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*Original Article*

# Detection of genomic variants in BRCA1 and BRCA2 across gastric cancer patients using next generation sequencing

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**Abstract:** Objectives: To explore the landscape of BRCA1/2 mutations in gastric cancer patients. Methods: Next-generation sequencing (NGS), Sanger sequencing, reverse transcription quantitative polymerase chain reaction (RT-qPCR), Immunohistochemistry, The Cancer Genome Atlas (TCGA), gnomAD, and DAVID. Results: With 95% of bases boasting a phred score surpassing 30 and a minimum coverage depth of 500X, our NGS approach ensures high-quality data acquisition. Analyzing BRCA1 and BRCA2 sequences revealed 11 and 4 mutations, respectively, with one pathogenic mutation identified in each gene. This emphasizes the prominence of BRCA1 mutations in gastric cancer. Sanger sequencing validation confirmed the presence of pathogenic mutations in select cases, consolidating our findings. Frequency analysis utilizing the gnomAD database elucidated the rarity of these mutations in the Asian population, underscoring their uniqueness. Exploring TCGA data further corroborated this rarity, emphasizing the distinctive nature of these mutations in gastric cancer. RT-qPCR analysis unveiled a significant reduction in BRCA1/2 expression in samples harboring pathogenic mutations, hinting at their potential role in down-regulating gene expression. Immunohistochemistry confirmed diminished protein expression in samples with pathogenic mutations, solidifying our observations. Kaplan-Meier survival analysis demonstrated significantly poorer survival outcomes for patients with pathogenic BRCA1/2 mutations compared to those without, emphasizing their potential role in prognosis. Additionally, KEGG pathway analysis highlighted the involvement of BRCA1/2 in critical cancer-associated pathways, emphasizing their role in tumorigenesis. Conclusion: Our comprehensive findings underscore the clinical significance of BRCA1/2 mutations in gastric cancer, advocating for further research to elucidate their mechanistic implications and therapeutic opportunities.

**Keywords:** Gastric cancer, NGS, BRCA genes

## Introduction

Gastric cancer (GC) stands as the third leading cause of cancer-related mortality worldwide, with the highest incidence noted in Eastern Asia, notably China, and the lowest in Northern America [1]. This disease is characterized by significant heterogeneity, encompassing various molecular subtypes, which can be categorized into intestinal and diffuse types according to the Lauren classification [2]. Recent studies utilizing next-generation sequencing (NGS) have uncovered an extensive repertoire of potential cancer-driving genes and have delineated the mutational landscape of gastric cancer. The Cancer Genome Atlas (TCGA) project classified gastric cancer into four subtypes: Epstein-Barr virus (EBV) positive, microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN) [3]. Chen et al. conducted

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whole-exome sequencing (WES) on paired normal-cancer tissues of 78 gastric cancer patients in northern China (Tianjin), identifying two GC subtypes characterized by either high-clonality (HiC) or low-clonality (LoC) [4, 5].

In the past decade, NGS has facilitated the integration of clinical genomics into the diagnosis and treatment of cancers [6-8]. Using lung cancer as an illustration, it has become customary to profile tumors for driver mutations, with target capture sequencing being able to pinpoint actionable mutated driver genes. In recent years, several studies focusing on gastric cancer have embraced target NGS technology [9-12]. The literature indicates that BRCA1/2 mutations exhibit high penetrance across various cancers, including gastric cancer, often manifesting in an autosomal dominant pattern [13-16].

Previous studies have explored the prevalence and implications of BRCA1/2 mutations in gastric cancer, with findings varying based on population demographics. For example, NGS of Russian patients with gastric cancer found that BRCA1/2 mutation carriers showed somatic loss of BRCA1/2 allele [17]. This highlights a possible association between BRCA1/2 mutations and a poorer prognosis, as these mutations appeared linked to more aggressive tumor characteristics in gastric cancer patients. Another study found that among 65 Russian patients with metastatic gastric cancer, two (3%) carried the BRCA1 5382insC germ-line mutation and exhibited a pronounced response to cytotoxic therapy [18]. These patients also showed loss of the remaining BRCA1 allele, indicating a causative role of BRCA1 heterozygosity in gastric predisposition. Similarly, another study conducted on a Chinese cohort of gastric cancer patients identified BRCA1/2 mutations in a small subset of cases [19, 20]. Their findings further supported the notion that BRCA1/2 mutations in GC patients might indicate worse clinical outcomes, including reduced overall survival.

The prevalence of BRCA1/2 mutations varies among different ethnic groups and geographical regions. Notably, significant variability exists across Latin American countries, attributed to the admixture of European, African, and Amerindian ancestries [21]. A founder mutation, known as ex9-12del, has been identified in the Hispanic population residing in the southern United States [22, 23], as well as in an unselected study population from central Mexico, where a mutation frequency of 29% was observed in individuals assessed for a family history of cancer [24].

In this extensive investigation involving genetic testing of 40 gastric cancer cases in Pakistan, both NGS and Sanger sequencing methods were employed to scrutinize the mutational landscape of BRCA1/2 genes. The implementation of NGS systems is poised to be established for genetic testing of gastric cancer. The primary objective of this study was to assess BRCA1/2 variants linked with gastric cancer cases by sequencing all exons and splice site regions of BRCA1 and BRCA2 genes via NGS.

## Methodology

### *Sample collection*

This study received approval from the ethical committee of Dera Ismail Khan Health Department, Khyber Pakhtunkhwa, Pakistan. We enrolled 40 fresh frozen gastric cancer tissue samples from patients from the Mufti Mahmood Memorial Teaching Hospital, Dera Ismail Khan, Pakistan, between June 2022 and December 2023. The study was conducted in compliance with the Helsinki guidelines, and informed consent was obtained from all patients before sample collection.

