Next-Generation Sequencing Analysis of Hereditary Ovarian Cancer Gene Panels in Turkish Population

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Research Article

Keywords: Next generation sequencing, Ovarian cancer, Hereditary cancer, BRCA1 & BRCA2, Multigene panels, Genetic test

Posted Date: January 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1241858/v1

Abstract

Purpose: Ovarian cancer is the seventh most frequent cancer in women worldwide and the eighth leading cause of death from cancer. Approximately 5–10% of all diagnosed cancer cases are caused by hereditary cancer risk syndromes. The aim of this study was investigate the prevalence of pathogenic variants associated with hereditary ovarian cancer propensity inTurkish people.

Methods:630 patients with personal or a family history of breast and/or ovarian cancer and other cancers included in the study. The Illumina NGS technology was used to enrich genomic DNA.

Results:118 of the 630 tested people have pathogenic mutation (20.0%). The mutation distribution detected in a total of 130 patients was 131. 1 mutation (1,315%) in *ATM* , 40 different mutations (52.630%) in *BRCA1*, 29 different mutations in *BRCA2* (38.165%), 2 different mutations (2,630) in *CHEK2*, 1 mutation (1,315%) in *ERCC2*, 1 mutation (1,315%) in *MUTYH*, 1 mutation (1,315%) in *RAD51C*, and 1 mutation (1,315%) were detected in *TP53*.Notably, in a patient with ovarian cancer both *BRCA1* and *BRCA2* mutations; *BRCA1* NM_007294.3Ex1c.135-2A>Gp.?rs80358065 and *BRCA2* NM_000059.3 Ex 11 c.6466_6469delTCTC p.Ser2156Asnfs*11rs879255330 were found, respectively.

Conclusion: Analysis of gene panels demonstrate the clinical importance of multigene panel analysis in hereditary cancer predisposition meeting the *BRCA1/2* NCCN criteria in Turkish population.

Introduction

In industrialized nations such as Poland, the United States, and the United Kingdom, breast cancer is the most prevalent cancer in women; ovarian cancer is the second most common gynaecological cancer [1]. Ovarian cancer is responsible for 150,00 fatalities, making it the seventh most prevalent cancer and the eighth most common cause of cancer mortality among women [2]. While age-standardised cancer rates are constant or declining in the majority of high-income nations,the cancer statistics are rising in many low- and middle-income countries [3]. According to Globocan 2018 data, 295.414 people are diagnosed with ovarian cancer, while 2.088.849 people are diagnosed with breast cancer each year. In Turkey, the rate of breast and ovarian cancer has virtually risen in recent years [4].

The ovarian cancer has such a high mortality rate may be due to its confusing, nonspecific, and often asymptomatic nature. When the disease has progressed outside the ovary, alarming symptoms generally emerge. Early-stage ovarian cancer limited to the ovary is frequently discovered by chance and has a five-year survival rate of 92% [5]. The mortality rates from ovarian cancer have remained largely unchanged for over five decades [6].

Approximately 5–10% of all cancer cases are caused by hereditary cancer predisposition syndromes [7, 8]. It is important to identify those syndromes for the patient as well as at-risk family members. Surgical management and, in certain situations, systemic treatment can be guided by genetic assessment of the inherited etiology of the diagnosis in affected patients. Furthermore, determining the underlying

syndrome can help lead a customised follow-up program for the patient and at-risk families, which can include surveillance and prevention process for secondary cancers linked to the specific syndrome.

Single-gene analysis of specific high-risk genes was previously utilized to determine the genetic cause of cancer heredity in specific families. The *BRCA1* and *BRCA2* genes for families with a breast/ovarian cancer history, the DNA mismatch repair (MMR) genes, *MLH1, MSH2*, and *MSH6*, for families suspected of having Lynch Syndrome, and the *APC* gene in patients with family history Familiar Adenomatous Polyposis (*FAP*) were all chosen based on the individual's personal familiy history.The advent of Next Generation Sequencing (NGS) has enabled multi-gene panel analysis, which is now frequently utilized in clinical practice to identify people who have an inherited cancer predispoisition [9, 10].

