like TP53, APC, and CDH1 have been well-documented in GC, the role of *BRCA1* and *BRCA2* mutations in gastric tumorigenesis has garnered increasing attention<sup>[8,13,14]</sup>. Mutations in these genes can lead to impaired DNA repair mechanisms, genome instability, and an increased susceptibility to cancer development.

The prevalence of *BRCA1* and *BRCA2* mutations in GC varies among different populations and geographic regions<sup>[15,16]</sup>. Studies have reported a wide range of mutation frequencies, suggesting that the contribution of *BRCA1* and *BRCA2* to GC susceptibility is complex and may be influenced by genetic and environmental factors<sup>[17,18]</sup>. Understanding the prevalence and functional consequences of these mutations is crucial for tailoring personalized treatment strategies and identifying individuals who may benefit from targeted therapies.

This comprehensive research study aims to investigate the prevalence, spectrum, and clinical implications of *BRCA1* and *BRCA2* mutations in GC patients. Using advanced Next-Generation Sequencing (NGS) technology, we conducted mutational analysis of these genes in a cohort of GC cases. By characterizing the mutational landscape of *BRCA1* and *BRCA2* in GC, we seek to elucidate their potential roles as molecular biomarkers and therapeutic targets.

## 2. Methodology

### 2.1. Sample collection and ethical approval

In our study, a total of 31 GC tissue samples were meticulously collected from patients undergoing surgical resection at the DHQ, Teaching Hospital, Dera Ismail Khan, KPK, a reputable healthcare facility in Pakistan. This collection of tissue specimens was undertaken in strict adherence to Helsinki ethical guidelines and protocols<sup>[19]</sup> to ensure the protection of patients' rights and privacy. Prior to the commencement of this research endeavor, ethical approval was diligently obtained from the Pakistan Agricultural Research Council (PARC), underscoring our commitment to conducting this study with the utmost integrity and respect for ethical standards.

The inclusion criteria for GC patients in this study encompassed individuals who had a confirmed diagnosis of GC, as histologically verified by biopsy reports, and were willing to participate in the research. Exclusion criteria comprised patients with a history of other malignancies, those undergoing treatment for GC, and individuals unable or unwilling to provide informed consent. Additionally, patients with incomplete medical records or insufficient tissue samples for genetic analysis were excluded from the study. Clinical information of the included patients is given in Table 1.

**Table 1.** An overview of GC patient's characteristics in the present study

| Sr.no | Characteristics | Sample count (n) |
|-------|-----------------|------------------|
| 1     | Sex             |                  |
|       | Male            | 25               |
|       | Female          | 06               |
| 2     | Age             |                  |
|       | >60             | 1                |
|       | <60             | 30               |
| 3     | Treatment       |                  |
|       | Pre-treatment   | 30               |
|       | Post-treatment  | 0                |

### 2.2. Nucleic acid extraction

In the present study, we employed a highly reliable kit-based method for DNA and RNA extraction. For



DNA extraction, GeneJET Genomic DNA Purification Kit (cat # K0721, Thermo Fisher) was used, while for RNA extraction, GeneJET Genomic RNA Purification Kit (cat # K0732, Thermo Fisher) kit was utilized. After the extraction of DNA and RNA, it was crucial to verify the genetic material's purity. To do so, the  $A_{260}/A_{280}$  absorbance ratio, measured at 260 nm and 280 nm, was employed as a criterion. Samples with an  $A_{260}/A_{280}$  ratio falling within the range of 1.8 to 2.0 were selected for subsequent downstream analyses.

### 2.3. Next generation sequencing (NGS)

The genomic DNA isolated was appropriately diluted to achieve the required concentration for polymerase chain reaction (PCR)-based library preparation. Targeted amplification of the coding regions and splicing sites of *BRCA1* (NM 007294) and *BRCA2* (NM 000059) genes was carried out using the AmpliSeq for Illumina BRCA Panel. The uniquely indexed libraries specific to the BRCA panel were then prepared following the guidelines outlined in the AmpliSeq for Illumina BRCA Panel Reference Guide. Subsequently, paired-end sequencing by synthesis was performed using the MiSeq sequencer from Illumina, located in San Diego, CA, USA.

The base quality and amplicon coverage of the raw sequencing reads were analyzed using the local run manager of the MiSeq sequencer. Following this, the cleaned reads, meeting the quality threshold of a phred score greater than 30, were aligned to the human reference genome hg19/GRCh37. This alignment was accomplished using the BWA-MEM Whole-Genome Aligner, version 0.7.9a-isis-1.0.2. Subsequently, mismatched calls were identified as mutations using the PISCES Variant Caller, version 5.2.9.23. These genetic mutations were then characterized and annotated with the assistance of the Illumina Annotation Engine, version 2.0.11-0-g7fb24a09.

### 2.4. Interpretation of the mutations

The interpretation of genetic mutations followed the guidelines established by the American College of Medical Genetics (ACMG) and the Association for

Molecular Pathology (AMP)<sup>[20]</sup>. To classify these mutations into categories such as pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, or benign, a combination of in-silico prediction tools and curated external databases was employed. In line with the results generated by in-silico prediction software, including SIFT (<http://sift.jcvi.org/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), and Mutation Taster (<http://www.mutationtaster.org/>), the functional impact of these variants on the *BRCA1* and *BRCA2* protein products was determined. Furthermore, the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) was consulted to assess the clinical significance of the respective mutations.

