BMC
Research Notes

Analysis of *BRCA1* and *BRCA2* large genomic rearrangements in Sri Lankan familial breast cancer patients and at risk individuals

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Abstract

Background: Majority of mutations found to date in the *BRCA1/BRCA2* genes in breast and/or ovarian cancer families are point mutations or small insertions and deletions scattered over the coding sequence and splice junctions. Such mutations and sequence variants of *BRCA1* and *BRCA2* genes were previously identified in a group of Sri Lankan breast cancer patients. Large genomic rearrangements have been characterized in *BRCA1* and *BRCA2* genes in several populations but these have not been characterized in Sri Lankan breast cancer patients.

Findings: A cohort of familial breast cancer patients (N = 57), at risk individuals (N = 25) and healthy controls (N = 23) were analyzed using multiplex ligation-dependent probe amplification method to detect *BRCA1* and *BRCA2* large genomic rearrangements. One familial breast cancer patient showed an ambiguous deletion in exon 6 of *BRCA1* gene. Full sequencing of the ambiguous region was used to confirm MLPA results. Ambiguous deletion detected by MLPA was found to be a false positive result confirming that *BRCA1* large genomic rearrangements were absent in the subjects studied. No *BRCA2* rearrangement was also identified in the cohort.

Conclusion: Thus this study demonstrates that *BRCA1* and *BRCA2* large genomic rearrangements are unlikely to make a significant contribution to aetiology of breast cancer in Sri Lanka.

Keywords: BRCA1, BRCA2, Large genomic rearrangements, Breast cancer

Findings

Background

Germ-line mutations in *BRCA1* and *BRCA2* tumor suppressor genes cause a hereditary predisposition to breast and ovarian cancer [1]. Such mutations account for 15-20% of familial breast cancer [2]. Although familial breast cancer contributes to 5-10% of all breast cancers, individuals carrying mutations in one of these genes have a 40-80% chance of developing breast cancer [3]. At present, in Sri Lanka women are diagnosed with breast cancer at a median age of 50 years contributing to approximately 25% of all cancers [4]. Majority of mutations found to date in the *BRCA1* and *BRCA2* genes in breast and/or ovarian cancer families are point mutations or small insertions

and deletions scattered over the whole coding sequence and the splice junctions. Point mutations and sequence variants in *BRCA1* [5] and *BRCA2* [6] genes were previously identified in this cohort of Sri Lankan breast cancer patients. More recently, large genomic alterations have been described in *BRCA1* and *BRCA2* genes. Such large alterations lead to change in genomic copy number and cannot be detected by conventional methods [7]. Rearrangements have occasionally been reported in patients who are negative for *BRCA1/BRCA2* mutations [8].

There is a difference in the degree of *BRCA1/BRCA2* rearrangements found in different ethnic groups and populations. The prevalence of *BRCA1/BRCA2* genomic rearrangements in Asians is thought to be low. However studies done in these populations are limited. Several deletions and duplications have been reported from Singapore [9], Korea [8], Malaysia [10] and China [11]. There are