The association between *BRCA1* and *BRCA2* germline mutations and the risk of ovarian cancer is widely known. Although it is assumed that germ-line *BRCA*1/2 mutations cause about 5–10% of all ovarian cancers and new research suggests that this figure is likely underestimated [11]. Germline *BRCA1/2* mutation testing is now available based on family history in the context of assessing and managing cancer risks in family members, and is currently varied both within and between nations [12] .

Patients with germ-line *BRCA1/2* mutations have shown better response to platin-based chemotheraphy and survival for ovarian cancer. The promise activity of poly(ADP-ribose) polymerase inhibitors in *BRCA*1/2 mutation carriers has gained interest in early *BRCA1/2* testing in the treatment process. Despite this, the value of taking a patient's *BRCA1/2* mutation status into account when planning treatment is unknown and is not commonly utilized in the treatment of patients with ovarian cancer. In this study, we examine a population-based cohort of 630 Turkish women diagnosed with ovarian/breast cancer to determine mutation frequency and to demonstrate the size of the targeted gene sequencing panels enhanced the number and diversity of mutations discovered.

Materials And Methods

Study group

Patients who applied to Department of Cancer Genetics, Istanbul Faculty of Medicine, Oncology Institute for genetic test and met the NCCN *BRCA1/2* test requirements were evaluated for hereditary cancer panel between 2016 and 2021. During this study, all blood samples were gathered from the cancer patients. All patients were informed about the importance of the genetic test. Prior to molecular genetic testing, the patients completed an informed consent form and gave perimision use of their personal and family history data for research and/or scientific publications. Test forms were used to obtain data on demographics, clinical history, and family history of cancer.

Gene selection:

In this study, 3 different gene panels with combinations of 60 genes were used. Three panels were used to conduct NGS analysis of inherited cancer susceptibility genes including, Multiplicom BRCA MASTR Plus Dx 2 gen, SOPHIA Hereditary Cancer Solutions 27 genes and SOPHIA Hereditary Cancer Solutions 60 genes. The genes studied were chosen due to their association to genetic cancer predisposition. Fanconi anemia is linked to the genes *BRCA2, BRIP1, PALB2*, and *RAD51C. ATM* and *MRE11A* are linked to ataxia-telangiectasia-like condition (*ATLD*) and ataxia-telangiectasia, respectively. Constitutional mismatch repair deficiency is linked to the genes *MLH1, MSH2, PMS2*, and *MSH6 (CMMR-D). MUTYH* is linked to MUTYH-related polyposis (*MAP).* Nijmegen breakage syndrome and Nijmegen breakage syndrome-like disorder (NBSLD) are linked to *NBN* and *RAD50*, respectively. The majority of genes studied in this study are related with higher risk of Breast and/or Ovarian cancer, as the most of patients who required hereditary cancer testing had a personal or family history of Breast and/or Ovarian cancer.

DNA Isolation

The QIAamp DNA Blood Mini Kit (QIAGEN) Genomic DNA Whole Blood Kit was used to extract genomic DNA from peripheral blood, and the NanoDrop 2000c Spectrophotometer was used to quantify it (Thermo Fisher Scientific).

Next generation sequencing

TruSight Rapid Capture target enrichment process and the TruSight Cancer panel, both from Illumina, Inc., (San Diego, CA, USA) apply previously developed NGS pipeline were used during the study [13].Library preparation, sequencing, bioinformatics, and data analysis were carried out [14, 15].TruSight Enrichment DNA Sample techonolgy (Illumina, San Diego, CA) was used for enrich the samples and Illumina MiSeq® v3 (2x300bp) (Illumina, San Diego, CA) for sequencing.

