

Prevalence of Mutation in BRCA1 gene, exons-2, 3, 5, 11 and exons-20 in breast cancer for early-onset detection in Tehran City

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Abstract

Breast cancer is the most common cancer among women, accounting for more than 30% of all cancers in world wide. The incidence rate among women younger than 25 and older than 79 is 1/20,000 and 1/9, respectively. Breast cancer caused by a mutation in BRCA1 gene has a higher incidence, higher mitotic rate, and more lymphatic penetrance than sporadic breast cancer. The purpose of present study is to investigate genetic mutations in Exons 2, 3, 5, 11 and 20 of BRCA1 gene and also the distribution of these mutations and the relationship between BRCA1 gene mutations with the prevalence rate of inherited breast cancer in the blood samples of women suspected of breast cancer in Tehran city. After the breast cancer genes BRCA1 were identified and genetic mutations in Exons 2, 3, 5, 11 and 20 of BRCA1 gene were investigated. The distribution of these mutations and the relationship between exons 2, 3, 5, 11 and 20 of BRCA1 gene mutations with the prevalence rate of inherited breast cancer in the blood samples of women suspected of breast cancer in Tehran city evaluated. Results of pathological of the breast cancer patients showed that 37 women (57%) were suffering from invasive cancer with ductal origin, 31 women (31%) were suffering from invasive cancer with milk cells origin, 5 women (5%) were suffering from in situ carcinoma with lobular origin, 7 women (7%) were suffering from in situ carcinoma with ductal origin. Among different exons of BRCA1 causing breast cancer that studied in this research, the changes in exons 2 and 3 had the highest distribution and exons 20 and 5 had the least distribution.

Keywords: Breast Cancer, BRCA1, Genetic Mutations, Exon

INTRODUCTION

Breast cancer is the main cause of cancer mortality among females worldwide each year and accounts for about 14% of cancer-related deaths [1]. The incidence of breast cancer among women has increased over the past decades and is expected to rise substantially in the coming years [2]. According to recent studies breast cancer is identified as the most common cancer among Persian women [3]. The incidence rate among women younger than 25 and older than 79 is 1/20,000 and 1/9, respectively. Genetic factors play an important role in the emergence of this types of cancer and the studies indicate that the risk of cancer is high for the close relatives of impacted person and 15% of total breast cancer cases depend on the strong genetic vulnerability toward this illness that is inherited as a sensible inheritance pattern [10]. The researches performed on Iranian population imply the 17% prevalence of one thousand women with prevalence peak occurring in age group 45-54 years old. The age average of breast cancer affected persons in Iran equals to 48.8 years old; while in other countries this figure is higher than 55 years old [11-12].

Mutation carriers within one of the two breast cancer susceptibility genes, *BRCA1* and *BRCA2*, are at high risk of breast and ovarian cancer [5-6-7]. Hereditary breast cancer is responsible for only 5-10% of all breast cancer cases, individuals carrying mutations in one of these genes have a 40-80% chance of developing breast cancer, making these mutations the strongest breast cancer predictors

known [8- 9]. *BRCA1* gene is located on chr17q, and any changes or mutations in this gene can lead to an increased risk of developing breast, ovarian, and prostate cancer. *BRCA1* gene has been identified as a tumor suppressor gene and its absence may lead to various damages to genome. Therefore, any change in *BRCA1* gene expression level may cause breast cell vulnerability in relation to the performance of oncogenes [13]. Breast cancer caused by a mutation in *BRCA1* gene has a higher incidence, higher mitotic rate, and more lymphatic penetrance than sporadic breast cancer (62). The purpose of present study is to investigate genetic mutations in Exons 2, 3, 5, 11 and 20 of *BRCA1* gene and also the distribution of these mutations and the relationship between *BRCA1* gene mutations with the prevalence rate of inherited breast cancer in the blood samples of women suspected of breast cancer in Tehran city.

MATERIAL AND METHODS

2.1. Clinical Samples

One hundred whole blood samples were collected from women suffering from breast cancer who had referred to Sina Hospital and Dr. Lobat Geranpayeh clinic in Tehran, Iran. The samples were collected prior to a clinical examination that includes diagnostic mammography and biopsy or fine needle aspiration in the case of a positive mammographic finding. Peripheral blood samples were collected in EDTA, heparin and citrate-coated vials. The blood sample was frozen at -70°C till further use.

Table 1: Primers used in this study.