### 2.5. Frequencies of the observed *BRCA1/2* mutations in gnomAD database

The gnomAD database is a valuable resource in the realm of genomics and genetics<sup>[21]</sup>. It compiles and integrates a vast collection of genomic data from diverse populations, offering a comprehensive repository of genetic variation information. GnomAD encompasses data from both exome and whole-genome sequencing, making it a robust platform for the exploration of genetic variants, including single nucleotide polymorphisms (SNPs) and insertions/deletions (indels). Researchers and clinicians rely on gnomAD to assess the frequency and distribution of genetic variants in human populations, aiding in the interpretation of genomic findings in various fields, from rare disease diagnosis to population genetics. In this study, we utilized the gnomAD platform to assess the prevalence of the identified *BRCA1/2* mutations in Asian individuals diagnosed with GC.

### 2.6. Analysis of the observed *BRCA1/2* mutations in The Cancer Genome Atlas (TCGA)

The cBioPortal is a pivotal resource in the field of cancer research, providing a user-friendly platform for the exploration and analysis of large-scale cancer genomics datasets<sup>[22]</sup>. Researchers worldwide rely on cBioPortal to gain insights into the genomic alterations and molecular profiles of various cancer types. This

valuable tool offers interactive visualizations, enabling the study of genetic mutations, copy number variations, and gene expression patterns in the context of cancer. In the present study, cBioPortal database was utilized to check the presence of the observed *BRCA1/2* mutations in GC TCGA dataset.

## 2.7. RT-qPCR analysis

Initially, the synthesis of first-strand cDNA was carried out using the cDNA Synthesis SuperMix from TransGen Biotech Co., Ltd., following the manufacturer's recommended protocols. Subsequently, RT-qPCR was conducted using the TB Green® Premix EX Taq™ II (Takara Bio, Inc.) on an Applied Biosystems 7900 Real-Time PCR System (Thermo Fisher Scientific, Inc.). The RT-qPCR protocol involved an initial denaturation step at 95 °C for 30 seconds, followed by 40 cycles of denaturation at 95 °C for 5 seconds and annealing/extension at 55 °C for 34 seconds. A final extension step was performed at 72 °C for 5 minutes, followed by a 15-minute extension at 72 °C. The expression levels of RNA were determined using the comparative  $2^{-\Delta\Delta C_q}$  method<sup>[23]</sup>, with GAPDH expression as the internal RNA standard. The primers used for RT-qPCR are listed in Table 2. All reactions were performed in triplicate.

## 2.8. Receiver operator characteristic (ROC) curve

Using the RT-qPCR expression data, we constructed ROC curves for *BRCA1/2* expression levels utilizing the SRPLOT web resource available at <https://bioinformatics.com.cn/srplot>.

## 2.9. Immunohistochemistry (IHC) analysis

The tissue sections underwent deparaffinization, and for antigen retrieval, they were subjected to heat treatment in an EDTA (ethylenediaminetetraacetic acid) solution at pH 8.0. Protein expression levels of the mutated genes in GC tissue samples were evaluated using 4-μm-thick sections obtained from formalin-fixed, paraffin-embedded (FFPE) specimens. Monoclonal antibodies targeting *BRCA1* (EPR19433, abcam) and *BRCA2* (EPR23442-43, abcam) were utilized, and staining was carried out using the Ventana BenchMark

XT staining system (Roche, Tokyo, Japan). In this analysis, non-pathogenic mutated tissue samples were used as the comparative reference. The assessment of tumor positivity was determined by a pathologist based on the presence of nuclear staining in tumor tissue or negativity in the absence of nuclear staining. Protein expression was evaluated, taking into account staining intensity.

## 2.10. Gene enrichment analysis

Metascape is a powerful and user-friendly bioinformatics tool designed for functional enrichment analysis<sup>[24]</sup>. It streamlines the interpretation of large-scale omics data by identifying enriched biological processes, pathways, and molecular functions. Metascape leverages a comprehensive gene annotation database, making it an invaluable resource for researchers seeking to unravel the biological significance of their datasets. In the present study, we used Metascape tool to perform gene enrichment analysis of *BRCA1/2* genes.

# 3. Results

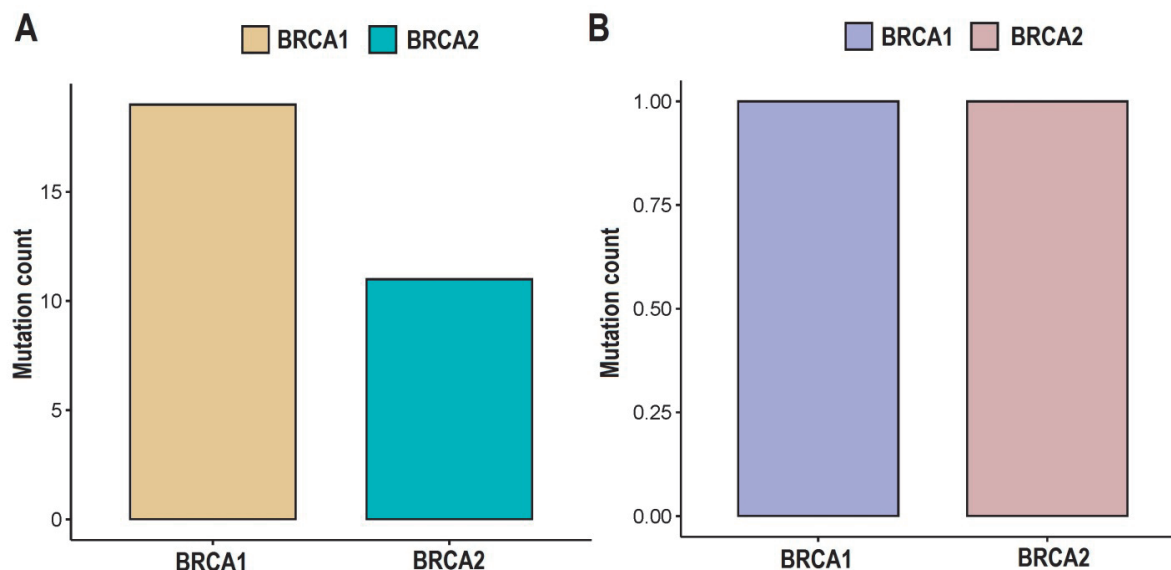
## 3.1. Next generation sequencing (NGS) and mutation detection

