A novel *BRCA1* duplication and new insights on the spectrum and frequency of germline large genomic rearrangements in *BRCA1/BRCA2*

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Research Article

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Abstract

Heritable breast cancers account for 5 to 10% of all breast cancers, and monogenic, highly penetrant genes cause them. Around 90% of pathogenic variants in *BRCA1* and *BRCA2* are observed using gene sequencing, with another 10% identified through gene duplication/deletion analysis, which differs across various communities. In this study, we performed a next-generation sequencing panel and MLPA on 1484 patients to explain the importance of recurrent germline duplications/deletions of *BRCA1-2* and their clinical results and determine how often *BRCA* gene LGRs were seen in people suspected of hereditary breast and ovarian cancer syndrome. The large genomic rearrangements (LGRs) frequency was approximately 1% (14/1484). All the 14 mutations were heterozygous and detected in patients with breast cancer. *BRCA1* mutations were more predominant (n = 8, 57.1%) than *BRCA2* mutations (6, 42.9%). The most common recurrent mutations were *BRCA2* exon three and *BRCA1* exon 24 (23) deletions. To the best of our knowledge, *BRCA1* 5'UTR-exon11 duplication has never been reported before. Testing with MLPA is essential to identify patients at high risk. Our data demonstrate that *BRCA1-2* LGRs should be considered when ordering genetic testing for individuals with a personal or family history of cancer, particularly breast cancer. Further research could shed light on *BRCA1-2* LGRs' unique carcinogenesis roles.

Introduction

Hereditary breast and ovarian cancer syndrome (HBOC) is characterized by an increased incidence of male and female breast cancer, ovarian cancer (Fallopian tube and peritoneal cancers), and, to a lesser extent, other cancers such as prostate cancer, pancreatic cancer, and melanoma. For breast and ovarian cancers, germline genetic testing has long been used to assess the likelihood of hereditary cancers. Heritable breast cancers account for 5 to 10% of all breast cancers, and they are caused by monogenic, highly penetrant genes [1].

HBOC is caused by germline *BRCA1* and *BRCA2* mutations [2]. *BRCA1* and *BRCA2* germline mutations are responsible for up to 30% of all inheritable breast cancers [3]. More than 2000 pathogenic variants in the *BRCA1* and *BRCA2* genes have been identified [4]. Carriers of the *BRCA1* mutation have a 60%-65% risk of developing breast cancer (BC) until the age of 70 and a 40%-60% risk of developing ovarian cancer (OC). The corresponding percentages for *BRCA2* mutation carriers are 45–55 percent for BC and 11–16.5 percent for OC [5]. The most prevalent malignancy in individuals with a germline *BRCA1* or *BRCA2* pathogenic variant is breast cancer, which varies from 46–87%. *BRCA* germline pathogenic variants raise the incidence of ovarian cancer from 16.5–63%. *BRCA1* and *BRCA2* germline pathogenic forms are inherited in an autosomal dominant pattern. Each person with a *BRCA1* or *BRCA2* germline pathogenic variant has a 50% risk of transmitting the variant to their offspring. Around 90% of pathogenic variants in *BRCA1* and *BRCA2* are observed using gene sequencing, with another 10% identified through gene duplication/deletion analysis, which differs across various communities [6]. The effects of *BRCA* mutations have also been discovered to be connected to prostate, pancreatic, stomach, and colorectal

the level of harm [7].

The majority of *BRCA1* and *BRCA2* mutations are small deletions, insertions, nonsense mutations, or splice variants that result in a truncated protein. Despite this, a number of significant large genomic rearrangements (LGRs) involving these genes have been reported [8]. These modifications are generally pathogenic since deletions or insertions of broad genomic sequences inside a coding area result in out-of-frame translation, resulting in a nonfunctional mutant protein. *BRCA1* LGRs may account for a sizable proportion of all disease-causing mutations in various populations, while *BRCA2* LGRs are less commonly found [9].

Various techniques were used to identify large deletions and duplications, including southern blotting, semiquantitative multiplex PCR, real-time PCR, restriction analysis, long-range PCR, and sequencing [10]. Multiplex Ligation-dependent Probe Amplification (MLPA) has been the most frequently used strategy for detecting these *BRCA1/2* gene mutations [8]. The most successful technique for routine *BRCA1/2* molecular screening was a diagnostic molecular algorithm focused on NGS (as the first step) and MLPA (as the second step). Large genomic rearrangements (LGRs) have been extensively studied in breast and ovarian cancer patients from various countries during the last several years. Numerous experiments demonstrate their presence in the genetic predisposition to gynecological tumors, and a large number of novel *BRCA1/2* gene rearrangements have been recorded [11].