### *Molecular analysis*

*DNA isolation:* Genomic DNA extraction from gastric cancer tissue samples was performed using a commercially available kit (Isolate II Genomic DNA Kit, Bioline). The concentration and purity of the extracted DNA were assessed using a NanoDrop 2000 Spectrophotometer and a Qubit 3.0 Fluorometer (Thermo Fischer Scientific, Waltham, MA, USA).

*NGS analysis:* For each sample, 10 ng of DNA was utilized to construct the sequencing library employing the Ion PGM™ sequencing system and the Oncomine™ BRCA Research Assay (Thermo Fisher Scientific, Waltham, MA, USA). This assay comprises two pools featuring 265 primer pairs that cover the entire coding sequence of the BRCA1 and BRCA2 genes, including splice site sequences at intron/exon

junctions. Following PCR amplification, the amplicons were partially digested using FuPa enzyme and subsequently ligated to barcoded adapters. Purification of the generated amplicons was carried out using AMPure™ XP Reagent (Beckman Coulter, Brea, CA, USA). Subsequently, the libraries were quantified, diluted to 100 pM, and subjected to emulsion PCR amplification using the Ion OneTouch™ 2 System and Ion PGM™ Hi-Q™ View OT2 Kit (Thermo Fisher Scientific, Waltham, MA, USA). Finally, NGS sequencing was performed on the Ion PGM™ sequencer utilizing the Ion PGM™ Hi-Q™ View Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA).

The sequencing data underwent quality control and alignment to the HG19 human genome using the Ion Torrent Suite™ Software 5.0.5 (Thermo Fisher Scientific). Subsequently, the data were analyzed utilizing the Torrent Variant Caller plugin version 5.0 (Thermo Fisher Scientific) to identify genetic variants. Variant annotation was performed using Ion Reporter™ software (Thermo Fisher Scientific). The coverage depth threshold was set at ≥250X.

The variants identified were classified into categories such as pathogenic, common polymorphisms, or variants of uncertain significance (VUS) based on information from various databases including ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), BRCA Exchange (https://brcaexchange.org), Universal Mutation Database (http://www.umd.be/BRCA1/ and http://www.umd.be/BRCA2/), and Leiden Open Variation Database (LOVD) (http://www.lovd.nl/3.0/home).

*Analysis of the BRC1/2 mutational frequencies:* gnomeAD, or the Genome Aggregation Database (https://gnomad.broadinstitute.org/), is a comprehensive collection of genetic variants derived from exome and genome sequencing data [25]. It serves as a valuable resource for researchers and clinicians to study human genetic variation across diverse populations. gnomeAD offers insights into the frequency and distribution of variants in the general population, aiding in the interpretation of genetic findings in research and clinical settings. This database plays a crucial role in understanding the genetic basis of various diseases and traits. In the current study, gnomeAD database was used to analyze mutation frequencies of BRCA1/2 genes across the Asian population.

*Analysis of the mutations in the TCGA database:* cBioPortal (https://www.cbioportal.org/) is an open-access resource that facilitates exploration and analysis of multidimensional cancer genomics data sets [26]. It aggregates data from various cancer studies, including genomic profiling, clinical information, and patient outcomes. Users can interactively visualize genetic alterations, such as mutations, copy number variations, and gene expression changes, across different cancer types and subtypes. In the current work, cBioPortal database was utilized to analyzed BRCA1/2 mutations across gastric cancer samples from TCGA.

*RT-qPCR analysis of BRCA1/2:* Total RNA extraction was performed using the Eastep® SuperTotal RNA Extraction kit (Promega), with subsequent cDNA synthesis using 1 μg of total RNA and the Reverse Transcription Kit (Biosharp). For cDNA amplification, Taq Pro Universal SYBR qPCR Master Mix from Vazyme was utilized. Gene expression levels were normalized to actin employing the $2^{-\Delta\Delta Ct}$ method. Student t-test was used to compare expression between the two groups of samples. PCR conditions included initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 30 s. Primer sequences used for RT-qPCR were: Actin sense: 5'-TGGCACCCAGCACAATGAA-3', Actin antisense: 5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'; BRCA1 sense: 5'-CTGAAGACTGCTCAGGGCTATC-3', BRCA1 antisense: 5'-CTGAAGACTGCTCAGGGCTATC-3'; BRCA2 sense: 5'-GAAAATCAAGAAAAATCCTTAAAGGCT-3', BRCA2 antisense: 5'-GTAATCGGCTCTAAAGAAACATGATG-3'.

*Immunohistochemistry analysis:* Immunohistochemical staining was conducted on selected cases having pathogenic mutations using formalin-fixed paraffin-embedded (FFPE) tissue blocks. Tissue sections of 4 microns thickness were cut using a microtome and mounted onto charged slides (Starfrost), followed by overnight drying at 38°C. Deparaffinization was performed in two changes of xylene for 5 minutes each, followed by hydration in two changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each. Subsequently, the sections were rinsed in distilled water.

Antigen retrieval was carried out by immersing the sections in 0.01 M Tris buffer solution (TBS), pH 6.0 antigen retrieval solution until the temperature reached 95°C for 2 minutes, followed by rinsing in phosphate buffer solution (PBS). Endogenous peroxidase blocking was carried out by immersing the sections in 3% $H_2O_2$ for 30 minutes. Subsequently, the sections were incubated overnight at 4°C with BRCA1 (polyclonal MS110, Abcam, USA) and BRCA2 (polyclonal ab27976, Abcam, USA) antibodies simultaneously, diluted to 1:10.