In this investigation, a cohort of 31 GC patients was studied, consisting of 25 males and 6 females (Table 1). The age of these individuals ranged from 19 to 62 years, with an average age of 37 years. Through comprehensive genetic analysis, we detected a total of 19 mutations in the *BRCA1* gene and 11 mutations in the *BRCA2* gene among the GC samples (Table 2). Notably, all of these identified mutations exhibited a high mutation quality score of 100. The sequencing data demonstrated an impressive coverage rate of 98.6%, and the average Quality score (Q30) reached a notable 98%. In-depth in silico analysis and interpretation based on ClinVar data revealed that within the *BRCA1* gene, there were 1 pathogenic mutation (5%) and 18 benign mutations (95%) among the subjects (Figure 1 and Table 2). Similarly, within the *BRCA2* gene, the analysis uncovered 1 pathogenic mutation (9%) and 10 benign mutations (91%) within the studied cohort (Figure 1 and Table 2).

**Table 2.** Incidence and categorization of mutations detected in the *BRCA1/2* genes among GC patients.

| Sr. no | Gene         | NM:c.DNA                     | Protein      | Nature (ClinVar) | Nature (In silico analysis) | No. patients |
|--------|--------------|------------------------------|--------------|------------------|-----------------------------|--------------|
| 1      | <i>BRCA1</i> | NM_007294.4:c.5467G>T        | p.Ala1823Ser | Pathogenic       | DC                          | 7            |
| 2      |              | NM_007294.4:c.5158A>G        | p.Thr1720Ala | Benign           | Non-DC                      | 15           |
| 3      |              | NM_007294.4:c.5117G>C        | p.Gly1706Ala | Benign           | Non-DC                      | 9            |
| 4      |              | NM_007294.4:c.5044G>A        | p.Glu1682Lys | Benign           | Non-DC                      | 9            |
| 5      |              | NM_007294.4:c.5024C>T        | p.Thr1675Ile | Benign           | Non-DC                      | 11           |
| 6      |              | NM_007294.4:c.4985T>C        | p.Phe1662Ser | Benign           | Non-DC                      | 10           |
| 7      |              | NM_007294.4:c.4956G>A        | p.Met1652Ile | Benign           | Non-DC                      | 14           |
| 8      |              | NM_007294.4:c.4955T>C        | p.Met1652Thr | Benign           | Non-DC                      | 19           |
| 9      |              | NM_007294.4:c.4913A>T        | p.Glu1638Val | Benign           | Non-DC                      | 14           |
| 10     |              | NM_007294.4:c.4910C>T        | p.Pro1637Leu | Benign           | Non-DC                      | 11           |
| 11     |              | NM_007294.4:c.4840C>T        | p.Pro1614Ser | Benign           | Non-DC                      | 4            |
| 12     |              | NM_007294.4:c.4837A>G        | p.Ser1613Gly | Benign           | Non-DC                      | 12           |
| 13     |              | NM_007294.4:c.4816A>G        | p.Lys1606Glu | Benign           | Non-DC                      | 14           |
| 14     |              | NM_007294.4:c.4729T>C        | p.Ser1577Pro | Benign           | Non-DC                      | 12           |
| 15     |              | NM_007294.4:c.4691T>C        | p.Leu1564Pro | Benign           | Non-DC                      | 17           |
| 16     |              | NM_007294.4:c.4682C>T        | p.Thr1561Ile | Benign           | Non-DC                      | 5            |
| 17     |              | NM_007294.4:c.4636G>T        | p.Asp1546Tyr | Benign           | Non-DC                      | 16           |
| 18     |              | NM_007294.4:c.4636G>A        | p.Asp1546Asn | Benign           | Non-DC                      | 13           |
| 19     |              | NM_007294.4:c.4600G>A        | p.Val1534Met | Benign           | Non-DC                      | 2            |
| 1      | <i>BRCA2</i> | NM_000059.4:c.275_276insCCAT | p.Gln92fs    | Pathogenic       | DC                          | 7            |
| 2      |              | NM_000059.4:c.1662T>G        | p.Cys554Trp  | Benign           | Non-DC                      | 18           |
| 3      |              | NM_000059.4:c.1744A>C        | p.Thr582Pro  | Benign           | Non-DC                      | 21           |
| 4      |              | NM_000059.4:c.1786G>C        | p.Asp596His  | Benign           | Non-DC                      | 12           |
| 5      |              | NM_000059.4:c.1792A>G        | p.Thr598Ala  | Benign           | Non-DC                      | 13           |
| 6      |              | NM_000059.4:c.1796C>T        | p.Ser599Phe  | Benign           | Non-DC                      | 11           |
| 7      |              | NM_000059.4:c.1798T>C        | p.Tyr600His  | Benign           | Non-DC                      | 9            |
| 8      |              | NM_000059.4:c.2350A>G        | p.Met784Val  | Benign           | Non-DC                      | 17           |
| 10     |              | NM_000059.4:c.2755G>A        | p.Glu919Lys  | Benign           | Non-DC                      | 12           |
| 11     |              | NM_000059.4:c.2927C>T        | p.Ser976Phe  | Benign           | Non-DC                      | 10           |

DC = Disease causing



**Figure 1.** A complete tally of detected mutations, both overall and pathogenic, within the *BRCA1/2* genes among GC patients through Whole Exome Sequencing (WES). (A) This subfigure represents the comprehensive count of identified mutations in *BRCA1/2* genes among GC patients, and (B) This subfigure B specifically focuses on the count of pathogenic mutations in *BRCA1/2* genes among GC patients.

