BRCA1 and BRCA2 Large Genomic Rearrangements Screening in Thai Familial Breast Cancer Patients by Multiplex Ligation-dependent Probe Amplification (MLPA)

Sunichya Jadsri[a], Takol Chareonsirisuthigul[a*], Budsaba Rerkamnuaychoke[a], Donniphat Dejsuphong[b], Atchara Tunteeratum[c] and Surakameth Mahasirimongkol[d]

[a]Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand 10400

[b]Research Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand 10400

[c]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand 10400

[d]Medical Genetics Section, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand 11000

[*] Corresponding author. E-mail address: takol.cha@mahidol.ac.th

Abstract

Breast Cancer has now become the most frequently diagnosed cancer and the leading cause of cancer death in females. BRCA1 and BRCA2 inherited mutations account for 5%-10% of all female breast cancers. However, prevalence of BRCA genes mutation is vary in between difference populations. In Thailand, there are no previous studies of BRCA1 and BRCA2 large genomics rearrangement (LGRs) have been reported. In this study, we screened the ''high-risk'' group consisted of 100 individuals who met clinical criteria for genetic examination of BRCA1/2 using Multiplex Ligation-dependent Probe Amplification (MLPA). Among a total of 100 selected cases, one duplication of BRCA1 exon 15 was determined but none of any LGRs were found in BRCA2. Similar to the other studies in Asian population, the prevalence of LGRs in Thailand likely to be low. The information of BRCA1 and BRCA2 LGRs from this study can be used as a nationwide of Thai database which will be useful for further study of the familial breast cancer.

Keywords: Breast Cancer, BRCA1/2 Mutation, Multiplex Ligation-dependent Probe Amplification (MLPA)

Introduction

Breast cancer is one of the most important public health problems. It has now become the most frequently diagnosed cancer; accounts for 1 in 4 cancers diagnosed and the leading cause of cancer death among females (International Agency for Research on Cancer, 2013). In 2012, estimated 1.7 million breast cancer new cases and 5.2 million breast cancer deaths were reported worldwide (International Agency for Research on Cancer, 2012; Ferlay et al., 2015). When comparing with previous world cancer statistics in 2008, the rise of more than 20% of incidence and 14% of mortality have been noticed (International Agency for Research on Cancer, 2013). In Thailand, estimated 13653 new cases and 5092 deaths were reported in 2012. (Youlden et al., 2014). The breast cancer new case accounts for more than 39% of all cancer new cases (National Center Institute Department of Medical Services Ministry of Public Health, 2014).

BRCA1 and BRCA2 are human genes that produce tumor suppressor proteins. Genetic alterations of these two genes account for 5%-10% of all breast cancers and estimate 15%-20% of familial breast cancers worldwide (Petrucelli, 2013). A germline pathogenic alterations in this two gene are well-known as a cause of hereditary breast and ovarian cancer syndrome (HBOC) which

characterized by an increased risk for breast cancer, ovarian cancer, prostate cancer and pancreatic cancer.

Two patterns of BRCA1 or BRCA2 gene mutation consist of 1) small mutations which refers to a single or multiple nucleotide sequence alteration and 2) large genomic rearrangements (LGRs) which refer to a large part of nucleotide sequence have new arranging such as deletion or duplication. Recently, BRCA genes mutation testing is available in many countries. The first choice of the methods is DNA sequencing for small mutation and Multiplex Ligation-dependent Probe Amplification (MLPA) for LGRs.

The Prevalence of BRCA 1 and BRCA 2 genes mutation is vary in between difference populations. A few studies in BRCA 1 and BRCA 2 mutation in Asian population have been reported (Lim et al., 2007; Kang et al., 2010; Kwong et al., 2012; De Silva, Tennekoon, Karunanayake, Amarasinghe, & Angunawela, 2014; Seong et al., 2014). In view of to date, there are no frequency reports of BRCA1 and BRCA2 LGRs in Thai population. The aim of this study is to investigate whether a similar situation holds for BRCA1/2 LGRs in Thailand. We accordingly screened for BRCA1/2 LGRs among the high-risk group of Thai familial breast cancer patients using MLPA testing.