In this study, we performed a next-generation sequencing panel and MLPA on 1484 patients to explain the importance of recurrent germline duplications/deletions of *BRCA1-2* and their clinical results and determine how often *BRCA* gene LGRs were seen in people suspected of HBOC syndrome. Our data broadens the spectrum of *BRCA* gene LGRs and provides insights for genotype-phenotype correlations for hereditary breast and ovarian cancer syndrome.

Materials And Methods

Patients

Consent for the publication and any additional related information was taken from the patients or their parents involved in the study. Most of the patients came to our clinic in their first few years of diagnosis. Clinical histories and molecular results were reviewed for all unrelated patients who examined at the Department of Medical Genetics, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey. According to National Comprehensive Cancer Network (NCCN) guidelines for breast-ovarian cancer, patients were evaluated. Patients underwent *BRCA1-2* NGS panel test between January 2017 and December 2020 at Ankara Central Genetic Laboratory (Ankara, Turkey). Negative patients and patients with NGS results suspected of possible deletion/duplication underwent MLPA test. All have a strong family history with at least three cancers in relatives (1st, 2nd, 3rd degree). Patients with uncertain/missing data have been filtered. Participants who underwent the MLPA test for the *BRCA1*/ *BRCA2* deletions or duplications have been chosen for the study..

DNA Panels And NGS

Blood samples were collected in EDTA tubes. The patients' DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) by QIAcube (Qiagen Inc Mississauga, ON, Canada). The DNA samples were quantified with a NanoDrop 1000 (Thermo Fisher Scientific Inc., MA, USA) spectrophotometer.

Qiaseq targeted DNA panel (DHS-102Z, Human BRCA1 and BRCA2 Panel) or Multiplicom BRCA MASTR Dx (Multiplicom N.V., Niel, Belgium) kits have been used for BRCA sequencing. The sequencing was performed on the Illumina MiSeq system (Illumina Inc., San Diego, CA, USA). The data were analyzed on QIAGEN Clinical Insight (QCI™) Analyze software (QIAGEN, Hilden, Germany) and Sophia DDM software (Sophia Genetics, Saint- Sulp). Visualization of the data was performed with IGV 2.7.2 (Broad Institute) software.

For MLPA, SALSA MLPA probemix P002 BRCA1, SALSA MLPA probemix P087 BRCA1, SALSA MLPA probemix P045 BRCA2/CHEK2, SALSA MLPA probemix P077 BRCA2 (MRC Holland, Amsterdam, Netherlands) kits were used. The study was performed on the ABI 3130 Genetic Analyser system (Applied Biosystems, Carlsbad, California, USA). For data analysis, the Coffalyser.Net tool was used (MRC Holland, Amsterdam, The Netherlands). The BRCA1 exon numbering used in this P002 BRCA1 product description is the traditional exon numbering (exons 1a, 1b, 2, 3, and 5–24), wherein no exon 4 is present; the BRCA1 exon numbering in the BRCA1 LRG_292 sequence and the NCBI NG_005905.2 reference sequence (mentioned within round brackets in manuscript) is different (https://www.mrcholland.com/).

Interpretations, Descriptive Statistics And Graphics

The LRGs were named using Human Genome Variation Society (http://www.HGVS.org/varnomen) guidelines and classified using the American Society of Medical Genetics and Genomics (ACMG) criteria for the interpretation and reporting of single gene copy number variants [12]. Descriptive statistical calculations have been made, and graphics have been prepared with Python (version 3.9.2).

Results

Females (1442, 97.17%) were more than males (42, 2.83%). The mean age was 49 (in females 48.6 and males 57.8) with a minimum age of 19 and a maximum of 88. Most of the patients were between 40–60 ages (Fig. 1).

