Comparison of the Diagnostic Accuracy of Next Generation Sequencing and Microarray Resequencing Methods for Detection of BRCA1 and BRCA2 Gene Mutations

BRCA1 ve BRCA2 Gen Mutasyonlarının Saptanmasında Yeni Nesil Dizi Analizi ile Mikroarray Tekrar Dizileme Yötemlerinin Tanısal Doğruluklarının Karşılaştırılması

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ABSTRACT

Objective: Breast cancer constitutes 29 % of estimated new cases of cancer in women, and it is also one of the major cause of death in all cancer types. In this study, DNA samples of familial breast cancer patients with BRCA1 and BRCA2 mutations which had been analyzed using conventional DNA sequencing method, were also analyzed with new methods including microarray and next generation sequencing (NGS) in order to compare their results

Methods: Seven patients with BRCA1 mutation, one patient with BRCA2 mutation, and two controls were included. All samples for the microarray method were studied on the GeneChip 3000 Scanner (Affymetrix) system and then analyzed on the Affymetrix GeneChip Resequencing Analysis Software (GSEQ® v4.0) system. Four patients from the patient group were selected for next generation sequencing and were analyzed on GS Junior 454 (Roche, Prague, Czech Republic) system. The raw data had been analysed by SeqPilot SeqNext module (v4.0, JSI medical systems, Kippenheim, Germany).

Results: Microarray resequencing analysis did not detect the mutations defined by conventional sequencing in patients, but mutations were detected in all of the 4 patients in the next generation sequencing.

Conclusion: Our study detected the NGS to be reliable as conventional DNA sequencing method for studying BRCA1/BRCA2 gene mutations. However, we suggest to confirm the NGS results with a conventional method because of homopolymer sequences which may cause false positive results.

Key Words: BRCA1, BRCA2, breast cancer, microarray, next generation sequencing

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INTRODUCTION

Breast cancer is not only the most common malignancy in women throughout the world but also constitutes 29% of the estimated new cases of cancer in women, but it is also one of the major causes of death in all cancer types (26%) (1). Together, the overall BRCA1/2 mutation prevalence was 24.0% in families with breast cancer and ovarian cancer history (2). So far, 1933 pathogenic variants have been identified in BRCA1 gene and 1795 pathogenic variants have been identified in BRCA2 gene. The common mutation forms are small insertion, small deletion, nonsense mutation, missense mutation, premature transcription termination, and splicing troubles. Deletion and insertion mutation also leads to a frame shift mutation (3).

Next-generation sequencing (NGS) allows for simultaneous sequencing of multiple cancer susceptibility genes and, for an individual, may be more efficient and less expensive than traditional testing. Different molecular methods are used for mutation analysis of BRCA1 and BRCA2 genes. Direct sequencing allows specification of the sequence alteration and is considered as gold standard. Because direct sequencing is time-consuming, there is necessity to perform alternative faster methods for BRCA1 and BRCA2 routine diagnostics with same accuracy.

MATERIALS and METHODS

This study was approved by the National Local Research Ethics Committee, Gazi University, Ankara, Turkey with an approval of # 221. Eight of the 10 individuals studied were found to have mutations in the BRCA1 or BRCA2 gene, two of which belonged to individuals who were found to have no mutations in the these genes. Eight patients and one control DNA sample were selected from patients who had previously undergone conventional sequencing analysis and/or MLPA mutation analysis for BRCA1/2 genes at Acibadem Hospital Genetik Diagnos Ctr, Istanbul. Other control DNA used in the study belonged to a case in which a mutation in the BRCA1/2 genes was not detected by a conventional sequence analysis within the scope of a research at Bilkent University Department of Molecular Biology and Genetics. Table 1 shows the previous analyzes results of the patients.

Table 1. Comparison of method results

| Gene/ Exon | Sanger Sequencing/ MLPA | Homozygote/ Heterozygote | Microarray | NGS |
|------------|-------------------------|--------------------------|------------|-----|
| Patient 1  | BRCA1/ Exon 10          | c.2863→2867delTCATC      | Heterozygote | not detected | not studied |
| Patient 2  | BRCA2/ Exon 11          | c.4987_4990delIGTCA      | Heterozygote | not detected | not studied |
| Patient 3  | BRCA1/ Exon 10          | c.788_789insG            | Heterozygote | not detected | not studied |
| Patient 4  | Deletion 13-22 exons (MLPA) | not detected            | Heterozygote | not detected | not studied |
| Patient 5  | BRCA1/ Exon 10          | c.2019delA              | Heterozygote | not detected | not studied |
| Patient 6  | BRCA1/ Exon 10          | c.843_846delCTCA         | Heterozygote | not detected | not studied |
| Patient 7  | BRCA1/ Exon10           | c.379delA               | Heterozygote | not detected | c.843_846delCTCA/wt |
| Patient 8  | BRCA1/ Exon10           | c.326delC               | Heterozygote | not detected | c.379delA/wt |

DISCUSSION

Genetic tests are required for treatment and follow-up of patients with breast cancer. Sanger sequence is still considered to be the gold standard method of genetic diagnosis in the identification of the human genome. However, the widespread use of newly discovered techniques has led to the search for new alternative diagnostic methods that are faster, cheaper and more reliable. For screening the entire sequence of genes with many exons, such as BRCA1 and BRCA2 recently developed these techniques is now being preferred as a priority.

Among patients with breast cancer and severe family histories of cancer, who test negative (wild type) for BRCA1 and BRCA2 can be expected to carry a large genomic deletion or duplication in one of these genes (6). Indeed, 12-15% of deleterious mutations in the BRCA1 gene correspond to large rearrangements ranging between 0.5 and 160 kb (7). Authors suggested that the high resolution of oligonucleotide array-CGH help to detect large rearrangements missed by other current methods, such as MLPA whose main limitation is a SNP (7). In a study including 33 familial breast and over cancer patients, mutations in the BRCA1 and BRCA2 genes were analyzed by microarray. They asserted that the accuracy of the microarray method for determining single nucleotide variations was calculated as 100% (8). Along with this, in our research, we could not detect any change in the patients with the microarray. We expected that the method could detect large deletions that existed in Patient 4, even if we could not catch the mutations found in other patients with the cause of technical limitations. It has been understood that researchers who have achieved success using this method have played an important role of their usage home-designed specific oligonucleotide primers in these achievements (7, 8). For this reason, the results show that the microarray method is not suitable for the use of mutation analysis of the BRCA1/ BRCA2 genes without using custom designed primers.
In the NGS analyzes performed on different platforms, it is stated that the method can detect large deletions according to the Sanger method but that the false positive results caused by the homopolymer sequences are the main limitations of the methods (9). In this reason, variations detected by this method and seen as suspicious should be verified by the Sanger method. In our study, Roche GS Junior 454 Sequencing NGS system was used. We observed that the mutations and polymorphisms previously determined by the Sanger method were also determined by the NGS system. However, we also detected some homopolymer sequence regions have resulted in erroneous readings and false positive results.

In the case of BRCA mutations, family history only accounts for 30–50% of mutations. Already, NGS may improve genetic testing in families with histories of high penetrance cancer genes. Economical NGS screening will also benefit patients with denovo mutations who would not otherwise undergo genetic screening based on family history (10). It should also be noted that the NGS system has an advantage over the Sanger method as it can detect large deletions and insertions, at the same time the analyzes can be performed quickly and inexpensively. In addition to its sensitivity and reliability, as a result of ability to scan large numbers of genes at the same time, the NGS generated data allows for more sophisticated analysis of gene interactions.

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Conflict of interest
No conflict of interest was declared by the authors.

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