After rinsing the sections in PBS for 4 minutes, they were stained with DAB chromogen (Envision Flex, Dako, Denmark) and subsequently counterstained with hematoxylin. Tissue not having pathogenic mutations served as the positive control. BRCA1 and BRCA2 status were deemed positive when tumor cells exhibited a golden brown staining. Positive results were reported based on the intensity of staining.

*Kaplan-Meier survival analysis of the impact of pathogenic BRCA1/2 mutations on prognosis of GC patients:* Survival analysis was conducted to assess the impact of BRCA1/2 mutations on the prognosis of GC patients using Kaplan-Meier survival analysis. Patients were stratified into two groups based on their BRCA mutation status: patients with pathogenic BRCA1/2 mutations and patients without pathogenic BRCA1/2 mutations. Overall survival (OS) was defined as the time from diagnosis to death or last follow-up. Kaplan-Meier curves were generated using the ggsurvplot function from the survminer package in R, with statistical differences between the groups assessed using the log-rank test (survdiff function). A *P*-value of less than 0.05 was considered significant. The analysis was performed in R (version 4.4.1).

*Gene enrichment analysis:* The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) is a web-based bioinformatics resource that aids researchers in comprehensively analyzing large gene lists [27, 28]. It provides functional annotation tools to uncover biological insights, such as gene ontology, pathway enrichment analysis, and protein-protein interaction networks, facilitating deeper understanding of experimental data. In the present study, DAVID was used to predict BRCA1/2-associated signaling pathways.

*Sanger sequencing:* The genomic DNA was extracted using the QIAamp DNA Mini Kit. Sanger sequencing was conducted as outlined below. Initially, PCR amplification was carried out utilizing F-Taq polymerase (Solgent, Korea). Each 25-μL reaction mixture comprised 1X PCR buffer, 1.5-mmol/L $MgCl_2$, 2 mmol/L of each dNTP, 5 pmol/L each of the forward and reverse primers, 0.5 U F-Taq polymerase, and 100-ng genomic DNA. The thermal cycling program consisted of the following steps: (1) Initial denaturation at 94°C for 5 minutes, (2) Denaturation at 94°C for 30 seconds, (3) Annealing at an appropriate temperature for 30 seconds, (4) Extension at 72°C for 45 seconds, and (5) Final extension at 72°C for 3 minutes. Steps 2 to 4 were iterated for 30 cycles. The primers were synthesized based on published sequences or custom-designed [29]. Conventional PCR was employed to amplify the full coding regions of BRCA1 and BRCA2 genes. The targeted region encompassed the complete coding regions of BRCA1 and BRCA2, along with approximately 20 bp of noncoding DNA flanking the 5' and 3' ends of each exon. Each PCR amplicon underwent treatment with a 20-μL reaction mixture consisting of 3 U exonuclease I, 5X exonuclease I buffer, and 1.7 U FastAP thermosensitive alkaline phosphatase (Fermentas, Waltham, Massachusetts, USA), followed by incubation at 37°C for 45 minutes and heat-inactivation at 80°C for 10 minutes. Cycle sequencing was carried out using the BigDye Terminator kit v1.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Sequencing products were analyzed on a 3130xl Genetic Analyzer (Applied Biosystems), and visualization and sequence alignment of Sanger data were performed using SeqScape software v2.7 (Applied Biosystems).

## Results

### *Landscape of BRCA1/2 mutations in gastric cancer patients*

We utilized NGS techniques to detect BRCA1/2 genetic mutations in gastric cancer patients. In the DNA sequences of BRCA1 and BRCA2 from all patients, 95% of bases exhibited a phred score exceeding 30, ensuring high-quality data. Moreover, a minimum coverage depth of 500X was achieved, guaranteeing robust sequencing depth across the regions of interest. Additionally, the uniformity of coverage reached an

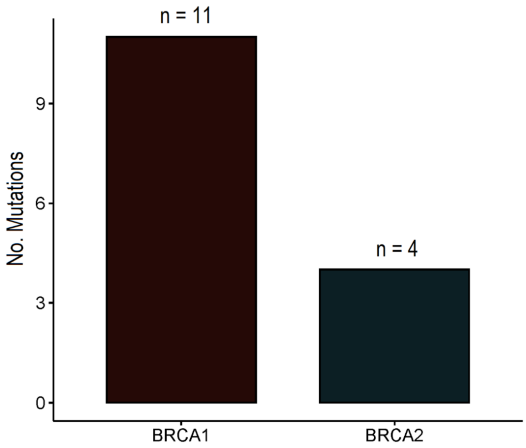


**Figure 1.** This figure illustrates the cumulative number of Breast Cancer 1 and 2 (BRCA1/2) mutations identified in the gastric cancer patients analyzed using next-generation sequencing (NGS) analysis.

impressive 99%, indicating consistent and reliable sequencing across the targeted regions of BRCA1 and BRCA2. Our results revealed a total of 11 mutations in BRCA1 and 4 mutations in BRCA2 (**Figure 1** and **Table 1**). Among the BRCA1 mutations, 1 (9.1%) was identified as pathogenic (p.Tyr1853Ser), while the remaining 10 were benign (90.9%). In BRCA2, 1 (25%) mutation (p.Trp31Ser) was deemed pathogenic out of the total 4 mutations, with the remaining 3 (75%) classified as benign. Regarding the frequency of the observed mutations classified as benign versus pathogenic in the analyzed cohort, the frequency of BRCA1 benign mutations was 37.5%, while the frequency of pathogenic mutations was 25%. Similarly, the frequency of BRCA2 benign mutations was 22.5%, while the frequency of pathogenic mutations was 25%.