Of particular interest is the observation that among the seven GC patients outlined in Table 1, we identified two pathogenic mutations, encompassing one mutations in *BRCA1* (p.Ala1823Ser) and other one in the *BRCA2* gene (p.Gln92fs). Given the direct association of pathogenic mutations with disease development, the subsequent phase of our investigation focused on scrutinizing the prevalence and functional implications of these pathogenic mutations among GC patients.

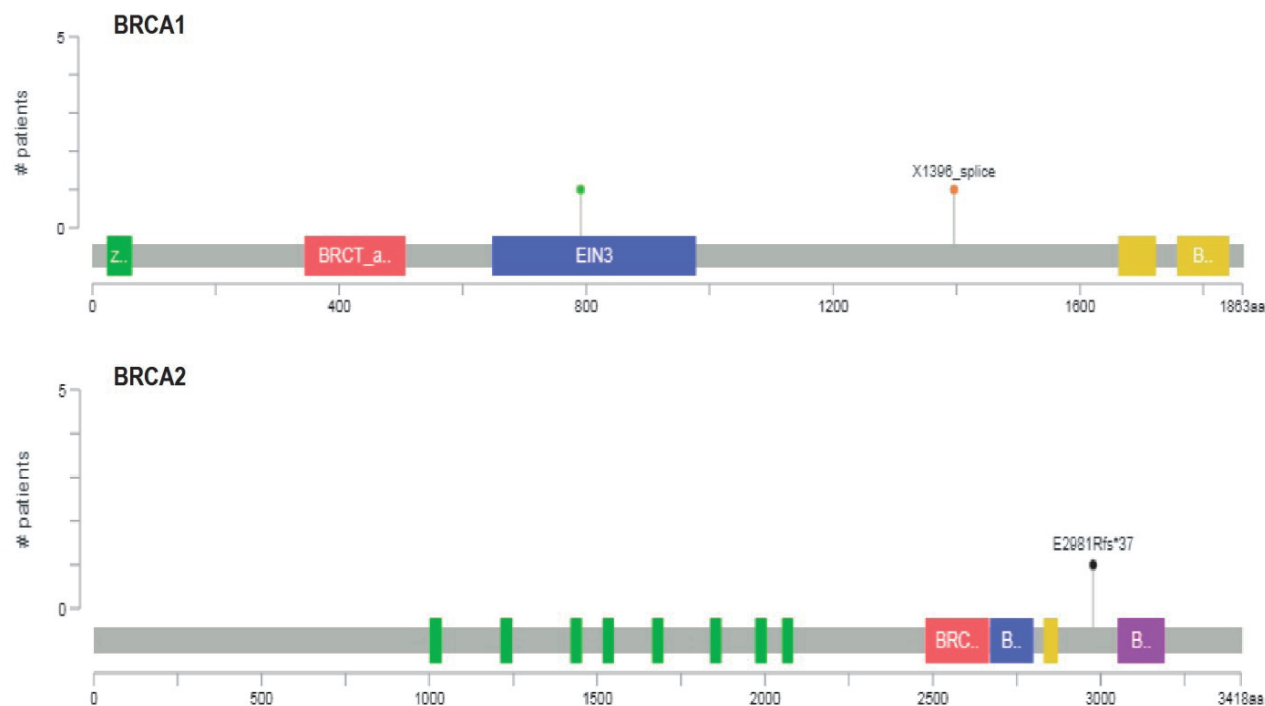
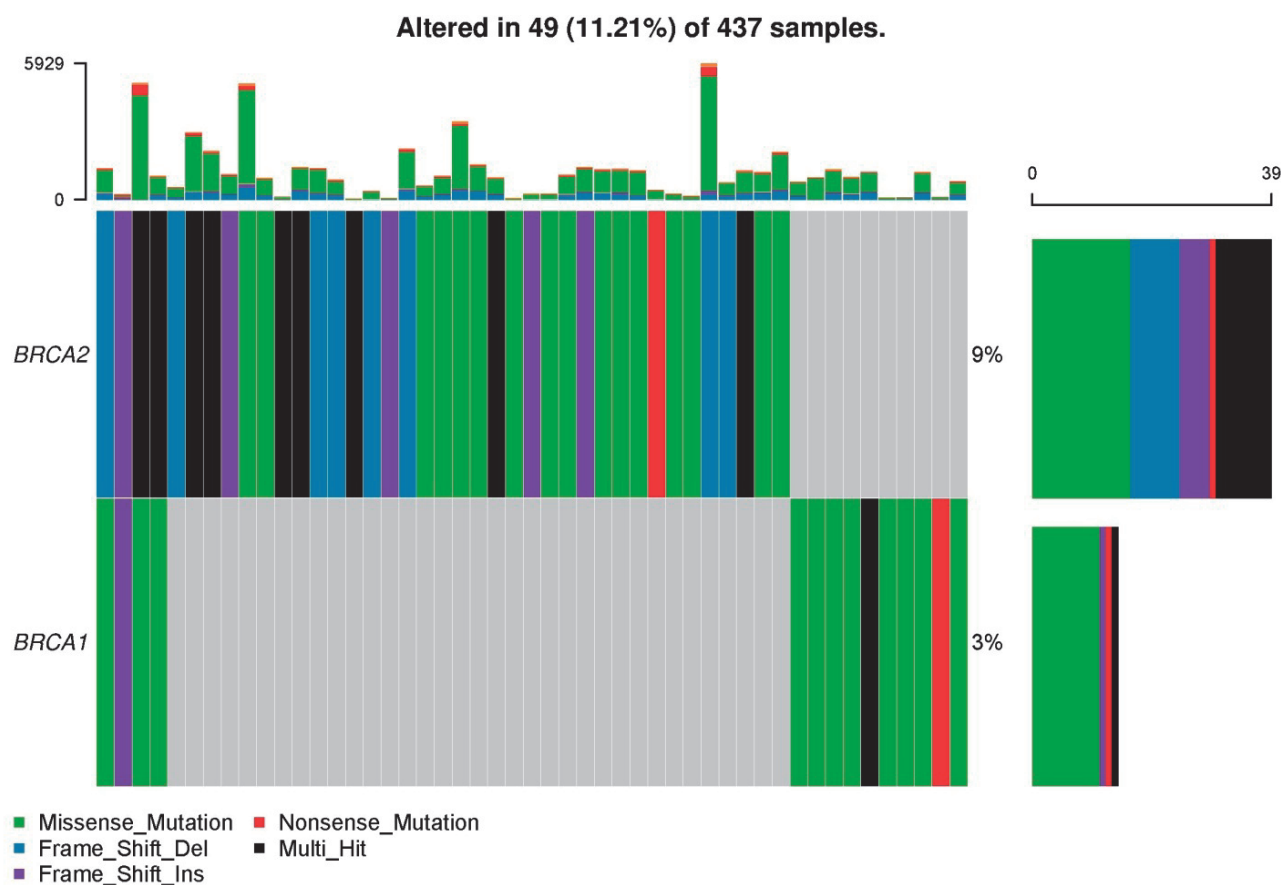
### 3.2. Frequencies of the observed *BRCA1/2* pathogenic mutations in gnomAD database