Gene Name	Gene Product	Primer Sequences (5' - 3')	Length(bp)	PCR Annealing Temperature (°C)
BRCA1	Exon2 FR	GAAGTTGTCATTTTATAAACCTTT TGCTTTTTCTTCCCTAGTATGT	315	57
BRCA1	Exon2 FR	TCCTGACAGAGCAGACATTTA TTGGATTTTCGTTCTCACTTA	308	53
BRCA1	Exon5 FR	CTCTTAAGGGCAGTTGTCAG TTCCTACTGTGGTTGCTTCC	278	58
BRCA1	Exon11 FR	CCAAGGTGTATGAAGTATGT GATCAGCATTCAGATCTACC	436	57
BRCA1	Exon20 FR	ATATGACGTGTCTGCTCCAC GGGAATCCAAATTACACAGC	259	57

2.2. DNA Extraction

G-DEX™ Iib Genomic DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea) were used for extracting genomic DNA from whole blood, and proceeded as follows. At first 300 µL whole blood centrifuged at 2,000 g for 5 minutes after mixing with 900 µL RBC lysis buffer, and then discarded the supernatant, leaving just a small amount of the cell pellet. After adding 300 µL cell lysis buffer and 1.5 µL RNase A solution, then soaked the tubes in a 37°C water bath. Once the tubes returned to room temperature, then added 100 µL protein precipitation buffer and centrifuged at 2,000 g for 5 minutes. Using the supernatant, after that genomic DNA pellet obtained by inverting the container gently with 300 µL 100% isopropanol. By centrifuging at 2,000 g for 3 minutes, the supernatant were discarded, leaving the genomic DNA pellet. In next step the pellet were suspended by gently inverting the container with 300 µL 70% ethanol and centrifuged again. After discarding the supernatant and drying the pellet for 10-15 minutes, the dried genomic DNA pellet were dissolved with 50 µL DNA rehydration buffer and stored at -20°C. The purity of the DNA was checked by 260/280 optical density using a Nanodrop ND-1000® Spectrophotometer (Cecil, BioQuest CE 2501, UK).

2.3. PCR and Sanger sequencing

At least 200 ng of DNA was PCR amplified using forward and reverse primers that targeted, exons 2, 3, 5, 11 and 20 of the *BRCA1* gene resulting in a 291 bp PCR product. The sequences of the primers were used in this study presented in table 1 (Lai et al., 2015). The PCR reactions were carried out in a reaction volume of 25 µl containing genomic DNA 30–40 ng, 1 mol/l dNTP 2 µl, 25 mM MgCl₂ 1.5 µl, 10x PCR Buffer 2.5 µl, 10 pM primer 0.5 µl each, 1 U Taq polymerase (CinnaGen) 0.4 µl, and DDH₂O. The reactions were carried out in the following thermocycler conditions: denaturation at 94 °C for 5 min, 36 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s, and final elongation step at 72 °C for 10 min. . PCR products were visualized by gel electrophoresis and staining with ethidium bromide in a digital camera system. PCR product was sent to Microsynth Company in Germany through Topaz Gene Kavosh Co. (Karaj- Iran) in order that its sequence to be determined. After the determination of sequences, sequence analysis was performed by BLAST program in NCBI database.

RESULTS

A total of one hundred whole blood samples were collected from 100 different women suffering from breast cancer who had referred to Sina Hospital and Dr. Lobat Geranpayeh clinic in Tehran, Iran. DNA was extracted and purified using a DNA Extraction Kit (Cinnagen, Tehran, Iran). The quality of DNA extraction method using electrophoresis in agarose gels and stained with ethidium bromide were studied. Nucleic acid sequences of the different *exons* of *BRCA1* gene were compared with those available in the GenBank database using NCBI/BLAST to search for related sequences.

Results of homology comparison in in the GenBank database using NCBI/BLAST shows that *exon 2* were 99% homologous with *exon 2 from UK*; (Gene Bank ACCESSION Number: NG005905.2) and 99% homologous with *exon 2 from USA*; (Gene Bank ACCESSION Number: DQ478408.1) (Table 2). *Exon 3* were 99% homologous with *exon 3 from USA*; (Gene Bank ACCESSION Number: AY273801.1) and 99% homologous with *exon 3 from HongKong*; (Gene Bank ACCESSION Number: KJ625175.1) (Table 2) (fig 3). *Exon 5* were 99% homologous with *exon 5 from India*; (Gene Bank ACCESSION Number: AY093486.1) and *exon 5* were 99% homologous with *exon 5 from HongKong*; (Gene Bank ACCESSION Number: KP272102.1) (Table 2). *Exon 11* were 99% homologous with *exon 11 from China*; (Gene Bank ACCESSION Number: AY304547.1), *exon 11* were 99% homologous with *exon 11 from USA*; (Gene Bank ACCESSION Number: BC115037.1). *Exon 20* were 99% homologous with *exon 20 from USA*; (Gene Bank ACCESSION Number: L78833.1) (Table 2) and *exon 20* were 94% homologous with *exon 20 from USA*; (Gene Bank ACCESSION Number DQ478408.1) (Table 2).