*Sanger sequencing analysis*

The verification of pathogenic mutations in BRCA1 (5558A>C) and BRCA2 (c.92G>C) genes in two gastric cancer cases was confirmed through Sanger sequencing analysis, as depicted in **Figure 2**. This analysis served to validate the presence of these mutations within the genetic makeup of the gastric cancer specimens under investigation.

*Frequency analysis of the BRC1/2 pathogenic mutations in the gnomAD database*

Pathogenic mutations hold greater clinical relevance than benign mutations, directly influencing disease progression. Focusing research efforts on them facilitates understanding disease mechanisms and developing targeted therapies. To uncover population-specific characteristics, we analyzed the frequency of two pathogenic mutations, BRCA1 (p.Tyr1853Ser) and BRCA2 (p.Trp31Ser), in the Asian population using the gnomAD database. The analysis revealed a frequency of 0 for both mutations in Asian gastric cancer patients, indicating their uniqueness in the Pakistani population.

*Analyzing the presence of BRCA1/2 pathogenic mutations in the TCGA*

In this part of our study, we analyzed the uniqueness of the detected pathogenic mutations, BRCA1 (p.Tyr1853Ser) and BRCA2 (p.Trp31Ser), in gastric cancer samples in the TCGA project. Utilizing the cBioPortal web portal, we examined the mutational spectrum of BRCA1/2 mutations among gastric cancer patients in the TCGA dataset. As depicted in **Figure 3**, a diverse array of BRCA1/2 mutations was evident among gastric cancer patients. However, notably, neither BRCA1 (p.Tyr1853Ser) nor BRCA2 (p.Trp31Ser) pathogenic mutations were observed in the analyzed gastric cancer samples. This absence within the TCGA dataset serves to underscore the uniqueness of these mutations.

*RT-qPCR analysis of BRCA1/2*

Subsequently, we examined disparities in BRCA1/2 gene expression between two cohorts: gastric cancer samples with pathogenic mutations in BRCA1/2 genes (n = 10) and those without (n = 30). Our analysis revealed a significant (*P*-value <0.05) reduction in BRCA1/2 gene expression levels in gastric cancer samples harboring pathogenic mutations compared to those lacking such mutations (**Figure 4**). These findings suggest that pathogenic mutations may contribute to the downregulation of BRCA1/2 expression in gastric cancer patients.

*Immunohistochemistry analysis*

Next, we conducted immunohistochemistry to validate the protein expression of BRCA1/2 genes. A total of six samples were analyzed: three without pathogenic mutations in BRCA1/2 genes, two with pathogenic mutations in BRCA1 gene, and two with pathogenic mutations in BRCA2 gene. The immunohistochemistry results revealed notably lower protein expres-

**Table 1.** Detailed overview of Breast Cancer 1 and 2 (BRCA1/2) mutations identified among gastric cancer patients

| Sr. no | Gene | NM:c.DNA | Protein | Nature (ClinVar) | Nature (In silico analysis) | No. patients |
|---|---|---|---|---|---|---|
| 1 | BRCA1 | NM_007294.4:c.5580C>A | p.His1860Gln | Benign | Non-DC | 11 |
| 2 | | NM_007294.4:c.5576C>G | p.Pro1859Arg | Benign | Non-DC | 09 |
| 3 | | NM_007294.4:c.5572A>C | p.Ile1858Leu | Benign | Non-DC | 10 |
| 4 | | NM_007294.4:c.5566C>T | p.Pro1856Ser | Benign | Non-DC | 11 |
| 5 | | NM_007294.4:c.5565A>G | p.Ile1855Met | Benign | Non-DC | 12 |
| 6 | | NM_007294.4:c.5531T>G | p.Leu1844Arg | Benign | Non-DC | 01 |
| 7 | | NM_007294.4:c.5518G>T | p.Asp1840Tyr | Benign | Non-DC | 01 |
| 8 | | NM_007294.4:c.5510G>T | p.Trp1837Leu | Benign | Non-DC | 09 |
| 9 | | NM_007294.4:c.5506G>A | p.Glu1836Lys | Benign | Non-DC | 11 |
| 10 | | NM_007294.4:c.5464C>G | p.His1822Asp | Benign | Non-DC | 15 |
| 11 | | NM_007294.4:c.5558A>C | p.Tyr1853Ser | Pathogenic | DC | 10 |
| 1 | BRCA2 | NM_000059.4:c.24G>T | p.Arg8Ser | Benign | Non-DC | 04 |
| 2 | | NM_000059.4:c.85C>T | p.Leu29Phe | Benign | Non-DC | 03 |
| 3 | | NM_000059.4:c.91T>A | p.Trp31Arg | Benign | Non-DC | 09 |
| 4 | | NM_000059.4:c.92G>C | p.Trp31Ser | Pathogenic | DC | 10 |