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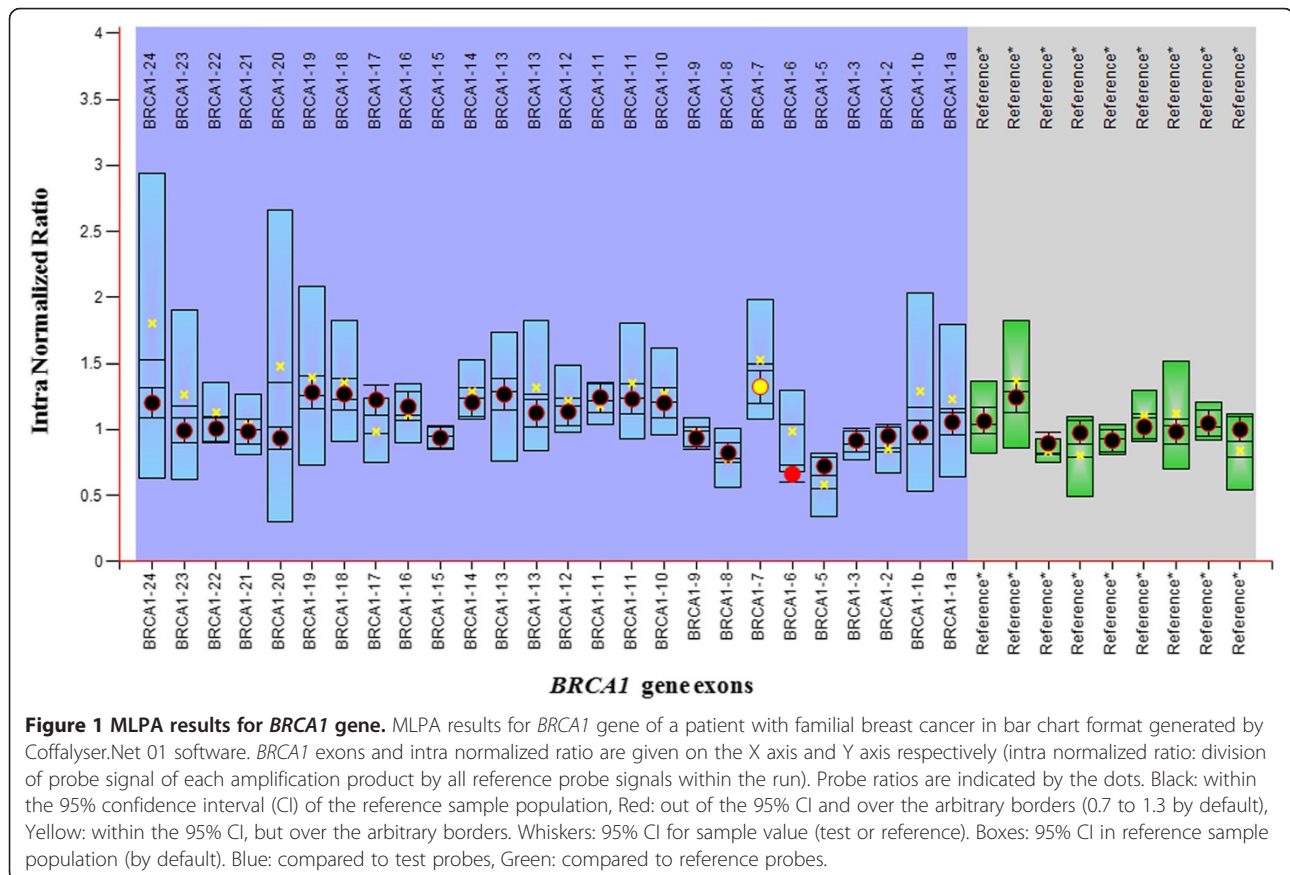Full list of author information is available at the end of the article