DISCUSSION

Molecular diagnostics for early-onset detection of breast cancer is a rapidly evolving field. After the breast cancer genes *BRCA1* were identified and genetic mutations in Exons 2, 3, 50, 11 and 20 of *BRCA1* gene were investigated. The distribution of these mutations and the relationship between exons 2, 3, 50, 11 and 20 of *BRCA1* gene mutations with the prevalence rate of inherited breast cancer in the blood samples of women suspected of breast cancer in Tehran city evaluated. Germ-

line *BRCA1* mutations confer a high risk for developing breast cancer by age 70 years for women. Results of initial studies based on multiple-case families, reported a female breast cancer risk at age 70 years in *BRCA1* and *BRCA2* mutation carriers of 85% and 84%, respectively [24, 25, 26, 27]. Results of the molecular and pathological investigation of samples showed that the mean age of the breast cancer patients were 51.24 years and their age range 36 to 68 years old and mean age of the control 49.75 years old. The maximum number of patients at the age of 36 to 55 age category with the abundance of % 53.2. Results of pathological of the breast cancer patients showed that 37 women (57%) were suffering from invasive cancer with ductal origin, 31 women (31%) were suffering from invasive cancer with milk cells origin, 5 women (5%) were suffering from in situ carcinoma with lobular origin, 7 women (7%) were suffering from in situ carcinoma with

ductal origin. On the other hand 52 women were in metastasis stage, 23 women experiencing grade III, 14 women experiencing grade II/III and 11 patients experiencing grade II. Among different exons of *BRCA1* causing breast cancer that studied in this research, the changes in exons 2 & 3 had the highest distribution and exons 20 & 5 had the least distribution. The results of polygenetic study on different exons of *BRCA1* indicated that breast cancer emergence in Tehran women is genetically similar to the breast cancer patients of USA, Britain, India, China and Hong Kong. Given the high prevalence of this type of cancer in Iran, early diagnosis and quick treatment on early stages may prevent its progression, molecular methods such as PCR and sequencing is a quick and reliable diagnostic method on early stages of the illness.

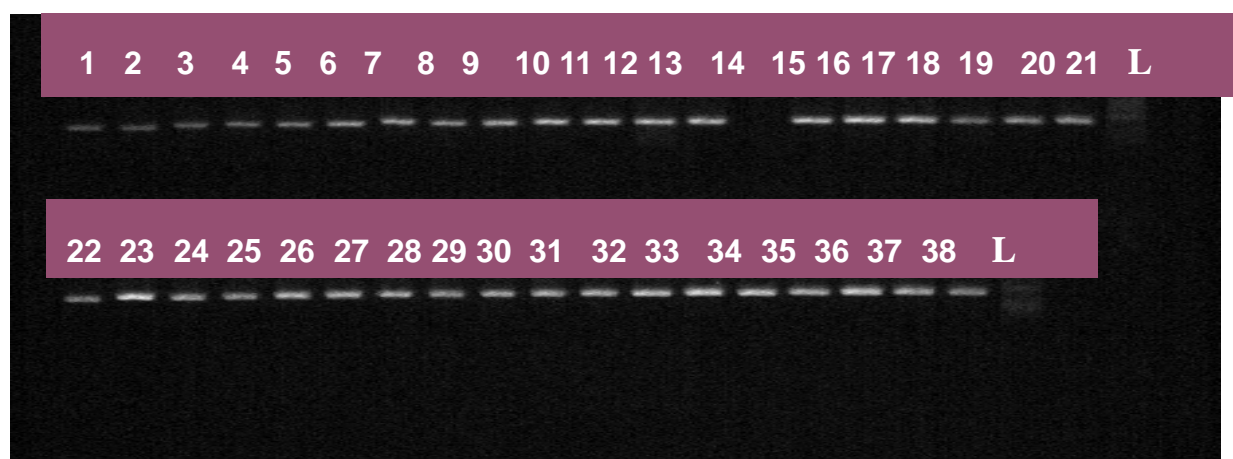


Fig. 2 Agarose gel electrophoresis of *BRCA1* gene exon 3 polymerase chain reaction amplification products.

