

RESEARCH ARTICLE

Haplotype Analysis of BRCA1 Gene D17S855 and D17S1322 Markers in Iranian Familial Breast Cancer Patients

Sayed Mohsen Miresmaeili¹, Dor Mohammad Kordi Tamandani^{1*}, Seyed Mehdi Kalantar², Seyed Mohammad Moshtaghiun³

Abstract

Background: Breast cancer molecular analysis by linkage analysis has the advantage of facilitating early diagnosis in asymptomatic genetic carriers, with a view to the preventive follow-up of these subjects and genetic counseling. The aim of this study was to evaluate BRCA1 gene D17S855 and D17S1322 markers in breast cancer patients. **Materials and Methods:** A series of 85 BC patients and 85 unrelated healthy women were recruited for haplotype analysis performed using two short tandem repeat markers located within the BRCA1 gene locus. Each marker was amplified with PCR genomic DNA from each individual and fluorescently end-labeled primers. **Results:** Both D17S855 and D17S1322 markers included 12 kinds of alleles. Results indicate that most of the BC patients shared two common 121-150 (11.2%, RR=1.56 and p=0.02) and 121-146 (5.6%, RR=1.9 and p=0.02) haplotypes. **Conclusions:** Our results should be helpful to understand the haplotype phase in the BRCA1 gene and establish a genetic screening strategy in the Iranian population.

Keywords: Breast cancer - BRCA1 haplotype - STRs - D17S855 - D17S1322.

Asian Pac J Cancer Prev, 17 (7), 3615-3617

Introduction

Breast cancer is one of the most frequent malignancies in females, Worldwide. Approximately 5-10% of breast cancers and ovarian cancers are due to germline mutations in the BRCA1 (OMIM 113705) and BRCA2 (OMIM 600185) genes (Forat-Yazdi et al., 2015). Mutations in these genes are responsible for familial breast and ovarian cancer (Haiman et al., 2003; Forat-Yazdi et al., 2015). It has been estimated that women carrying deleterious mutations in BRCA1 and BRCA2 genes confer a high lifetime risk, of up to 87%, of developing breast cancer and up to 68% of developing ovarian cancer. Among white women in the United States, 5% to 10% of breast cancer cases and 10% to 15% of ovarian cancer cases are due to inherited mutations in BRCA1 and BRCA2 (Neamatzadeh et al., 2015). In addition, women and men carrying BRCA2 mutations have heightened risks of pancreatic cancer, prostate cancer, and melanoma (Friedenson et al., 2005). It has been reported risks for young women with inherited BRCA1 or BRCA2 mutations are particularly increased (Walsh et al., 2006).

Haplotype-based analyses have been proposed as a powerful comprehensive approach to identify causal genetic variation underlying complex diseases and early diagnosis (Haiman et al., 2003). Screening of large genes is difficult to select patients carrying mutations (Osorio et al., 2003). Screening of BRCA1 mutation is faster by

short tandem repeat (STR) polymorphism. Individual polymorphism of STR is good evidence for following inheritance of repeat polymorphism. Several markers were existed within and flanking BRCA1. D17S1323 (intron 12), D17S1322 (intron 19), and D17S855 (intron 20); were genotyped in the 3' of BRCA1 gene D17S855 and D17S1323 were di-nucleotide STR and D17S1322 was trinucleotide repeat (Mefford et al., 1999; Nowacka-Zawisza et al., 2008).

The current study was planned to investigate evaluate haplotyping of BRCA1 two D17S1322 and D17S855 intragenic marker between BC patients and healthy women.

Materials and Methods

Participants

This study was carried out in Department of Biology, University of Science and Art, Yazd, Iran. A series of 85 patients with age range 36–51 years was recruited from among individuals referred to the one of three different hospitals Yazd, Iran. Participants recruited were either diagnosed with breast cancer or ovarian cancer. A total of 85 unrelated and age-matched healthy women recruited as controls. The study was approved by the Bioethics Committee of the University of Science and Art in agreement with the Helsinki statement. All participants provided written informed consents.

¹Department of Biology, Science Faculty, University of Sistan and Baluchestan, Zahedan, ²Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, ³University of Yazd, Yazd, Iran. *For correspondence: hn_1364@yahoo.com

Genotyping

To determine the haplotype structure of the BRCA1, the following markers were used: three intragenic short-tandem repeats, D17S855, D17S1322, and D17S1323. These markers span approximately 450 Mbp around the BRCA1 locus. Genomic DNA was extracted from peripheral blood using Qiagene kit (Genet Bio, Korea) and in according to the manufacture's protocol. The primer sequences for all markers were retrieved from the Genome Data Base online database. A genomic intragenic amplicons of di-nucleotide of BRCA1 genes were individually amplified by PCR amplification. For PCR amplification, three set of primer pairs were used to amplify three intragenic markers of BRCA1 gene. PCR amplification for a 25µL PCR reaction volume was used. The PCR mix consisted of 12.5µL of Taq 2x Master Mix RED (cat.A180301,Ampliqon), 8.5µL of water and 2.0 µL of primer mix. To make the final reaction, 1.0 µL of DNA was added.The PCR reaction was run with an initial denaturation at 94°C for 4 minutes, followed by 32 cycles with denaturing at 94°C for 30 seconds, annealing temperature was specific for each primer for 30 seconds and extension at 72°C for 1 minute. Final extension carries out for 10 minutes at 72°C.