no published reports on the analysis of *BRCA1* and *BRCA2* large genomic rearrangements in Sri Lankans and this study examined the possibility of such genomic rearrangements in a cohort in which point mutations and sequence variants in *BRCA1* and *BRCA2* were previously described [5,6].
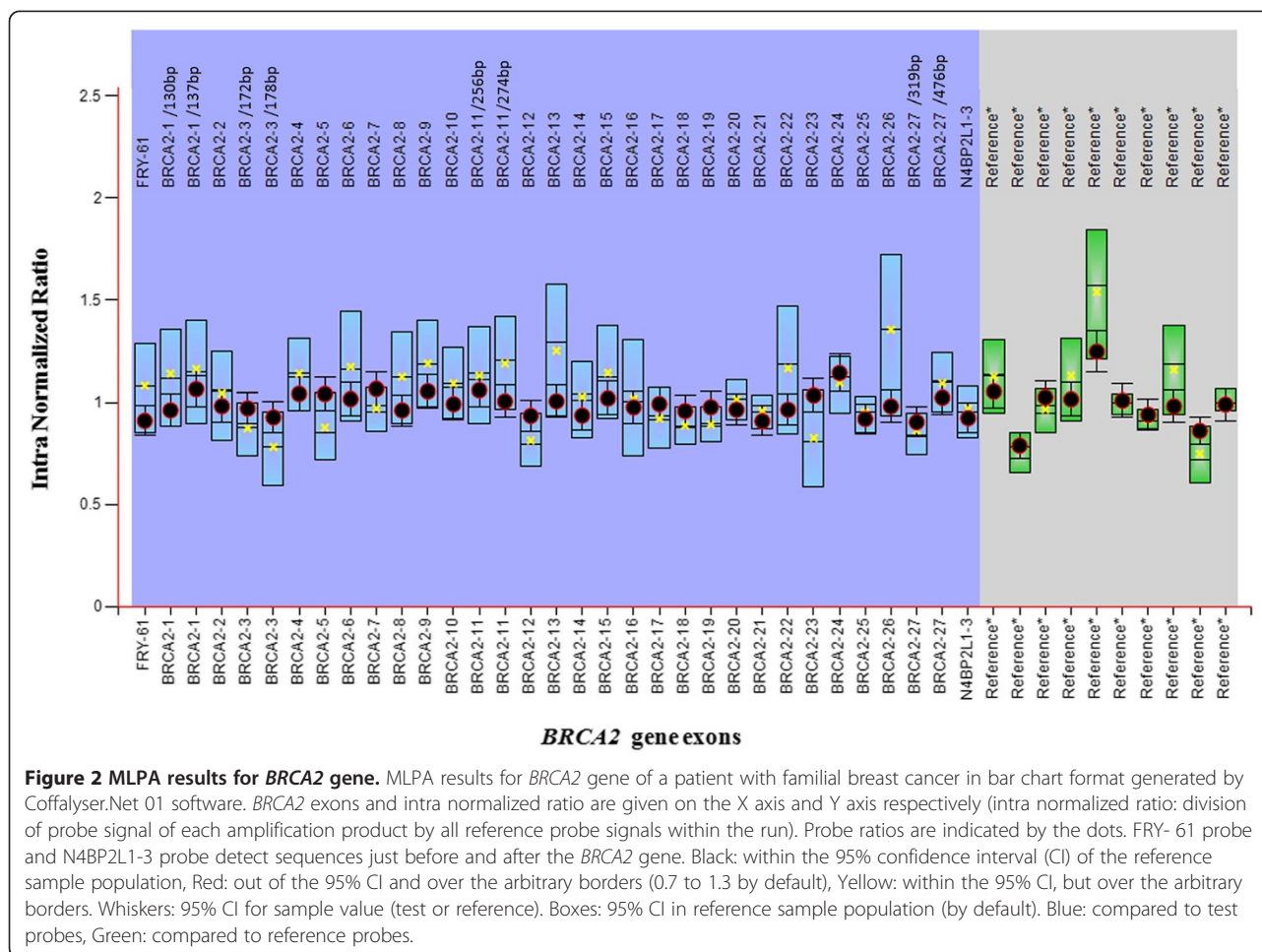
Results and discussion

MLPA analysis did not reveal any large genomic rearrangements in *BRCA2* gene in any of the subjects studied. However, according to MLPA analysis, one breast cancer patient was detected with an average intra normalized ratio (probe signal of each amplification product/ all reference probe signals within each sample run) of 0.64 in exon 6 of *BRCA1* gene which was predicted as an ambiguous deletion. An average ratio of 0.64 indicates a reduction in relative peak area of the amplification product by 36%. Figure 1 shows the MLPA results of the patient found to have an ambiguous exon 6 deletion for *BRCA1*. Figure 2 shows the MLPA results for the *BRCA2* gene of one of the familial breast cancer patients. The average ratios were within the 0.7-1.3 range indicating absence of exon deletions or duplications of the *BRCA2* gene in all samples analysed.

In the patient who showed an ambiguous deletion for *BRCA1* exon 6, the average ratio for exon 6 was outside the 95% confidence limits and outside the arbitrary range of 0.7 to 1.3. Although for exon 7, the average ratio exceeded the arbitrary range this was still within the 95% confidence limits. Thus we attempted to confirm the *BRCA1*/ exon 6 deletion by direct sequencing. The sample was sequenced (both forward and reverse strands) along with a healthy control sample for comparison. Sequence data were analysed using Mutation Surveyor DNA Variant Analysis Software – Softgenetics against reference sequence of *BRCA1* in the basic local alignment search tool (BLAST) published by National Centre for Biotechnology Information (NCBI), USA (accession no. L78833). Sequence data showed no change in the DNA sequence of exon 5, 6 and 7of the patient compared to reference sequence. Data also indicated that the MLPA probe hybridization site was intact.