Materials and Methods

Population

A total of 685 familial breast cancer patients were recruited to Ramathibodi Hospital for clinical analysis of BRCA1 and 2 mutation. All patient data regarding clinical history and ancestry were obtained by health care provider report on test requisition forms and questionnaire. The ''high-risk'' group consisted of 100 individuals who met at least one of clinical criterias predicting a relatively high probability of carrying a mutation in BRCA1 or BRCA2: (1) families with three or more first-degree relatives with breast and/or ovarian cancer in two successive generations, (2) individual or family history of bilateral breast cancer, (3) two first-degree relatives diagnosed with breast cancer including at least one case diagnosed before the age of 50, (4) two affected first-degree relatives consisting of one case diagnosed with premenopausal breast cancer under the age of 50 years, and another with ovarian cancer, (5) one woman with ovarian cancer and breast cancer diagnosed before the age of 60 years, (6) breast cancer before the age of 36 years, (7) a male individual diagnosed with breast cancer, or (8) two first-degree relatives diagnosed with ovarian cancer regardless of age (NCCN, 2015).

Research ethics has been reviewed and approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, based on the Declaration of Helsinki.

DNA extraction and quantification

Briefly, DNA were extracted from whole blood specimen using QuickGene-810 automation system (Fujifilm, Japan). DNA concentration and absorbance at 280 and 260 nm were measured using NanoDrop™ 2000 Spectrophotometers (Thermo Scientific, USA).

Multiplex ligation-dependant probe amplification (MLPA)

Between 50 and 100ng of DNA was used for the analysis of each case as suggested by the manufacturer's instructions for the SALSA® MLPA® probemix P002-D1 BRCA1 and SALSA® MLPA® probemix P045/P045B BRCA2 (MRC Holland, Netherlands). SD024 DNA Sample (MRC Holland, Netherlands), an artificial positive duplication DNA sample were used as a positive control. Analysis was

as described previously by Seong et al., 2014. The peak heights of each probe were normalized by dividing each peak height by the total peak heights of all probes in the sample of interest. This normalized value was divided by the average normalized value of that probe (obtained from all samples in the experiment). Data were normalized to 1 from the control probes; thresholds of 1.3 and 0.7 were set for identifying single copy gain or loss respectively.

Results

From a total of 685 Thai familial breast cancer patients, 100 individuals of high-risk group were selected for BRCA1 and BRCA2 LGRs detection using MLPA. Mutation screening was performed if an index case or family fulfilled one of the following criteria. Simply stated, most patients met these high-risk criteria if they had invasive or in situ breast cancer diagnosed under age 50 years, or ovarian cancer or male breast cancer diagnosed at any age, in conjunction with 2 or more relatives similarly affected criteria. The characteristics of the cases and their family histories are summarized in Table 1. Among these patients, the median age was about 58 years, ranging from 38 to 84 years. There were 22% under 50 years, 41% between 50-60 years and 37% over 60 years of age, respectively.

MLPA had been performed in all 100 individuals. After analysis of normalized data, we observed 1% (1/100) of cases that positive for BRCA1 large genomic rearrangements. The calculated probe ratios of this patient showed an increased copy number for BRCA1-15-332 nt segment probe which indicated duplication in exon 15 of BRCA 1 gene (Figure 1). However, none of any large genomic rearrangements was detected in BRCA2 gene. The breakpoints of the duplication in BRCA1 exon 15 should be further confirmed and fully characterized at the sequencing level.

Table 1 The information of 100 high-risk Thai familial breast cancer patients which were screened for BRCA1 and BRCA2 large genomic rearrangements by MLPA

Age	<50	22%
	50-60	41%
	>60	37%
	Median age (yrs)	58
History of ovarian cancer in Family	Yes	15%
	No	85%
No. of 1st Degree relative affected with breast cancer	0	7%
	1	70%
	2	14%
	3	6%
	5	3%
History of early onset < 50 breast cancer in family	Yes	63%
	No	37%

Table 1 (Cont.)

History of bilateral breast cancer in family	Yes	3%
	No	97%
> 3 family member with HBOC*	Yes	23 %
	No	77 %
No. of family member affected with HBOC*	0	1%
	1	57%
	2	28%
	3	7%
	4	1%
	5	3%