Data Analaysis

The Sophia Genomic Alignment and Variant Calling software was used to match sequences to the reference genome (GRCh37/hg19) for variant analysis. Version 5.10.6 of the SOPHiA DDM software (Saint-Sulpice, France) for independents read alignment and variant calling. VariantStudio software version 2.1 (Illumina), SeqNext, and SOPHiA DDM software were used to analyze the generated variant call files for annotation and first-step filtering of the variants. dbSNP, G1000, ExAC, GnomAD, SIFT, POLYPHEN2,MUTATION TASTER, ClinVar, HGMD data bases are used to identify the pathogenic variants.

Results

With the development and use of high-throughput next-generation sequencing during the last decade, sequencing technology has advanced quickly. Thanks to improvements in bioinformatics analysis, in

comparison to traditional germline testing for mutation in a single gene method, the use of targeted NGS-based multigene testing panels to give complete analysis of cancer susceptibility genes has shown to be a feasible option [16–18].

Using Illumina's NGS technology, we designed and validated totally 3 gene panels including Multiplicom BRCA MASTR Plus Dx 2 gen, SOPHİA Hereditary Cancer Solutions 27 genes and SOPHİA Hereditary Cancer Solutions 60 genes. Out of 630 patients, 562 (89.20%) patients were Multiplicom BRCA MASTR Plus Dx, 43 (6.83%, 6.83) patients SOPHİA Hereditary Cancer Solutions 27 genes, and 25 (3.97%) patients were tested with SOPHIA Hereditary Cancer Solutions 60 genes. The Supplementary Material 1 showes Pathogenic germline mutations in Turkis Breast/Ovarian Cancer Families.

Patient demographics: Between 2016 and 2021, 630 ovarian patients applied to Department of Cancer Genetics, Istanbul Faculty of Medicine, Oncology Institute labratuary for genetic test. All patients referred for genetic counseling are from all seven region of Turkey. The median age of the patients was 51 (Age range: 25-84 years old.) In this study, we used Next-Generation Sequencing (NGS) to verify three different gene panels for evaluating ovarian cancer mutations in Turkish population. These panels will sequence and analyze the protein-coding areas of the targeted genes to look for cancer-causing mutations in 8 distinct malignancies: breast, ovarian, prostate, uterine, colorectal, pancreatic, stomach cancers, and melanoma. The aim of this research was to evaluate the hereditary cancer panel's analytical and functional capabilities.

The participants in this prospective research were 630 Turkish people, with 546 (86.6%) having ovarian cancer, 84 (13.33%) having breast and ovarian cancer together. The total number of patients was 630 (100%). 500 patients out of 630 patients did not carry any mutations in the investigated genes (79.36%), whereas 130 patients (20.64%) carried mutations.

Out of 130 patients with mutation, 81 of them substantially have *BRCA1* pathogenic variants, while *BRCA2* pathogenic variants were detected 43 in patients. 40 different *BRCA1* mutations (52.630%) were detected in patients. In the *BRCA1* gene, the three most frequent mutations are as follows: BRCA1 NM_007294.3,EX 19 c.5266dupC p.Gln1756Profs*74 rs431825413 (detected in 13 ovarian patients and 2 breast+ovarian cancer patients), *BRCA1* NM_007294.3 EX4 c.181T>G p.Cys61Gly rs28897672(in 5 ovarian+2 breast+ovarian patients), and *BRCA1* NM_007294.3 EX 23 c.5444G>A p.Trp1815* rs80356962(in 5 ovarian patients). According to the Breast Cancer Information Core database *BRCA1*:c.5266dupC is one of the most commonly reported mutations in *BRCA1* and in the Ashkenazi Jewesh population, it is recognized founder muatation. c.5266dupC, on the other hand, is less common in the AJ population (0.13 percent) [PubMed] [Google Scholar] and has been found in a variety of other groups, especially in Europe. These mutations in the *BRCA1* gene have been identified as the most prevalent alterations in our country's population that cause ovarian cancer. In Turkish ovarian cohort this mutation also can be used as a founder mutation in accordance with the literature.