Examination of pathogenic mutations in the *BRCA1* gene (p.Ala1823Ser) and in the *BRCA2* gene (p.Gln92fs), as listed in Table 2, across the gnomAD database yields significant insights. Remarkably, these specific mutations have not been previously documented among Asian GC patients, consistently displaying a frequency of 0 in this database. This intriguing observation not only underscores the rarity of these mutations within the Asian GC population but also

suggests a distinctive prevalence pattern unique to the Pakistani population in the context of gastric GC.

### 3.3. Analysis of the observed *BRCA1/2* mutations in The Cancer Genome Atlas (TCGA)

In the subsequent phase of this study, an extensive mutational exploration of the *BRCA1/2* genes was conducted within GC samples obtained from the TCGA dataset, utilizing the cBioPortal platform. The primary objective was to identify potential genetic variations and assess their prevalence across TCGA dataset. The findings of this analysis revealed a distinct trend: the pathogenic mutations in *BRCA1* gene (p.Ala1823Ser) and in the *BRCA2* gene (p.Gln92fs) that were identified in GC patients of Pakistani origin were notably absent within the TCGA GC samples (Figure 2). This observation further highlights the exceptional nature of these particular pathogenic mutations within the context of GC among individuals of Pakistani descent. In sum, these mutations appear to represent distinct genetic markers associated with susceptibility to GC in the Pakistani population.



**Figure 2.** Visual representations using oncoplots and lollipop plots to depict the observed *BRCA1/2* mutations among TCGA GC patients.

Two rows illustrate the percentage of samples samples that exhibit *BRCA1/2* mutations, while the lollipop plots highlight the specific amino acid alterations resulting from these mutations at the protein level.