The large genomic rearrangements (LGRs) frequency was approximately 1% (14/1484). All the 14 mutations were heterozygous and detected in patients with breast cancer. *BRCA1* mutations were more predominant (n = 8, 57.1%) than *BRCA2* mutations (6, 42.9%) (Fig. 2). The most common recurrent mutations were *BRCA2* exon 3 [NG_012772.3(LRG_293):g(8697_8847)del] and *BRCA1* exon 24 (23) [NG_005905.2(LRG_292):g.(172181_172307)del] deletions (Fig. 2). The only *BRCA2* mutation, *BRCA2* exon 3 deletion, has been observed six times. *BRCA1* exons 18–19 (17–18) [NG_ 005905.2(LRG_292):g.(154032_154652)del] deletion, *BRCA1* exon 11 (10) [NG_ 005905.2(LRG_292):g.(123123_126550)del] deletion, *BRCA1* exon 13 (12) [NG_005905.2(LRG_292):g.(170280_170342)del] deletion, *BRCA1* exons

1a-11 (5`UTR_ex10) [NG_ 005905.2(LRG_292):g.(?_126550)dup] duplication, and *BRCA1* exons 3–8 (3–7) [NG_ 005905.2(LRG_292):g.(102204_118210)dup] duplication were observed once. Duplications were observed only in *BRCA1* gene. Majority of the LGRs were one exon deletion in either *BRCA1* or *BRCA2*. Even though *BRCA1* LGRs were predominant, the most recurrent mutation was observed in *BRCA2* (Fig. 2).

Discussion

This is the first study mentioning the importance of recurrent *BRCA* large genomic rearrangements (LGRs) in patients with breast cancer phenotype in the Turkish population. Recurrent and other LGRs were observed only in the patients with breast cancer. The principal finding of our study is that *BRCA1* LGRs were more predominant in the Turkish population, but *BRCA2* mutation was more recurrent. The mean age in males and females were 57.8 and 48.6, respectively. Most of the cancers observed between 40–60 years of age.

Currently, the MLPA procedure is the gold standard for establishing a conclusive molecular diagnosis. The *BRCA* Tumor (Multiplicom, Niel, Belgium) Panel and Sophia DDM framework (Sophia Genetics SA, Saint Sulpice, Switzerland) were found to be the most appropriate combination for concomitant and optimal CNV, SNV, and indel detection in this situation[13]. In this study, it was also observed that analyzing LGRs with Sophia DDM was very helpful and informative. Researchers have also shown that *BRCA1* gene mutations occur more often in high-risk cancer databases than *BRCA2* gene mutations [14]. The present study's findings corroborate current information in the literature [15]. CNVs are more prevalent in the *BRCA1* gene because of the proliferation of intronic Alu repeat sequences [16].

The most recurrent rearrangement detected in this study was *BRCA2* exon three deletions, which was found to be associated with a high risk of breast and ovarian cancer [17] (Fig. 2). This mutation and *BRCA1* exon 24 deletion were detected in both female and male breast cancers. Both deletions and *BRCA1* exons 18–19 deletion reported many times across Europe [18–20]. We observed high *BRCA* LGRs frequency in triple-negative breast cancer patients (9/14), as mentioned in the literature [21]. LGRs were not detected in patients with the ovarian, pancreas, or other cancers except for breast cancer. Because the respective exons are not in the frame, the altered transcript will degrade through nonsense-mediated decay. As a result, *BRCA1* would not colocalize with *BARD1* and *BACH1*, preventing DNA repair. The detected LGRs were classified as pathogenic based on these criteria.

To the best of our knowledge, *BRCA1* $5UTR - exon11(5$UTR_ex10) [NG_ 005905.2(LRG_292):g.(?_126550)dup] duplication has never been reported before (Fig. 3). It was observed in a 57-year-old female patient with bilateral invasive ductal carcinoma. Bilateral tumor status was ER+, PR+, and c-erbB2 scores were 2+. Bilateral axillary lymph node metastases were detected. Her deceased father was diagnosed with gastric cancer at the age of 86. She has two female cousins with breast cancer connected with her mother. No cancer was detected in her mother, her two brothers, and her two sisters.

Ten percent of the pathogenic variants in *BRCA1* and *BRCA2* were established through deletion/duplication tests, which may differ between populations [6, 22]. The frequency of the LGRs was approximately 1% in this study. Among all the *BRCA* mutations detected in the center, the frequency of the LGRs was 6%. The authors experienced an average LGR rate of 7.9 percent in the Myriad data [6]. According to Smith et al., the overall CNV rate for *BRCA1* and *BRCA2* was marginally higher, with an average of 11.9 percent in European families [23].

The results of this study help provide current information about the relationship between *BRCA1* and *BRCA2* LGRs status and the development of breast cancer. The main strength of our study is that it involved a diverse, well-defined community of participants, many of whom had symptoms that manifested in the clinical sense, allowing us to generalize our findings to patients. We used a specialized diagnostic center with extensive cancer gene testing experience to do an exhaustive and functional cancer gene analysis.