Fragment analysis

We determined STR genotypes as described by Barker and Fain (1993). A volume of 2 µl of each PCR product

Table 1. The D17S1322 Marker Alleles Frequency between BC Patients and Controls

STR D17S1322	Frequency		
	Patients (n=85)	Control (n=85)	p-value
109	12(7.1)	10(5.9)	0.41
112	60(35.3)	55(32.4)	0.32
115	44(25.9)	38(22.4)	0.26
118	39(22.9)	40(23.5)	0.50
121	5(3.5)	14(8.2)	0.03
124	4(1.8)	0.0	0.06
127	0.0	2(1.2)	0.25
139	0.0	1(0.6)	0.50
142	0.0	5(2.9)	0.03
148	0.0	1(0.6)	0.50
151	3(1.8)	1(1.2)	0.50
157	3(1.8)	2(1.2)	0.50
160	0.0	1(0.6)	0.50

Table 2. The D17S855 Marker Alleles Frequency between BC Patients and Controls

STR D17S855	Frequency		
	Patients (n=85)	Control (n=85)	p-value
136	4(2.4)	0.0	0.61
138	1(0.6)	0.0	0.50
140	2(1.2)	3(1.8)	0.50
142	17(10.0)	18(10.6)	0.50
144	48(28.2)	45(26.5)	0.4
146	30(17.6)	17(10.0)	0.03
148	33(19.4)	32(18.8)	0.50
150	27(15.9)	40(23.5)	0.05
152	5(2.9)	7(4.1)	0.38
154	0.0	2(1.2)	0.25
156	3(1.8)	6(3.5)	0.25

Table 3. D17s1322 and D17s855 Different Possible Haplotypes

Haplotype	Patients (n=85)	Control (n=85)	RR	P-Value
121-150	12(11.2)	3(3.2)	1.56	0.02
121-146	6(5.6)	0	1.9	0.02
142-150	0	0	0	-
142-146	0	0	0	-

was mixed with 0.5 µl of the FAM, HEX and TAMRA 500 internal size standard (Applied Biosystems Inc., Foster City, CA, USA) for D17S1322 and D17S855 markers, respectively. Samples were read on the ABI Prism 3730 using the Gene marker Software (Applied Biosystems).

Statistical analysis

In this study all statistical analyses were carried out using the IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp). A non-parametric test, the Chi-square, was used to compare the groups. Results were judged as statistically significant at a p-value of 0.05 or less.

Results

Results showed that D17S1322 markers had 11 alleles with length range between 109 and 160 bp and D17s855 marker had 11 alleles with length range between 136 and 156 bp (Tables 1 and 2). Results showed that 4 possible haplotypes of D17s1322 and D17s855 STRs including 146-121, 146-142, 150-121, and 150-142 bp. Analysis revealed that only two 121-150 (RR=1.56, p=0.02) and 121-146 (RR=1.9, p=0.02) haplotypes are informative in this population. The 121-150 haplotype frequency was approximately 2 times more among BC patients than controls (Table 2).

Discussion

BRCA1 (MIM# 113705) and BRCA2 (MIM# 600185) are major predisposition genes in hereditary breast and ovarian cancer (Alsop et al., 2012; Clark et al., 2012). Frequency of germline BRCA1/2 mutations was estimated at 2-4.7% in unselected breast cancer (BC) patients from different populations (Ticha et al., 2010). D17s855, D17s1323, and D17s1322 microsatellites are the highly informative BRCA1 intragenic marker. The previous study reveals that the distribution of some three intragenic markers haplotype is different in healthy and patient women (Miresmaeili et al., 2016). The present study results have showed that the D17s855 and D17s1322 marker allele distribution in BC patients differs considerably from controls, while the presence of common disease-associated haplotypes in BC patients, suggested that disease-associated haplotypes have arisen only once in two distinct ancestors.

Our study results suggested four different possible bi-allelic haplotypes between D17s1322 and D17s855 markers including 146-121,146-142, 150-121,and 150-142.However, only two 121-150 (RR 1.56, p=0.02) and 121-146 (RR 1.9, p=0.02) haplotypes are informative

between BC patients and controls (table 5). In this study the 121-150 haplotype was approximately 2 times more frequent among BC patients than controls.