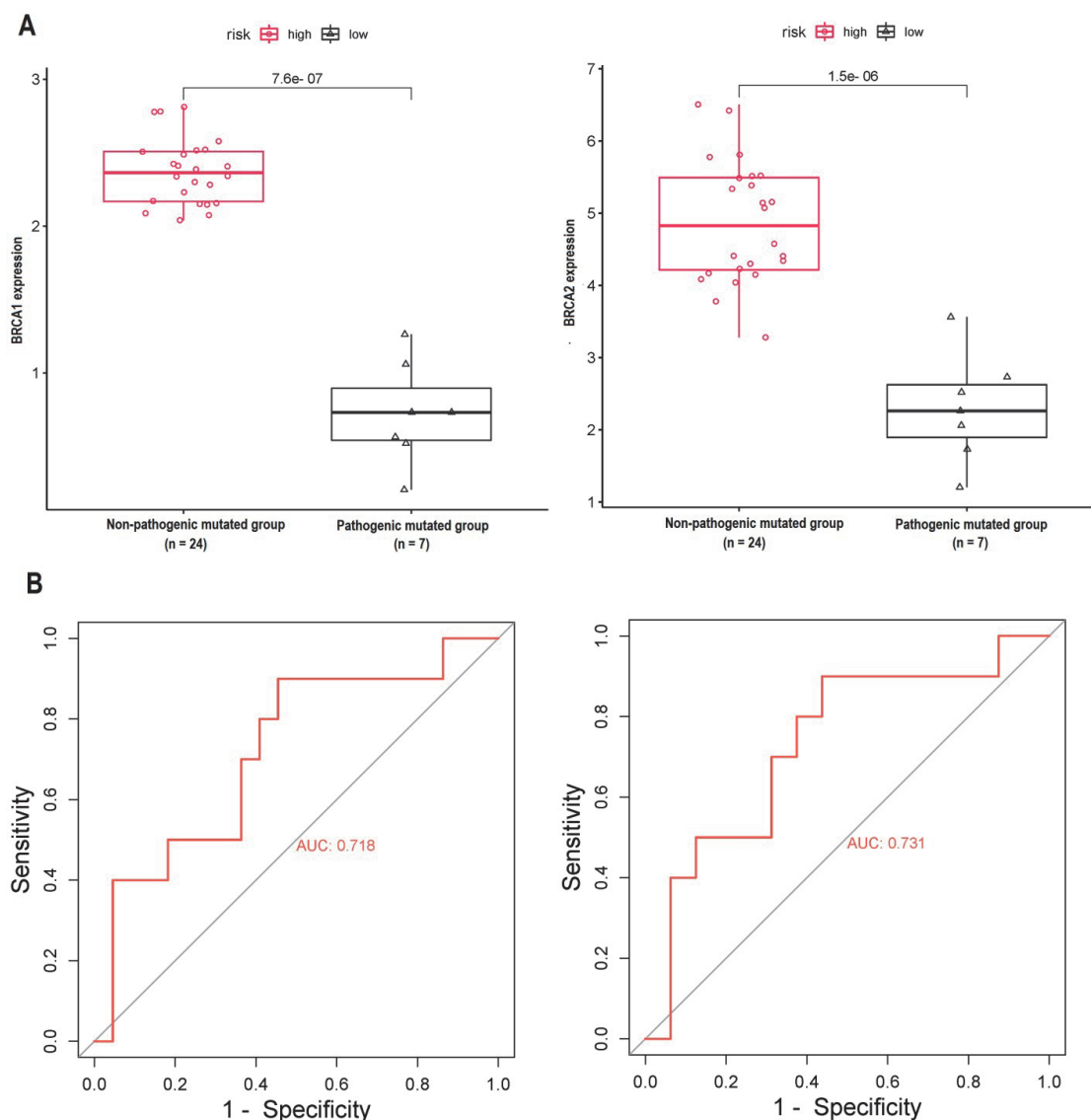


### 3.4. RT-qPCR-based expression analysis of *BRCA1/2* genes

The evaluation of *BRCA1/2* gene expression was carried out using RT-qPCR in two distinct subsets of GC samples. One subset consisted of samples harboring pathogenic mutations in in *BRCA1* gene (p.Ala1823Ser) and in the *BRCA2* gene (p.Gln92fs), while the other subset comprised samples without such mutations, representing the non-pathogenic mutation group. Upon analyzing the results of the RT-qPCR analysis, a noticeable trend became evident: the expression levels of *BRCA1/2* genes exhibited a significant decrease within the GC samples containing *BRCA1/2* pathogenic

mutations, in clear contrast to the non-pathogenic mutation group (Figure 3A). This intriguing finding suggests a potential association between *BRCA1/2* mutations and the heightened expression of these genes within the context of GC.

Moreover, the ROC curves derived from the RT-qPCR expression data of *BRCA1/2* genes offered further confirmation of their precision and sensitivity as prospective biomarkers (see Figure 3B). These curves illustrated the genes' capacity (Figure 3B) to effectively distinguish, underscoring their value as dependable biomarkers for GC diagnosis.

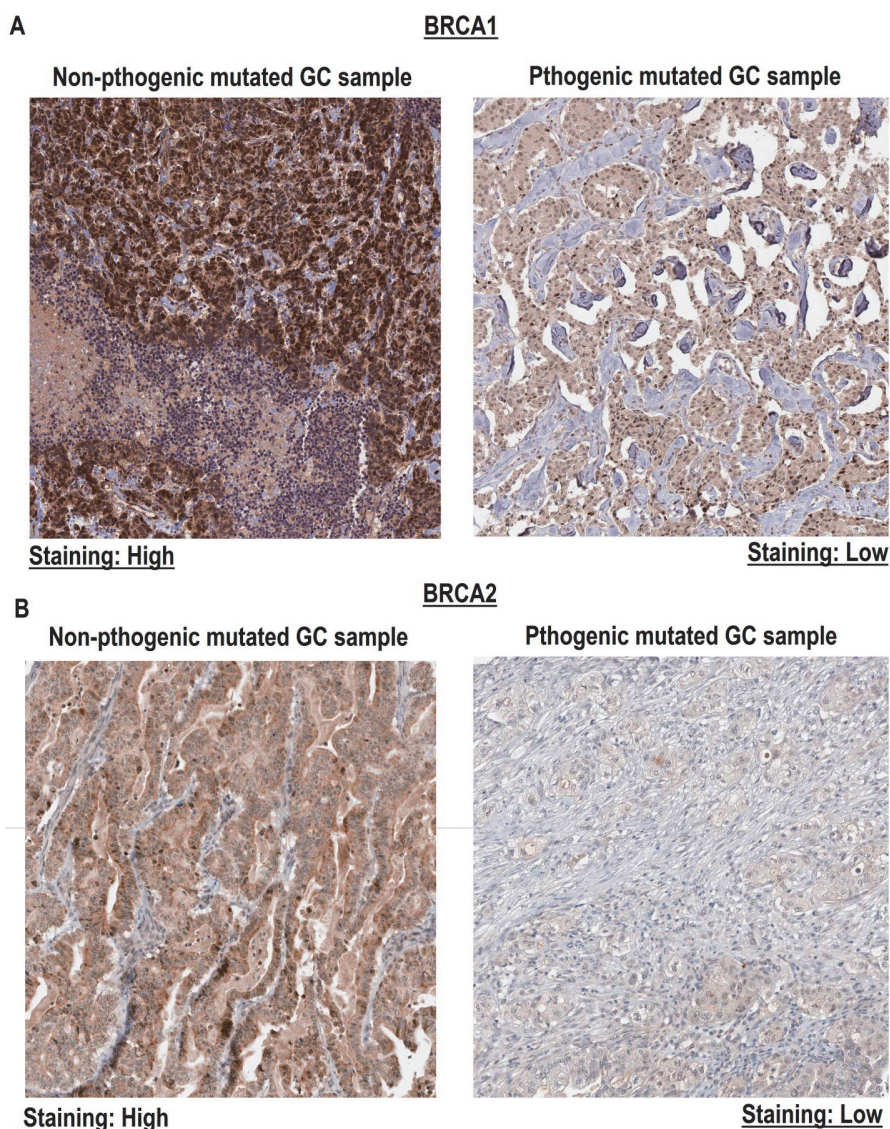


**Figure 3.** The analysis of relative expression and ROC curve assessments for *BRCA1/2* genes, comparing pathogenic mutated and non-pathogenic mutated groups of GC samples. (A) Subfigure A portrays the relative expression analysis of *BRCA1/2* genes via RT-qPCR, and (B) Subfigure B showcases ROC curves based on RT-qPCR expression data for *BRCA1/2* genes. A significance level of  $P < 0.05$  served as the threshold for classification.