In 43 of 130 patients, 29 different mutations (38.165%) were found in the BRCA2 gene. The three most common mutations are as follows: *BRCA2* NM_000059.3 Ex 25 c.9317G>A p.Trp3106* rs80359205 in 5

patients 4 of whom were diagnosed with ovary and 1 with ovary and breast cancer, *BRCA2* NM_000059.3 EX23 c.9097dupA p.Thr3033Asnfs*11 In 3 individuals, 1 of which is over and 2 is over + breast together, BRCA2 NM_000059.3 ex 2 c.67+1G> A rs81002796 was seen in 3 patients with ovarian ca. GeneKor MSA has identified a number of variations in the *BRCA2* gene that are pathogenic including *BRCA2* NM_000059.3 Ex 25 c.9317G>A p.Trp3106* rs80359205 [6] that we detected in 5 Turkish ovarian patients one with ovarian+breast together. According to literature, the *BRCA2* c.9097dupA mutation is the most prevalent in the Turkish population, and it is also highly common in eastern nations [19] in accordance with our study group. *BRCA2* NM_000059.3 ex 2 c.67+1G>A rs81002796 is registered as pathogenic variant in clin var database.

1 patient diagnosed with ovarian ca carries 2 different mutations in *BRCA1* and *BRCA2*. Mutations of this patient; BRCA1 NM_007294.3 Ex1 c.135-2A>G p.? rs80358065 and BRCA2 NM_000059.3 Ex 11 c.6466_6469delTCTC p.Ser2156Asnfs*11 rs879255330,respectively. In the Pakistani community, 539 breast cancer patients who were chosen based on their family history and diagnostic age were invesitgated and *BRCA1* NM_007294.3 Ex1 c.135-2A>G p.? rs80358065 was detected as deletirious mutataion [20].

84 patients diagnosed with both ovarian + breast cancer. A total of 21 different mutations were detected in 27diagnosed both breast+ovarian cancer carrier patients (32.14%). The mutations found in patients diagnosed both breast and ovarian ca are as follows: *BRCA1* NM_007294.3 EX 10 c.1151_1154dupAGTG p.Trp385*fs*1 (in 2 patients), *BRCA1* NM_007294.3 ex10 c.1016delA p.Lys339Argfs*2 rs80357618 (1 patient), *BRCA1* NM_007294.3 ex 10 c.1621C>T p.Gln541* rs80356904(1 patient), *BRCA1* NM_007294.3 ex10 c.3794delA p.Asn1265Ilefs*3 rs80357767 (in 1 patient), *BRCA1* NM_007294.3 ex16 c.4717delG p.(Asp1573Metfs*28) (in one patient), *BRCA1* NM_007294.3 ex19 c.5209A>T p.Arg1737* rs80357496 (in one patient), *BRCA1* NM_007294.3 ex2 c.66dupA p.(Glu23Argfs*18) rs80357783 (in one patient), *BRCA2* NM_000059.3 ex11 c.5576_5579delTTAA p.Ile1859Lysfs*3 rs770318608(in 2 patients), *BRCA2* NM_000059.3 ex 11 c.5722_5723delCT p.Leu1908Argfs*2 rs80359530 (in 2 patients), *BRCA2* NM_000059.3 ex 9 c.771_775delTCAA p.Asn257Lysfs*17 rs80359675 (in 1 patient), *BRCA2* NM_000059.3 ex27 c.9682delA p.Ser3228Valfs*21 rs398122618 (1 patient).