Table 2. Homology of the Nucleic Acid Sequences of the different exons of *BRCA1* gene in the GenBank Database (NCBI)

<i>BRCA1</i> Exons	Homology (%)	Accession No.	Country
<i>Exon 2</i>	99%	NG005905.2	UK Orban, <i>et al.</i> , (16), 2016
<i>Exon 2</i>	99%	DQ478408.1	USA Raymond, <i>et al.</i> , (17) 2016
<i>Exon 3</i>	99%	AY273801.1	USA Rieder, <i>et al.</i> , (18) 2003
<i>Exon 3</i>	99%	KJ625175.1	Hong Kong Kwong, <i>et al.</i> , (19) 2014
<i>Exon 5</i>	99%	AY093486.1	India Valarmathi, <i>et al.</i> , (20) 2003
<i>Exon 5</i>	99%	KP272102.1	Hong Kong Kwong, <i>et al.</i> , (19) 2014
<i>Exon 11</i>	99%	AY304547.1	China Zhao, <i>et al.</i> , (21) 2009
<i>Exon 11</i>	99%	BC115037.1	USA Strausberg, <i>et al.</i> , (22) 2006
<i>Exon 20</i>	99%	L78833.1	USA Smith, <i>et al.</i> , (23) 2005
<i>Exon 20</i>	94%	DQ478408.1	USA Raymond, <i>et al.</i> , (17) 2016

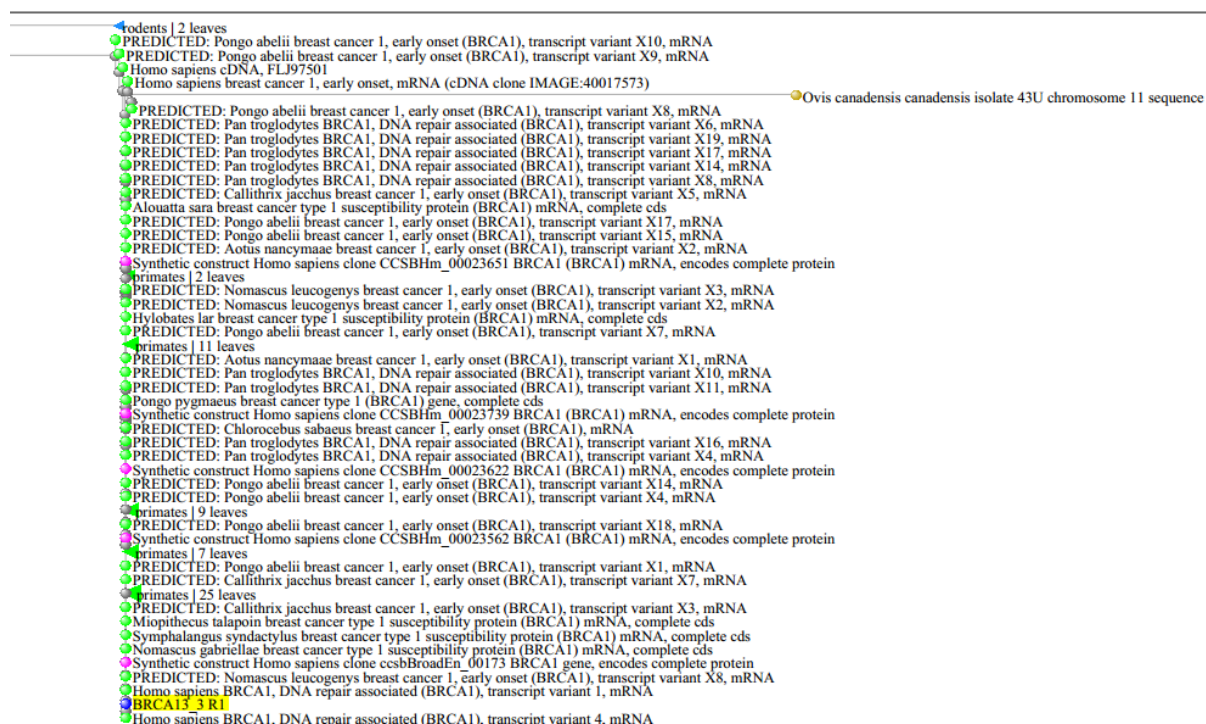


Fig 3. Phylogenetic Tree of BRCA1 gene exon 3

Results of homology comparison in the Gen Bank database using NCBI/BLAST shows that exon 2 were 99% homologous with exon 2 from UK; Gene Bank ACCESSION Number: NG005905.2 (16). Exon 3 were 99% homologous with exon 3 from USA; Gene Bank ACCESSION Number: AY273801.1 (18). Exon 5 were 99% homologous with exon 5 from India; Gene Bank ACCESSION Number AY093486.1 (20). Exon 11 were 99% homologous with exon 11 from China; Gene Bank ACCESSION Number: AY304547.1 (21). Exon 20 were 99% homologous with exon 20 from USA, Gene Bank ACCESSION Number: L78833.1 (23), Many studies have shown that BRCA1 and BRCA2 are the most important breast cancer susceptibility genes in high-risk families in the world (28).

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