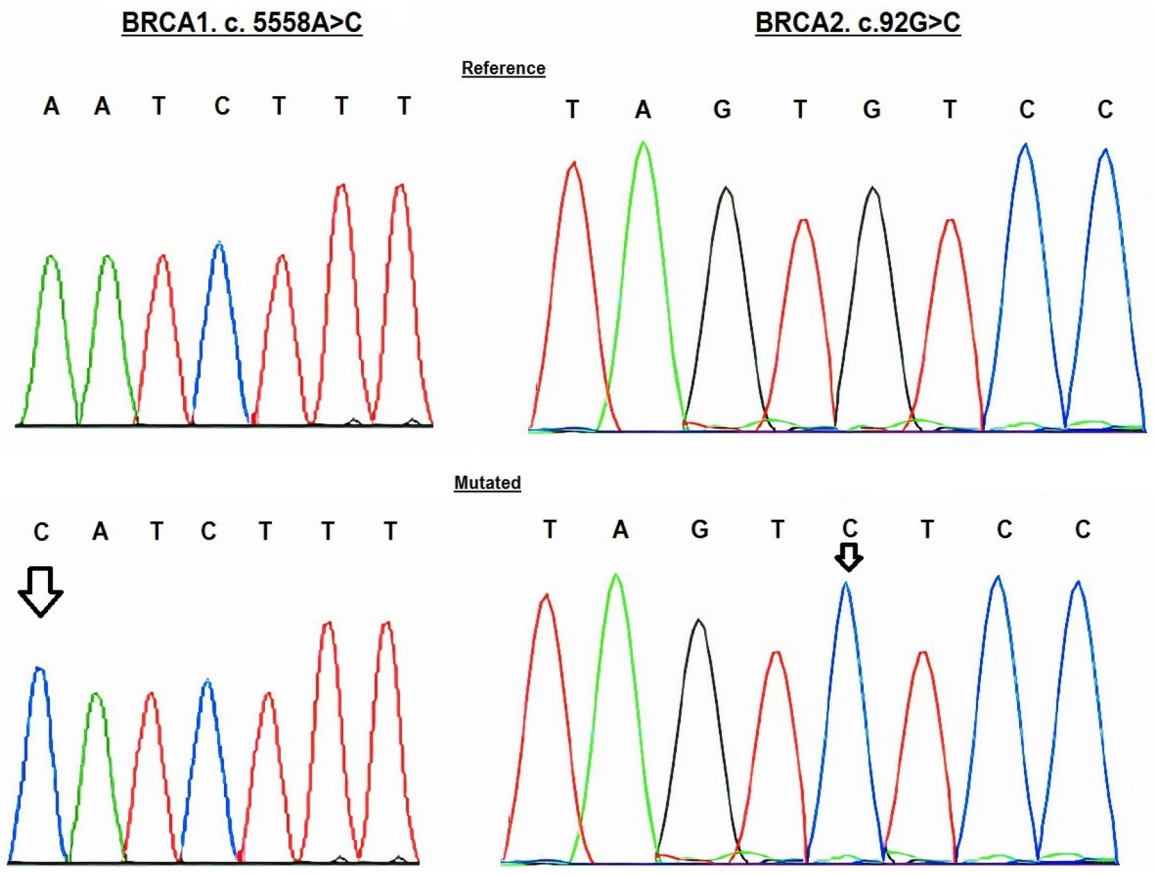


**Figure 2.** This figure displays the sequencing chromatograms representing the Breast Cancer 1 and 2 (BRCA1/2) mutations detected in gastric cancer patients through Sanger sequencing.

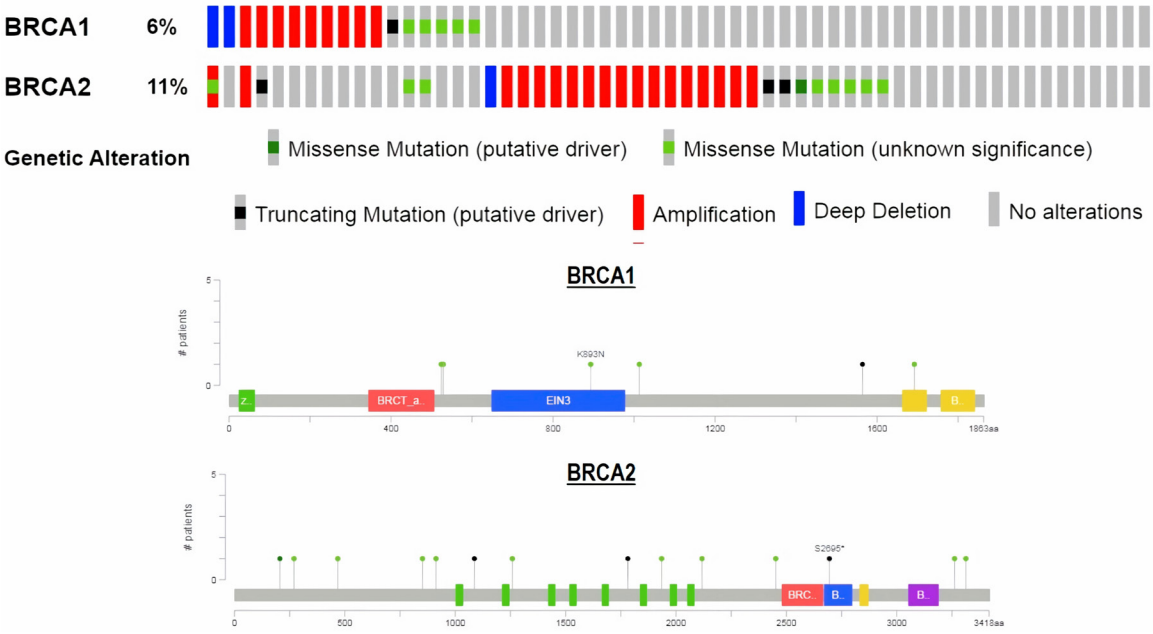


**Figure 3.** Frequency and nature of Breast Cancer 1 and 2 (BRCA1/2) mutations across gastric cancer patients in The Cancer Genome Atlas (TCGA) database.