Conclusion

Our study gives a novel insight into the diagnosis of patients suspected of having breast cancer. Testing with MLPA is essential to identify patients at high risk. Our data demonstrate that *BRCA1-2* LGRs should be considered when ordering genetic testing for individuals with a personal or family history of cancer, particularly breast cancer. Further research could shed light on *BRCA1-2* LGRs' unique carcinogenesis roles. To detect and explain the critical mutations, large cohorts are needed. A more detailed examination of cancer genetics research is needed to improve patient risk management, prognosis, and treatment decisions.

Declarations

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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No financial assistance was received.

Availability of data and material

The data (including patient demographic information and mutations) and the code of the current study is available from the corresponding author on reasonable request.

Authors' contributions

IS designed the research, analyzed and interpreted the results (including the coding part with Python version 3.9.2), wrote the manuscript, and approved the final manuscript.

HS collected the data, analyzed and interpreted the results, reviewed the manuscript, and approved the final manuscript.

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Figures

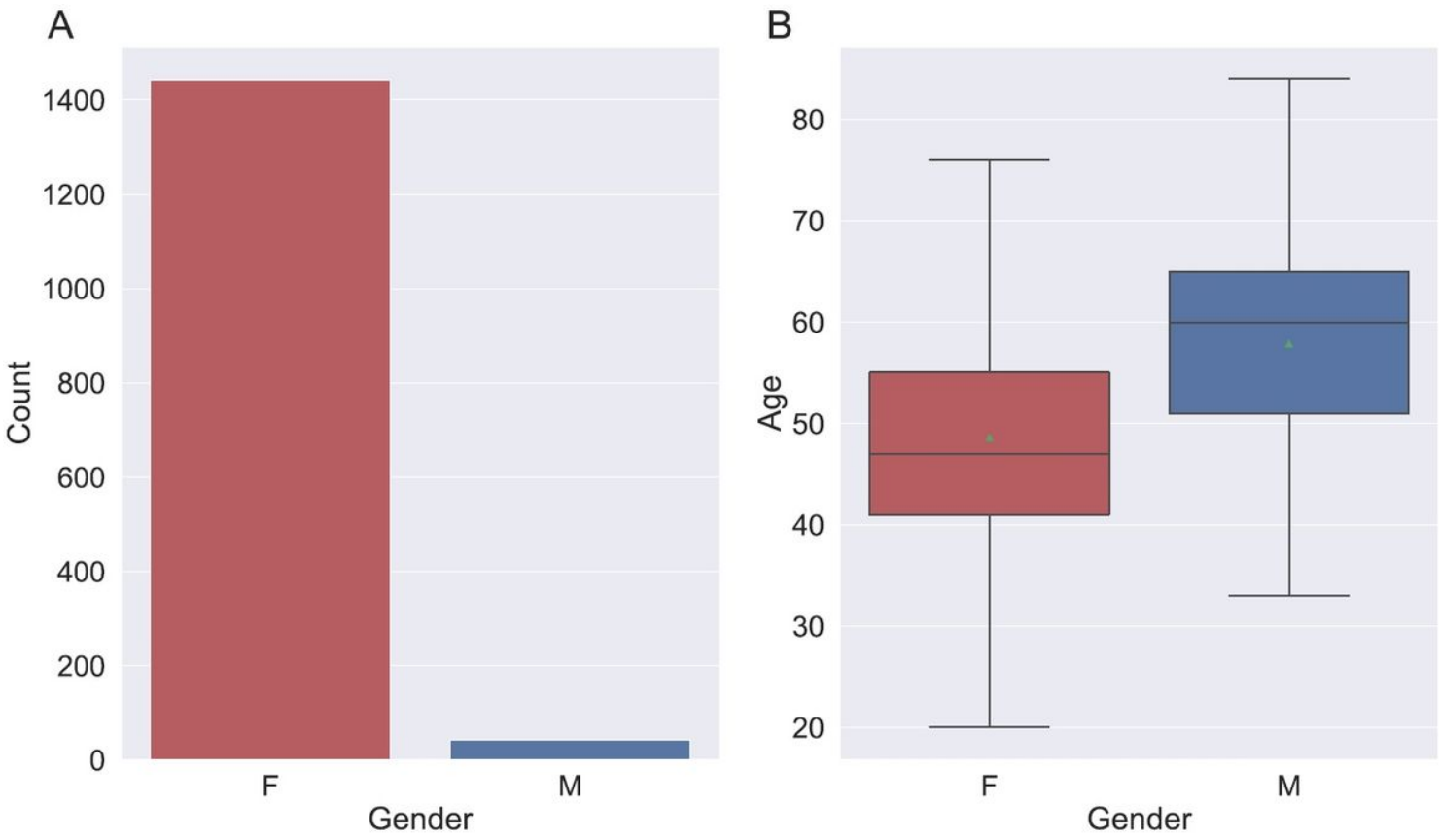


Figure 1

Patients characteristics a. Bar plot showing the number of patients in terms of gender. b. Boxplot showing mean (green triangle) and median (black line) age of the patients in terms of gender.