### 3.5. Immunohistochemistry-based expression analysis of *BRCA1/2* genes

We conducted an IHC analysis to assess the proteomic expression of BRCA1/2 proteins in GC tissue samples. Specifically, we examined one tissue sample with pathogenic mutations in *BRCA1* gene (p.Ala1823Ser) and in the *BRCA2* gene (p.Gln92fs), and another tissue sample without any pathogenic mutations. The goal was to investigate potential disparities in protein expression between these two categories of samples.

Upon scrutinizing the staining results, a significant pattern became evident (Figure 4). The GC tissue samples with pathogenic mutations displayed notably lower levels of BRCA1/2 protein expression when compared to their counterparts lacking these mutations (Figure 4). This finding suggests a possible correlation between the presence of pathogenic mutations in the *BRCA1/2* genes and down-regulation in the expression of these proteins within the context of GC.



**Figure 4.** Proteomic expression analysis of BRCA1/2 proteins was conducted using immunohistochemistry (IHC) to compare pathogenic mutated and non-pathogenic mutated GC samples. Differences in expression were assessed by evaluating staining intensities.

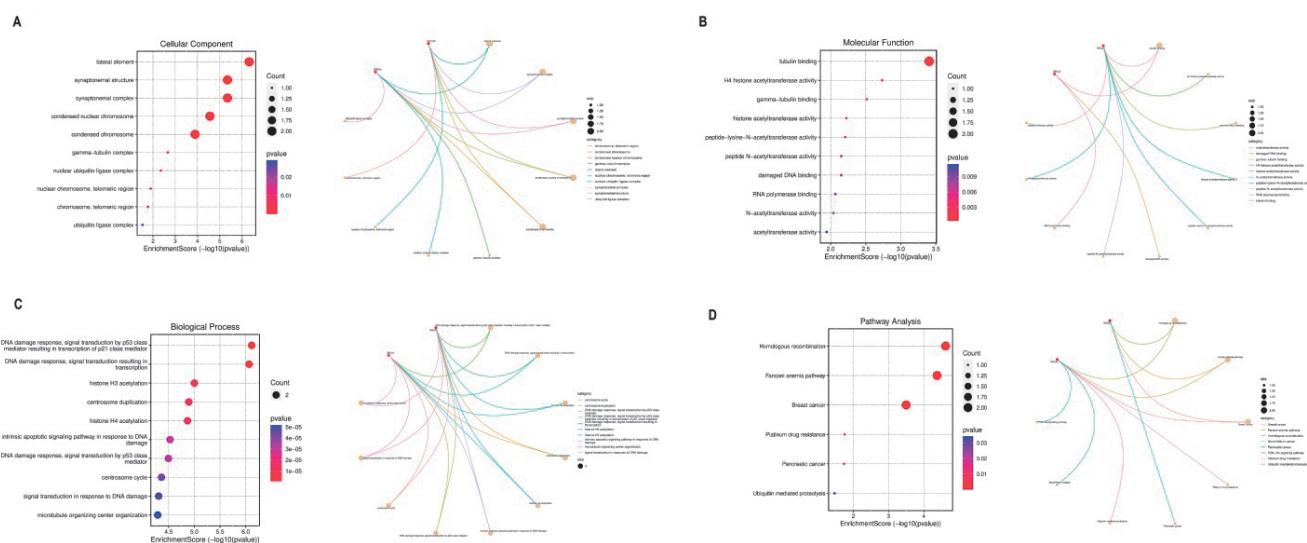
### 3.6. Enrichment analysis of *BRCA1/2* genes

Next, we conducted GO and KEGG enrichment analyses. Within the GO analysis, *BRCA1/2* genes exhibited enrichments in terms such as “lateral element,”

“synaptonemal structure,” “synaptonemal complex,” “condensed nuclear chromosome,” and “nuclear chromosome” among others (Figure 5A). In the case of molecular function (MF), they displayed enrichments in

terms like “tubulin binding,” “H4 histone acetyltransferase activity,” “gamma tubulin binding,” and “acetyltransferase activity” (Figure 5B). Furthermore, within biological processes (BP), these genes were associated with terms including “DNA damage response,” “signal transduction by p53 class mediator resulting in transcription of P21 class

mediator,” “DNA damage response,” “signal transduction resulting in transcription,” and “histone H3-acetylation” (5C). Additionally, in the KEGG pathway analysis, they were enriched in pathways such as “homologous recombination,” “Fanconi anemia pathway,” “breast cancer,” and “platinum drug resistance in cancer” (Figure 5D).



**Figure 5.** The results of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses performed on *BRCA1/2* genes using Metascape. (A) *BRCA1/2* genes-related CC terms, (B) *BRCA1/2* genes-related MF terms, (C) *BRCA1/2* genes-related BP terms, and (D) *BRCA1/2* genes-related KEGG terms. A  $P < 0.05$  was used as the cut-off criterion.