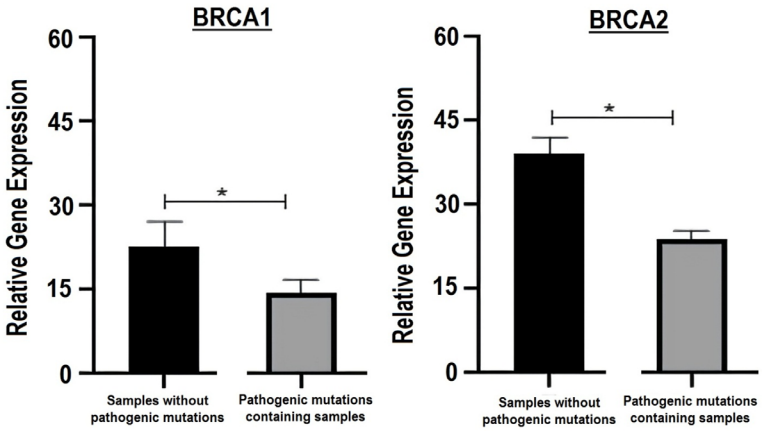


**Figure 4.** Expression analysis of Breast Cancer 1 and 2 (BRCA1/2) genes across gastric cancer tissue samples via the reverse transcription quantitative polymerase chain reaction (RT-qPCR). *$P$-value <0.05.

sion of BRCA1/2 in gastric cancer samples harboring pathogenic mutations compared to those lacking such mutations (**Figure 5**).

*Kaplan-Meier survival analysis of the impact of pathogenic BRCA1/2 mutations on prognosis of GC patients*

In this part of the study, we analyzed the survival differences between GC patients with and without pathogenic BRCA1/2 mutations using Kaplan-Meier survival analysis. The survival curves demonstrate that patients with pathogenic BRCA1/2 mutations (red curve) had significantly poorer survival outcomes compared to those without such mutations (blue curve) over a 20-week follow-up period (**Figure 6**). The statistical significance of this difference was evaluated using the log-rank test, yielding a $P$-value of 0.045, which indicates a significant difference in survival probability between the two groups (**Figure 6**). Specifically, the survival probability decreases more steeply for the mutation-positive group, suggesting that the presence of pathogenic BRCA1/2 mutations may be associated with a higher risk of mortality or disease progression.

*BRCA1/2-associated signaling pathways*

The KEGG enrichment analysis of BRCA1/2 genes suggested that these genes were positively associated with important cancer caus-