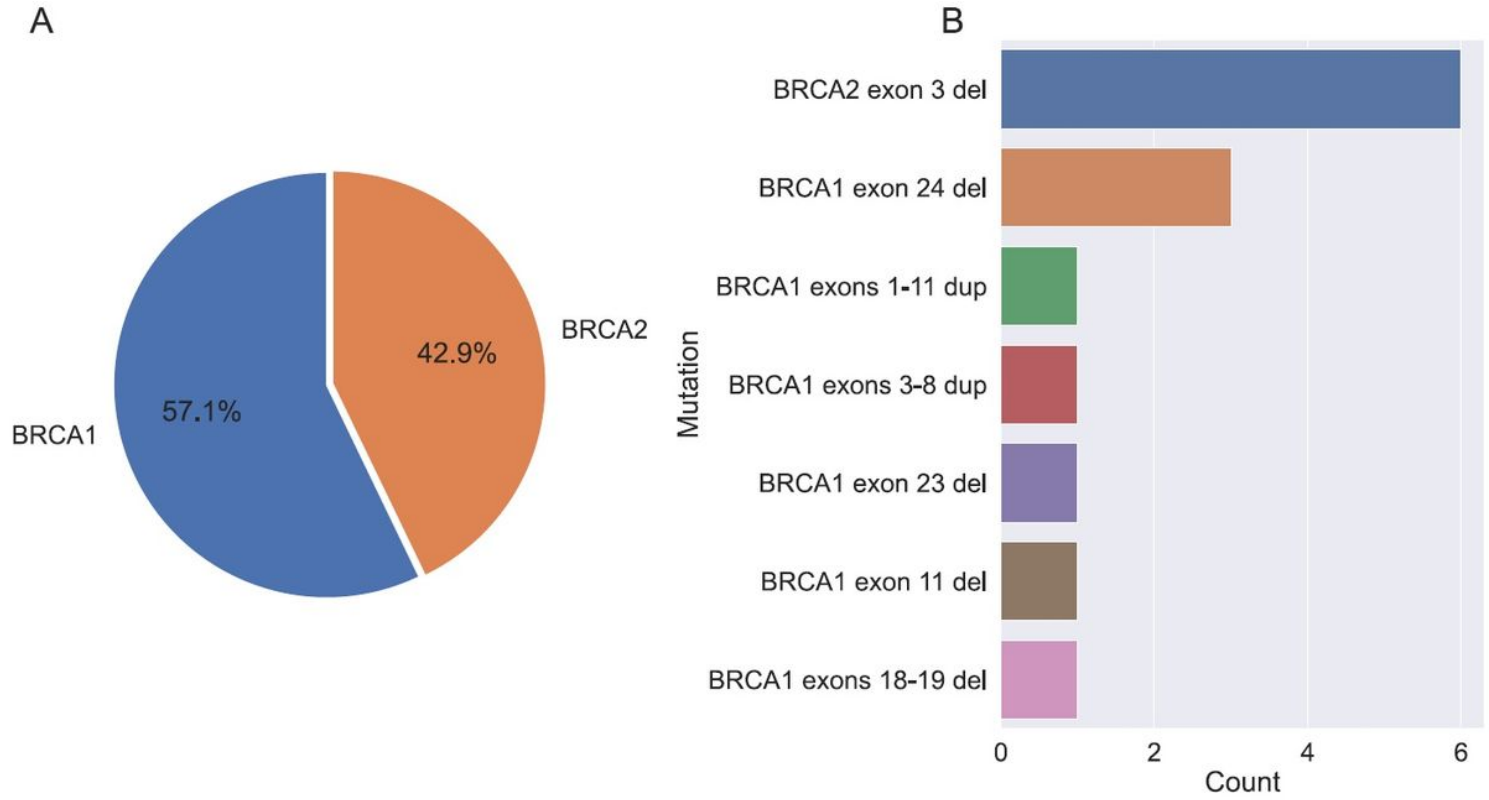
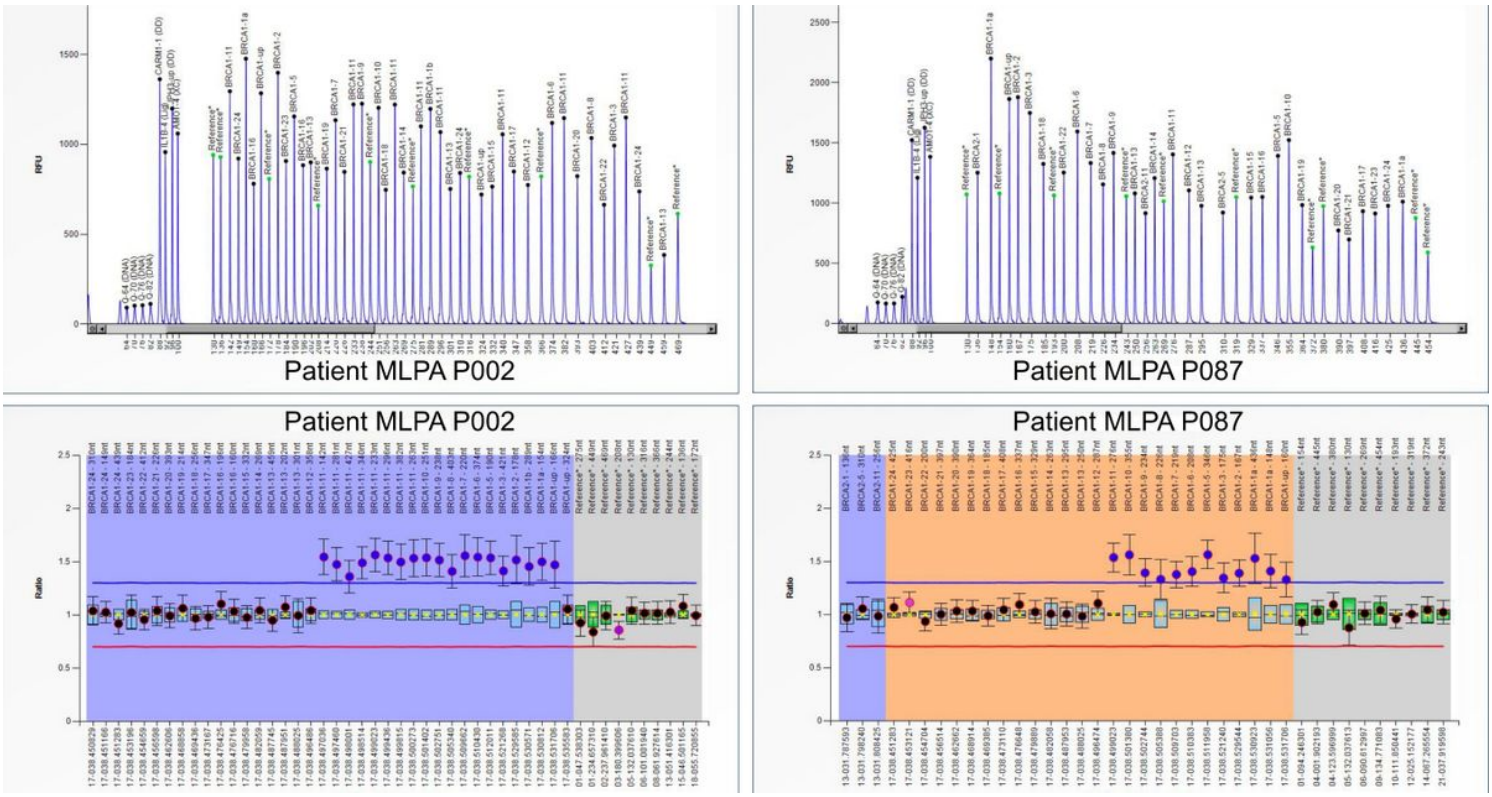


Figure 2

Spectrum of the genes and mutations a. Pie chart showing the spectrum of the mutated genes with percentages in the study. b. Bar plot showing the most common mutations in the study.



MLPA results of the novel mutation Confirmation of the presence of a novel duplication of 5'UTR-exon11 detected in BRCA1 using the SALSA® MLPA® P002 and P087 probe mix. Graphical representation of the results using Coffalyser.Net tool (MRC Holland, Amsterdam, The Netherlands).