Familial breast cancer patients and at risk individuals were previously investigated for *BRCA1* and *BRCA2* mutations and some were positive for *BRCA1/2* mutations. From this cohort, three at risk individuals and two familial breast cancer patients were positive for clearly pathogenic *BRCA1* mutations and only six familial breast cancer

Figure 1 MLPA results for *BRCA1* gene. MLPA results for *BRCA1* gene of a patient with familial breast cancer in bar chart format generated by Coffalyser.Net 01 software. *BRCA1* exons and intra normalized ratio are given on the X axis and Y axis respectively (intra normalized ratio: division of probe signal of each amplification product by all reference probe signals within the run). Probe ratios are indicated by the dots. Black: within the 95% confidence interval (CI) of the reference sample population, Red: out of the 95% CI and over the arbitrary borders (0.7 to 1.3 by default), Yellow: within the 95% CI, but over the arbitrary borders. Whiskers: 95% CI for sample value (test or reference). Boxes: 95% CI in reference sample population (by default). Blue: compared to test probes, Green: compared to reference probes.

Figure 2 MLPA results for *BRCA2* gene. MLPA results for *BRCA2* gene of a patient with familial breast cancer in bar chart format generated by Coffalyser.Net 01 software. *BRCA2* exons and intra normalized ratio are given on the X axis and Y axis respectively (intra normalized ratio: division of probe signal of each amplification product by all reference probe signals within the run). Probe ratios are indicated by the dots. FRY- 61 probe and N4BP2L1-3 probe detect sequences just before and after the *BRCA2* gene. Black: within the 95% confidence interval (CI) of the reference sample population, Red: out of the 95% CI and over the arbitrary borders (0.7 to 1.3 by default), Yellow: within the 95% CI, but over the arbitrary borders. Whiskers: 95% CI for sample value (test or reference). Boxes: 95% CI in reference sample population (by default). Blue: compared to test probes, Green: compared to reference probes.

patients were positive for clearly pathogenic *BRCA2* mutations. The same cohort was used for the detection of *BRCA1* and *BRCA2* genomic rearrangements in the present study.

Detection of *BRCA* rearrangements is very important in a population since in some populations the occurrence of large deletions and duplications in either *BRCA1* or *BRCA2* is substantial. A prevalence of 2.1% for *BRCA1* large genomic rearrangements has been detected in Spanish hereditary breast/ovarian cancer families testing negative for point variations and small insertions/ deletions in *BRCA1* and *BRCA2* [12]. *BRCA1/2* large genomic rearrangements have shown noticeable founder effect in certain European and American populations. One large genomic rearrangement, *BRCA1* exon 9–12 deletion, is considered as a mutation in Mexican population [13]. In Northern Finland, a large deletion of exons 1A-13 in *BRCA1* gene is currently identified to represent 14.3% (1/7) of the Finnish population [14].

Arthrobacter luteus (*Alu*) short stretches of repetitive DNA appear to be the main source of large genomic rearrangements by providing hotspots for unequal

homologous recombination [15,16]. Several large genomic rearrangements reported in *BRCA1* have been frequently recognized within intragenic *Alu* repeats [16] and *BRCA1* pseudogene (Ψ*BRCA1*) 30 kb upstream [17,18]. To date, at least 81 different large genomic rearrangements have been found in *BRCA1* gene and account for 8%–27% of all *BRCA1* mutations. Alternatively *Alu* sequences are less common in *BRCA2* gene, where only few large genomic rearrangements are reported, accounting for 0%–11% of all *BRCA2* mutations [19-21].