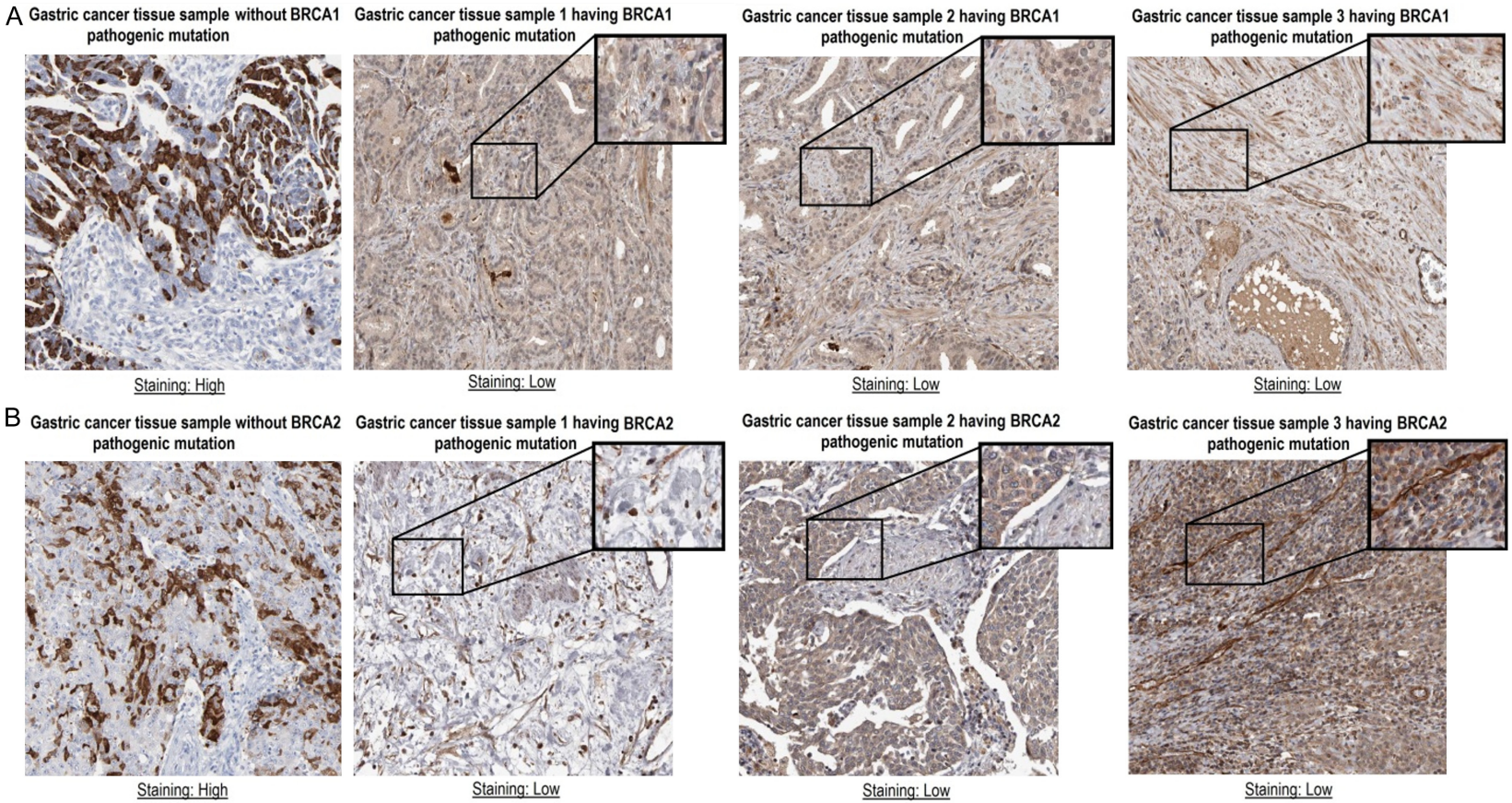


**Figure 5.** This figure illustrates the proteomic expression profiling of Breast Cancer 1 and 2 (BRCA1/2) proteins in gastric cancer samples using immunohistochemistry (IHC). A. Proteomic expression profiling of BRCA1 protein across gastric cancer samples without and with pathogenic mutations. B. Proteomic expression profiling of BRCA2 protein across gastric cancer samples without and with pathogenic mutations.

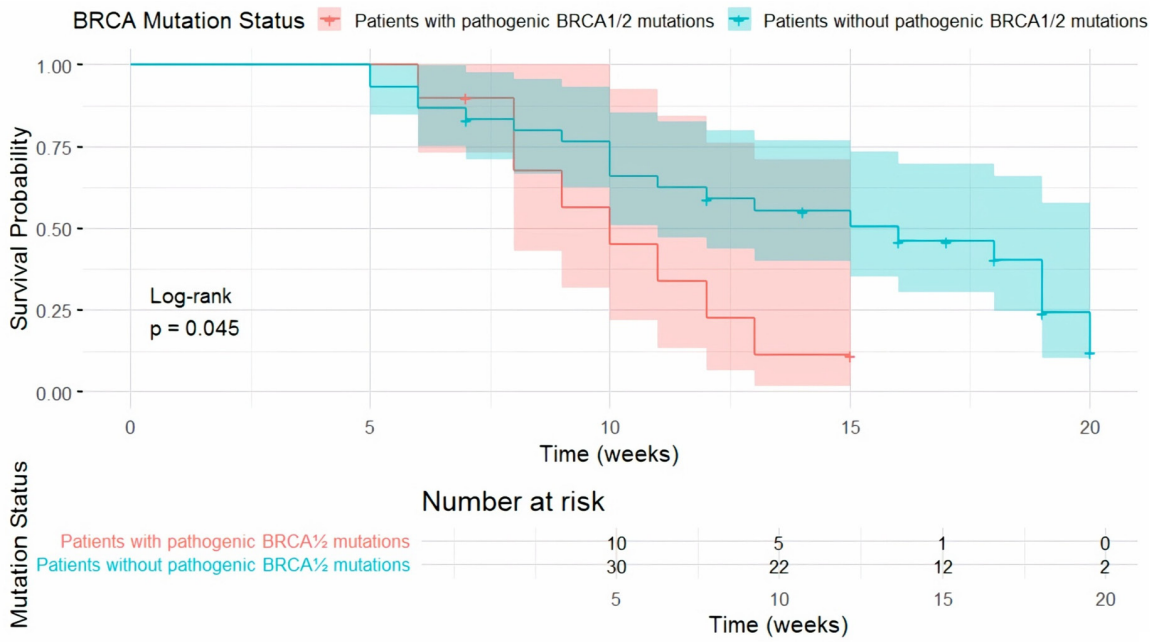


**Figure 6.** Kaplan-Meier survival analysis of gastric cancer patients stratified by Breast Cancer 1 and 2 (BRCA1/2) mutation statuses. Kaplan-Meier survival curves comparing survival probabilities between gastric cancer patients with pathogenic BRCA1/2 mutations (red curve) and those without pathogenic mutations (blue curve) over a 20-week follow-up period. *P*-value <0.05.

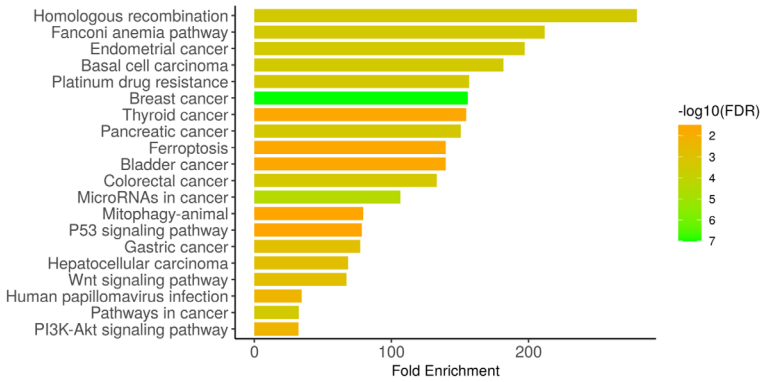


**Figure 7.** A Kyoto encyclopedia of genes and genomes (KEGG) analysis of the Breast Cancer 1 and 2 (BRCA1/2) genes was conducted using the database for annotation, visualization, and integrated discovery (DAVID) tool. FDR = False discover rate.