Studies show that all specific BRCA1 founder mutation carriers shared a common haplotype suggested common ancestor. For example BRCA1 185delAG founder mutation carriers shared a haplotype including specific alleles of D17S855 (146-148 bp), D17S1322 (116 bp), D17S1323 (145 bp), and D17S1327 (127 bp) markers (Laitman et al., 2012). De La Hoya et al., have been described that the shortest alleles (139 and 141 bp) of the D17S855 microsatellite are more frequent among individuals carrying BRCA1 mutations (De La Hoya et al., 2012). In addition, Osorio et al., have found that the 139 bp allele of the D17S855 was 3.5 times more frequent than expected in mutations linked to D17S855 microsatellite larger alleles (Osorio et al., 2003). In this study we have found 3 markers of the D17S855 microsatellite with larger length (144, 146 and 148 bp) was over-represented in the Iranian breast cancer patients. However, we did not examine BRCA1 gene mutations and not established the phase between BRCA1 mutation and D17S855 alleles, but these results contravene their findings, functionally. Taken together, we suggest that D17S855 marker alleles in Iranian breast/ovarian patients are not randomly distributed but clustered in the BRCA1 subsets that may be identified by genotyping the BRCA1 specific mutations. According to the results, it suggested that all of BRCA1 gene mutation carriers had a common haplotype with different alleles of D17S855 marker.

In conclusion, Haplotyping of the familial breast cancer along with members of X families revealed the presence of a di-allelic 121,150 haplotype between D17s1322 and D17s855 markers of BRCA1 in the Iranian BC patients. Our results will be helpful to understand the haplotype phase in BRCA1 gene and establish a genetic screening strategy in the Iranian population, as D17s1322 and D17s855 genotyping may become of use in selecting individuals for BRCA1 genetic testing. Although further studies will be necessary to confirm these findings in BRCA1 and establishment phases between BRCA1 mutations and microsatellites alleles.

Acknowledgments

The present project was supported by a grants from the Research Foundation of the University of Sistan and Baluchestan, Zahedan, Iran, Science and Technology Park, Yazd, Iran and department of biology of Science and Art University, Yazd, Iran.

References

Alsop K, Fereday S, Meldrum C, et al (2012). BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: A report from the Australian ovarian cancer study group. *J Clin Oncol*, **30**, 2654–63.

Clark SL, Rodriguez AM, Snyder RR, (2012). Structure-Function Of The Tumor Suppressor BRCA1. *Comput Struct Biotechnol J. Research Network Computational Structural*

Biotechnol, **1**, 1–8.

- De La Hoya M, Sulleiro S, Osorio A, et al (2002). Clustering of cancer-related mutations in a subset of BRCA1 alleles: A study in the Spanish population. *Int J Cancer*, **100**, 618–9.
- Haiman CA, Stram DO, Pike MC, et al (2003). A comprehensive haplotype analysis of CYP19 and breast cancer risk: the Multiethnic Cohort. *Hum Mol Genet*, **12**, 2679-92.
- Forat-Yazdi M, Neamatzadeh H, Sheikhha MH, et al (2015). BRCA1 and BRCA2 common mutations in Iranian breast cancer patients: a meta-analysis. *Asian Pac J Cancer Prev*, **16**, 1219-24.
- Friedenson B (2005). BRCA1 and BRCA2 Pathways and the risk of cancers other than breast or ovarian. *Med Gen Med*, **7**, 60.
- Laitman Y, Feng B-J, Zamir IM, et al (2012) Haplotype analysis of the 185delAG BRCA1 mutation in ethnically diverse populations. *Eur J Hum Genet. Nature Publishing Group*, **21**, 212–6.
- Mefford HC, Baumbach L, Panguluri RC, et al (1999) Evidence for a BRCA1 founder mutation in families of West African ancestry. *Am J Hum Genet*, **65**, 575–8.
- Miresmaeili M, Kordi D M, Kalantar M, Moshtaghioun M (2016) Haplotype analysis of BRCA1 intragenic markers in Iranian patients with familial breast and ovarian cancer. *Int J Reprod BioMed*, **14**, 271-4
- Neamatzadeh H, Shiryazdi SM, Kalantar SM (2015). BRCA1 and BRCA2 mutations in Iranian breast cancer patients: A systematic review. *J Res Med Sci*, **20**, 284-93.
- Nowacka-Zawisza M, Brys M, Romanowicz-Makowska H, et al (2008). Dinucleotide repeat polymorphisms of RAD51, BRCA1, BRCA2 gene regions in breast cancer: *Original Article. Pathol Int*, **58**, 275–81.
- Osorio A, de la Hoya M, Rodríguez-López R, et al (2003). Over-representation of two specific haplotypes among chromosomes harbouring BRCA1 mutations. *Eur J Hum Genet*, **11**, 489–92.
- Ticha I, Kleibl Z, Stribrna J, et al (2012). Screening for genomic rearrangements in BRCA1 and BRCA2 genes in Czech high-risk breast/ovarian cancer patients: high proportion of population specific alterations in BRCA1 gene. *Breast Cancer Res Treat*, **124**, 337-47.
- Walsh T, Casadei S, Coats KH, et al (2006). Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *JAMA*, **295**, 1379-88.