### 3.7. *BRCA1/2*-associated drugs

In our comprehensive investigation, we made use of the DrugBank database to systematically explore therapeutic possibilities aimed at modulating the expression of mutated *BRCA1* and *BRCA2*. Through our meticulous analysis, Cisplatin, Estradiol, Tretinoin,

Genistein, Acetaminophen, Quercetin, Quercetin, and Quercetin (Table 3) emerged as compounds with significant potential to effectively up-regulate the expression levels of *BRCA1/2* genes. This discovery holds substantial promise for innovative therapeutic approaches within the context of GC.

**Table 3.** DrugBank-based *BRCA1/2* associated drugs

| Sr. No | Hub gene     | Drug name     | Effect                                   | Reference | Group    |
|--------|--------------|---------------|--|-----------|----------|
| 1      | <i>BRCA1</i> | Cisplatin     | Increase expression of <i>BRCA1</i> mRNA | A22234    | Approved |
|        |              | Estradiol     |  | A21155    |          |
|        |              | Tretinoin     |  | A24464    |          |
|        |              | Genistein     |  | A22773    |          |
| 2      | <i>BRCA2</i> | Acetaminophen | Increase expression of <i>BRCA2</i> mRNA | A20418    | Approved |
|        |              | Quercetin     |  | A21498    |          |
|        |              | Estradiol     |  | A21155    |          |
|        |              | Resveratrol   |  | A23885    |          |
|        |              | Metribolone   |  | A23234    |          |
|        |              | Genistein     |  | A22773    |          |



## 4. Discussion

GC continues to pose a significant global health challenge due to its complex etiology and diverse genetic alterations<sup>[25]</sup>. GC stands as the fifth most prevalent cancer and the third primary contributor to cancer-related fatalities on a global scale. This emphasizes the critical need for a deeper exploration into the molecular foundations of this disease<sup>[26]</sup>.

In this study, we aimed to elucidate the role of *BRCA1* and *BRCA2* mutations in Pakistani GC patients and assess their functional consequences. The findings of this study shed light on the potential implications of these mutations in the context of GC development and progression.

Comprehensive analysis in the current study involved the use of NGS to scrutinize *BRCA1/2* genes in a cohort of 31 Pakistani GC patients. This approach revealed the presence of one, one pathogenic mutations in *BRCA1* gene (p.Ala1823Ser) and in the *BRCA2* gene (p.Gln92fs) along with various benign mutations. The pathogenic mutations are of particular significance as they have been well-documented for their association with hereditary breast and ovarian cancers<sup>[27,28]</sup>. To the best of our knowledge, this study marks the first documentation of such pathogenic mutations in GC within the Pakistani population.

To explore the functional consequences of these observed pathogenic mutations, we conducted expression analyses of *BRCA1* and *BRCA2* genes at both mRNA and protein levels. The results yielded a striking and consistent pattern - GC samples harboring pathogenic mutations in these genes displayed a down-regulation of *BRCA1/2* gene expression as compared other GC samples which do not harbor pathogenic mutations in those genes. Earlier studies have also indicated that pathogenic mutations typically result in the disruption and impaired function of the mutated gene in cancer patients<sup>[29,30]</sup>.

The observed down-regulation of mutated *BRCA1* gene (p.Ala1823Ser) and *BRCA2* gene (p.Gln92fs) in the current study is noteworthy for several reasons. Firstly, it underscores the impact of *BRCA1/2* mutations beyond their canonical role in DNA repair<sup>[31,32]</sup>. These mutations appear to exert an influence on gene

expression, potentially contributing to the molecular alterations associated with GC. Secondly, the down-regulation was observed not only at the mRNA level but also at the protein level, indicating a multifaceted effect on the cellular processes regulated by *BRCA1/2*. Prior investigations have also suggested that the decrease in protein-level expression of *BRCA1/2* genes leads to the disruption of crucial cellular processes related to DNA repair<sup>[33,34]</sup>. These *BRCA1/2* down-regulation-based finding underscores the potential clinical relevance of the pathogenic mutations in the context of GC. The observed down-regulation of *BRCA1/2* genes in GC samples with pathogenic mutations has far-reaching implications for the management of GC patients. It suggests a potential role for these mutations as biomarkers with diagnostic and therapeutic significance. Down-regulation of *BRCA1/2* genes in Pakistani GC patients may contribute to genomic instability and altered DNA repair mechanisms, which could impact the disease progression and response to therapy. Therefore, targeted therapies that exploit the underlying biology of these mutations may offer new avenues for treatment, potentially enhancing treatment response and patient outcomes. In addition, we have investigated potential drugs (Cisplatin, Estradiol, Tretinoin, Genistein, Acetaminophen, Quercetin, Quercetin, and Quercetin) from the DrugBank database that have the capability to enhance the expression of *BRCA1/2* genes when used in the treatment of GC patients.

Finally, pathway analysis revealed the involvement of *BRCA1/2* genes in various divers' pathways, including "homologous recombination," "Fanconi anemia pathway," "breast cancer," and "platinum drug resistance in cancer". The dysregulation of these pathways is already well document in various cancers<sup>[35-37]</sup>.

While our study provides compelling evidence of the functional consequences of *BRCA1/2* mutations in GC, it is not without limitations. The sample size, although informative, is relatively small, and further validation in larger cohorts is essential. Additionally, in-depth mechanistic studies are warranted to elucidate the precise molecular pathways through which these

mutations impact gene expression and GC progression.

## 5. Conclusion

In conclusion, our study highlights the presence of pathogenic mutations in *BRCA1* and *BRCA2* genes in Pakistani GC patients and underscores their functional consequences in terms of down-regulated gene expression. These findings open new avenues for research into the molecular underpinnings of GC and the development of targeted therapies for patients with these mutations. Further investigation is needed to harness the full clinical potential of these discoveries and improve the management of GC in affected populations.

## Data Availability

The data could be obtained by contacting corresponding author.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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