The aim of this study was to assess the contribution of *BRCA1* and *BRCA2* rearrangements for predisposing to breast cancer in familial breast cancer patients and at risk individuals in Sri Lanka. We did not observe any conclusive large genomic rearrangements of *BRCA1* and *BRCA2* among the subjects studied. However in other Asian countries like Singapore three novel *BRCA* rearrangements have been found [9]. These were exon 13 duplication and exon 13–15 deletion of *BRCA1,* and exon 4–11 duplication in *BRCA2. BRCA1* genomic rearrangement found in Korean population involved exons 13–15. This exon 13–15 deletion has also been identified in

three families with America/French-German, Danish, and Singaporean/Indian ethnicities [12,22,23]. In a Malaysian population, two genomic rearrangements in *BRCA1* (exon 13–15 deletion and exon 1–14 deletion) and one in *BRCA2* (exon 14–16 deletion) were detected [10].

According to the findings of the present study *BRCA1* and *BRCA2* large genomic rearrangements are unlikely to significantly contribute to breast cancer in Sri Lanka. This is the first report on the analysis of *BRCA1* large genomic rearrangements in Sri Lanka.

The importance of recognizing the large rearrangements with respect to *BRCA1/2* is explained by *BRCA* rearrangements showing apparent founder effect in some populations which can be used as diagnostic tools. BRAC Analysis Large Rearrangement Test (BART) is already established in the country like US and also has been introduced as new updates to HBOC (Hereditary Breast and Ovarian Cancer) guidelines by National Comprehensive Cancer Network (NCCN). So BART is especially recommended for the individuals with strong personal and family history of breast and ovarian cancer along with routine BRCA analysis. Under such circumstances, it is so important to undergo large genomic rearrangements analysis by the familial breast cancer patients and their at risk individuals in a particular population as a diagnosis tool for breast cancer.

Conclusion

Although we failed to find any conclusive *BRCA1* large genomic rearrangement and did not find any *BRCA2* large genomic rearrangement in familial breast cancer patients and at risk individuals in the current study, a large study sample especially including Eurasians and other ethnic groups may reveal novel or reported genomic rearrangements among Sri Lankans.

Methods

Study participants

A total of 105 participants (N = 57 with a family history of breast cancer, N = 25 at risk individuals and N = 23 healthy controls without a personal or family history of any cancer) were studied. Mean age at diagnosis was 47.76 ± 9.55 years for familial breast cancer patients.

Fourteen familial patients were diagnosed below 40 years of age. Mean age at the MLPA analysis was 36.88 ± 14.95 for at risk individuals. Among the familial cases 34, 17 and 4 patients had one, two and three affected family members respectively. Two patients had 4 affected family members. According to histopathlogical data of familial breast cancer patients, 48 had infiltrating (invasive) ductal carcinoma and data were not recorded for remaining 9 patients. None of the patients had metastasis.

The majority of the patients and controls and all at-risk individuals were ethnically Sinhalese. There were no descendents of Europeans. Ethical approval from the Research, Ethics and Higher Degree Committee, Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo and written informed consent from the study participants were obtained prior to the study.

Genomic DNA was extracted using the protocol described by Miller et al. [24] from aliquots of peripheral blood samples that had been stored at –20°C. MLPA was performed using the SALSA MLPA KIT P002-C1 BRCA1 probemix and SALSA MLPA KIT P090 BRCA2 probemix (MRC-Holland, Amsterdam, Netherlands) for *BRCA1* and *BRCA2* genes according to manufacturer's protocol. The processed data obtained via MegaBACE Genetic Profiler software suite® v2.2 as well as via ABI GeneMapper® v4.1 were analyzed by using Coffalyser. Net 01 software.

Exon 5, 6 and 7 specific primers were designed in order to confirm the predicted ambiguous deletion detected from MLPA data. These primers were able to amplify whole regions of exon 5, 6 and 7 and MLPA probe hybridization site as well as several intronic regions. Resultant PCR products were subjected to direct sequencing using Applied Biosystems™ 3500 DX Genetic Analyzer in order to locate the deletion site of exon 6 as well as to confirm the data obtained from MLPA analysis.

Authors' contribution

SDS carried out molecular genetic studies, sequence alignment and drafted the manuscript. EHK and KHT conceived and designed the study, helped molecular genetic studies, data analysis and revision of the manuscript. IA and PA provided clinical expertise, recruitment of study participants and supervised clinical data and sample collection. All authors read and approved the final manuscript.

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