*HBOC: Hereditary breast and ovarian cancer syndrome

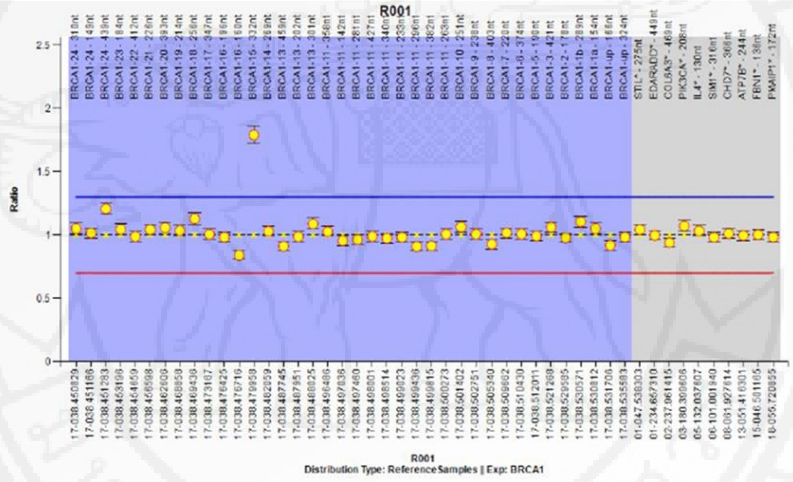

Figure 1 Calculated probe ratios of the positive sample: arranging probes by chromosomal location shows an increased copy number for BRCA1-15-332 nt segment probe in exon 15 of BRCA 1 gene.

Discussion

The prevalence, penetrance and spectrum of BRCA1/2 mutations all differ between populations and are influenced by ethnicity and geographical location. In some populations, genomic deletions and/or rearrangements of the gene reportedly represent about 10% of all deleterious BRCA1 mutations identified, and in one study from the Netherlands two rearrangements accounted for 23% of mutations (Mazoyer, 2005). In our MLPA screening study, only one case from high-risk group (1%) was found duplication in exon 15 of BRCA 1 gene. Although MLPA has the ability for detection of variations in the copy number of gene, but MLPA is just screening method. Therefore, the positive finding from MLPA need to be confirm with other methods for example, long-range PCR, Fluorescent in situ hybridization (FISH) or Comparative genomic hybridization (CGH) (Ewald et al., 2009; Youlden et al., 2014).

Similar to the previous studies in Asian country, the prevalence of LGR in Thailand likely to be low. The Prevalence of 14.51% of BRCA1/2 mutations, 6.79% of BRCA1 small mutations, 6.79% BRCA2 small mutations, 0.62% of BRCA1 LGRs and 0.31 % of BRCA2 LGRs were observed in 324 Malaysian patients considered in high-risk and medium-risk group (Kang et al., 2010). Study from 555 clinically high-risk breast and/or ovarian cancer probands in southern Chinese population showed that prevalence of 12.43% of BRCA1/2 mutations, 4.86% of BRCA1 small mutations, 6.85% of BRCA2 small mutations, 0.36% of BRCA1 LGRs and 0.36 % of BRCA2 LGRs were observed (Kwong et al., 2012). A cohort study in 57 familial breast cancer patients, 25 risk individuals and 23 healthy controls using MLPA to detect BRCA1 and BRCA2 large genomic rearrangements but none of any large genomic rearrangements were found in Sri Lankan familial breast cancer patients (De Silva et al., 2014). Seong et al. examined 221 Korean familial breast cancer patients and found 36.65% of BRCA1/2 mutations, 16.74% of BRCA1 small mutations, 18.55% BRCA2 small mutations, 1.36% of BRCA1 LGRs when BRCA2 LGRs were not found.

Lim et al. (2007) demonstrated 2 % of BRCA1 and 1% of BRCA2 LGRs in Singaporean unrelated early-onset and familial breast and/or ovarian patients who previously tested negative for deleterious BRCA mutations by conventional PCR-based mutation detection. Interestingly, this study was done in patients who previously tested negative for BRCA small mutations which induced the highest prevalence of both BRCA1 and BRCA2 LGRs among all studies in Asian population. This supports that the prevalence also seems difference depend on population group and selected criteria.

There are some limitation in this study including: 1) Family history information was collected from patients by the questionnaire which may misunderstood from unclear questions. So, inaccurate answer may affect familial breast-risk assessment and 2) Analysis of MLPA data is from a limited number of samples.

Conclusion and Suggestion

To our knowledge, this study is the first report of BRCA1 and BRCA2 large genomic rearrangements in Thai familial breast cancer population. The finding will be as a nationwide of Thai database which will be useful for further study of the familial breast cancer. However, because of a small sample size, more samples with highly selective breast cancer risk-assessment criteria should be done. For further study, we plan to confirm and characterize the duplication region in exon 15 of BRCA1 gene using long-range PCR and sequencing. The data may be correlated with prognosis or risk of developing familial breast cancer.

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