A total of 78 individuals were tested using broad panels excpet *BRCA1* and *BRCA2*, and 7 mutations in 6 distinct genes, other than *BRCA1* and *BRCA2*, were discovered in 7 of these patients. *ATM* NM_000051.3ex53 c.7889T>A p.(Leu2630*) rs1591178998 in one patient with ovarian cancer, *CHEK2* NM_001005735.1 ex14 c.1580delC p.(Pro527Argfs*8) rs1555913078 in 1 patient diagnosed with ovarian, *CHEK2* NM_001005735.1 ex5 c.609A>G p.(Ile203Met) rs575910805 in a ovarian cancer diagnosed patient, *MUTYH* NM_001048171.1 ex14 c.1395_1397delGGA p.(Glu466del) rs587778541 was found in one ovarian cancer patient, *RAD51C* NM_058216.2ex7 c.934C>T p.(Arg312Trp) rs730881932 in 1 patient with ovarian cancer, *TP53* NM_000546.5 ex10 c.1024C>Tp.(Arg342*) rs730882029 in 1 patient with ovarian cancer.

The number of total mutations found in 130 patients is 131 (100%).1 patient carries two mutations (0.77%).1 patient carries two mutations (0.77%) and 129 patients are carriers of a single mutation (99.23%). A total of 76 different mutations (100%) including, 1 mutation (1,315%) in *ATM*, 40 different mutations (52.630%) in *BRCA*1,29 different mutations in *BRCA2* (38.165%), 2 different mutations (2,630) in *CHEK2*, 1 mutation (1,315%) in *ERCC2*, 1 mutation (1,315%) in *MUTYH*, 1 mutation (1,315%) in *RAD51C*, 1 mutation (1,315%) in *TP53* were found.

Discussion

We developed and validated the hereditary cancer test panels, which is intended to discover clinically significant germline mutations linked to hereditary risk for common cancer types (including breast, ovarian, colorectal, pancreatic, prostate, uterine, stomach, and melanoma). To support clinical usage, the test's performance is evaluated across a broad range of variations in 3 different gene panels including, Multiplicom BRCA MASTR Plus Dx 2 gen, SOPHIA Hereditary Cancer Solutions 27 genes and SOPHIA Hereditary Cancer Solutions 60 genes. The goal of this study was to figure out the mutation distribution in ovarian cancer patients in the Turkish population. Different mutations in the *BRCA1* and *BRCA2* genes have been shown to be responsible for the development of ovarian cancer in our nation. In addition, the influence of various genes on the development of ovarian cancer in the Turkish population was assessed, as was the growth in gene variety in hereditary cancer gene panels used in the screening of ovarian cancer patients over time. Our rate of detecting mutations in different genes rose in direct proportion to the rise in gene diversity in genetic testing. Furthermore, because some of the variants we observed in breast + ovarian cancer patients were only seen in individuals with two cancer types, we believe the variations we discovered are a founder mutation for patients with both cancer. This study used several NGS-based gene panels, and it is the first time in our nation that *BRCA1* and *BRCA2* genes have been found in such high numbers of ovarian cancer patients.

Declarations

Author Contribution:

SB Tuncer: Protocol/project development, Data collection or management

B Celik: Project development, Manuscript writing

SK Erciyas: Data collection

Ozge Sukruoglu Erdogan: Manuscript writing/editing

DA Odemis: Data Collection

BK Gültaşlar: Protocol development

AA Ghafour: Materials and Methods Development

MP Saip: Project development

H Yazici: Management

Acknowledgments:

The study was approved by the Ethics Committee of Istanbul Medical Faculty at Istanbul University (Ethical Approval No:2016/4, 08.01.2016).

Statements & Declarations:

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the committee of Istanbul Medical Faculty at Istanbul University (Ethical Approval No:196, dated February 19, 2016).

Research funding:

This research was funded by Research Projects Coordination Unit of Istanbul University.

Competing Interests:

Authors state no conflict of interest.

Author Contributions:

SB Tuncer: Protocol/project development, Data collection or management

B Celik: Project development, Manuscript writing

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Consent to participate:

Informed consent was obtained from all individuals included in this study.

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