ing signaling pathways, including "homologous recombination, the Fanconi anemia pathway, endometrial cancer, basal cell carcinoma, breast cancer, thyroid cancer, pancreatic cancer, ferroptosis, bladder cancer, colorectal cancer, and gastric cancer pathways etc. (**Figure 7**)". Among these pathways, homologous recombination, the Fanconi anemia pathway, and endometrial cancer show the highest fold enrichment and are highly significant. These pathways are crucial for cancer development due to their roles in DNA repair and oncogenesis. The gastric cancer pathway shows moderate fold enrichment with a highly significant FDR (~7) **Figure 7**. This suggests that the genes or pathways analyzed are highly relevant in the context of gastric cancer, indicating a potential association with the disease's progression. Other pathways like microRNAs in cancer and Ferroptosis, which play roles in gene regulation and cell death, show lower fold enrichment but are still of interest due to their potential involvement in gastric cancer progression. In summary, the homologous recombination pathway emerges as one of the most significant pathways in the analysis, with a high level of statistical confidence, suggesting its critical role in gastric cancer development. Other pathways, such as P53 signaling and PI3K-Akt signaling, though important in general cancer biology, are less specifically tied to gastric cancer in this context.

## Discussion

In this study, the landscape of BRCA1/2 mutations in gastric cancer patients was explored utilizing the NGS technique. Our results demonstrated high-quality sequencing data with a phred score exceeding 30 for 95% of bases, a minimum coverage depth of 500X, and a uniformity of coverage reaching 99%. This robust sequencing approach allowed us to identify a total of 11 mutations in BRCA1 and 4 mutations in BRCA2 among gastric cancer patients. Notably, our findings revealed a higher frequency of mutations in the BRCA1 gene compared to BRCA2, suggesting its potential significance in gastric cancer pathogenesis. Results of the study further revealed one pathogenic mutation in BRCA1 (p.Tyr1853Ser) and one in BRCA2 (p.Trp31Ser), underscoring the clinical relevance of these mutations in gastric cancer. The BRCA1 (p.Tyr1853Ser) pathogenic mutation has previously been reported in a lung cancer patient [30]. However, to the best of our knowledge, no studies have reported the presence of this mutation in gastric cancer patients, highlighting its rarity in this type of cancer. Similarly, the BRCA2 (p.Trp31Ser) mutation has been detected in patients with Fanconi anemia, yet there is no available literature documenting this mutation in gastric cancer. The absence of reports on these mutations in gastric cancer suggests that they may be exceedingly rare or not commonly associated with the disease. Further studies are needed to explore the prevalence and clinical significance of these mutations in gastric cancer, as understanding their occurrence could have important implications for genetic screening and targeted therapies in this cancer subtype.

Pathogenic mutations in BRCA1 and BRCA2 genes are implicated in the pathogenesis of various cancers, including breast and colorectal cancers [31-34]. These mutations disrupt critical cellular functions, such as DNA repair mechanisms mediated by the homologous recombination repair pathway, leading to genomic instability and accumulation of genetic mutations [35-38]. Additionally, mutated BRCA1/2 genes impair tumor suppression mechanisms, deregulate signaling pathways involved in cell proliferation and survival, and confer a genetic predisposition to cancer development [39-41]. These combined effects are thought to significantly promote the initiation, progression, and aggressiveness of gastric cancer, making BRCA1/2 mutations a potential driver of tumorigenesis in this cancer type. Thus, the pathogenic mutations in BRCA1/2 not only predispose individuals to cancer development but also influence the malignancy's aggressiveness [42, 43]. Moreover, the absence of BRCA1 (p.Tyr1853Ser) and BRCA2 (p.Trp31Ser) pathogenic mutations in the TCGA dataset further suggests their uniqueness and potential relevance to specific Pakistani population. This emphasizes the importance of population-specific studies in elucidating the genetic landscape of cancer and guiding personalized treatment strategies [44-46].

The results from the RT-qPCR analysis demonstrated a significant reduction in BRCA1/2 gene expression levels in gastric cancer samples harboring pathogenic mutations, suggesting that these mutations may have a regulatory impact on gene expression. This reduction could be due to the presence of mutations that impair transcriptional activity or destabilize the mRNA, leading to decreased expression levels. The correlation between BRCA1/2 mutations and reduced gene expression aligns with previous studies in other cancers, such as breast and ovarian cancer [47-49], where BRCA1/2 mutations are known to disrupt normal gene function, often contributing to cancer progression. Furthermore, the immunohistochemistry (IHC) analysis revealed diminished BRCA1/2 protein expression in the same gastric cancer samples with pathogenic mutations, reinforcing the idea that these mutations not only affect gene transcription but also have a direct impact on protein expression. Furthermore, KEGG enrichment analysis highlighted the association of BRCA1/2 genes with key cancer-related signaling pathways, including homologous recombination, the fanconi anemia pathway, and various cancer pathways. This suggests a potential role of BRCA1/2 mutations in dysregulating these pathways, contributing to gastric cancer development and progression [50-53].

## Conclusion

In conclusion, our study sheds light on the landscape of BRCA1/2 mutations in gastric cancer patients, highlighting their clinical relevance and potential implications for diagnosis and therapy. The identification of pathogenic muta-

tions in BRCA1/2 genes underscores the importance of genetic testing in gastric cancer patients, facilitating personalized treatment strategies. Furthermore, our findings emphasize the need for population-specific studies to elucidate unique mutation patterns and their therapeutic implications. Future research focusing on the mechanistic roles of BRCA1/2 mutations in gastric cancer pathogenesis and exploring targeted therapies tailored to these genetic alterations holds promise for improving patient outcomes in the clinical management of gastric cancer.

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## Disclosure of conflict of interest

None.

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