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 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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ÖZET

Amaç: Meme kanseri kadınlarda yeni tanı alan kanserlerin %29'unu oluşturmakla birlikte, tüm kanser türleri içerisinde mortalite riski en yüksek kanser türüdür. Bu çalışmada ailesel meme kanseri olgularında geleneksel DNA dizileme yöntemi kullanılarak *BRCA1* ve *BRCA2* mutasyonu saptanmış bireylerin DNA örnekleri, mikrodizin ve yeni nesil dizileme yöntemleri ile analiz edilmiş ve tüm bu yöntemlerin tanıdaki başarıları karşılaştırılmıştır.

Yöntem: Çalışmamıza *BRCA1* mutasyonuna sahip yedi, *BRCA2* mutasyonuna sahip bir hasta ve iki kontrol dahil edilmiştir. Mikrodizin yöntemi için tüm örnekler GeneChip 3000 Scanner (Affymetrix) sisteminde çalışılmış, ardından Affymetrix GeneChip Resequencing Analysis Software (GSEQ® v4.0) sisteminde analiz edilmiştir. Hasta grubundan dört hasta yeni nesil dizileme için seçilmiş ve GS Junior 454 *(Roche, Prague, Czech Republic*) sisteminde çalışılmıştır. Yeni nesil dizileme çalışmasının sonuçları ise SeqPilot SeqNext module (v4.0, JSI medical systems, Kippenheim, Germany) sisteminde analiz edilmiştir.

Bulgular: Hasta grubunda daha önce tespit edilmiş mutasyonlar mikrodizin analizinde saptanmamıştır, fakat yeni nesil dizileme yöntemiyle çalışılan dört hastanın dördünde de daha önce belirlenmiş olan mutasyonlar tespit edilmiştir.

Sonuç: Çalışmamızda yeni nesil dizileme yönteminin *BRCA1/BRCA2* genlerinde görülen mutasyonları yakalamada, geleneksel DNA dizileme yöntemi kadar başarılı olduğunu göstermiştir. Yine de yeni nesil dizileme yöntemi homopolimer diziler sebebiyle yanlış pozitif sonuçlar verebileceğinden, bu yöntemle elde edilen sonuçların geleneksel yöntemle doğrulanması gerekmektedir.

Anahtar Sözcükler: *BRCA1, BRCA2*, meme kanseri, mikrodizin, yeni nesil dizileme

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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 1953 pathogenic variants have been identified in *BRCA1* gene and 1795 pathogenic variants have been identified in *BRCA2* gene. The most 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 sequential 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.

Multiplex Ligation Dependent Probe Amplification (MLPA) is a common method detecting large deletions/ duplications within the *BRCA1* or *BRCA2* genes, as this type of mutation cannot be detected by the standard direct sequencing. As an alternative to this latter method; also the array-based chip technology using SNP-specific oligonucleotides (Microarray) is used. Next Generation Sequencing (NGS) methods which is sequencing large DNA regions are much faster from currently available diagnostic techniques and based on their advantages it is currently replacing the previous tecniques.

Our results demonstrated that detection of the mutations including large or small deletions/insertions in *BRCA1* or *BRCA2* genes using microarray analysis were not successful, whereas next generation sequencing revealed more diagnostic and reliable results.

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 sequence analysis and/or MLPA mutation analysis for *BRCA1/2* genes at Acıbadem Hospital Genetic Diagnosis Center, 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	c2863¬2867delTCATC	Heterozygote	not detected	not studied
Patient 2	BRCA2/ Exon 11	c.4987_4990delGTCA	Heterozygote	not detected	not studied
Patient 3	BRCA1/ Exon 10	c.788_789insG	Heterozygote	not detected	not studied
Patient 4	BRCA1	Deletion 13-22 exons **(MLPA)**	Heterozygote	not detected	not studied
Patient 5	BRCA1/ Exon 10	c.2019delA	Heterozygote	not detected	c.2019delA/wt
Patient 6	BRCA1/ Exon 10	c.843_846delCTCA	Heterozygote	not detected	c.843-846delCTCA/wt
Patient 7	BRCA1/ Exon10	c.3794delA	Heterozygote	not deteced	c.3794delA/wt
Patient 8	BRCA1/ Exon10	c.5266insC	Heterozygote	not detected	c.5266insC/wt

Wt:wild type

The patient and control samples were studied with the GeneChip 3000 Scanner (Affymetrix) in accordance with the manufacturer's instructions. All study steps were carried out in accordance with the protocol using 10 ng/µl DNA samples (4). The obtained data were analyzed by the Affymetrix GeneChip Resequencing Analysis Software (GSEQ® v4.0). Only four patients selected due to financial limitations were studied with the next generation sequencing technology using GS Junior 454 System platform *(Roche, Prague, Czech Republic*). This assay had been used in order to amplify the coding regions of the *BRCA1* and *BRCA2* genes, including SNVs and CNVs. In the study, 25 ng/ml DNA was amplified with BRCA MASTR DX kit (*Multiplicom, USA*) including specific primers capable of amplifying all encoded exons in the *BRCA1* and *BRCA2* genes. The following steps of the study were carried out in accordance with the protocol (5).

The raw data had been provided in SFF file format. The SFF data had been analysed by SeqPilot SeqNext module (v4.0, JSI medical systems, Kippenheim, Germany). The SNVs were filtered by their occurrence in at least 25% of the reads. Distinct variations were checked against the in-house and mutation databases. Due to inaccurate sequencing of homopolymers by Roche 454 pyrosequencing, small indels in homopolymer stretches were filtered using stringent criteria (bidirectional occurrence in at least 20% of the forward reads and 40% of the reverse reads or vice versa) and visual inspection in the SeqNext software.

RESULTS

None of the 8 patients carrying the mutation in the study were able to detect these variations by microarray analysis. Mutations detected by conventional sequencing have been identified in all 4 patients in which we performed next generation sequencing analysis. None of the two control patients showed any change in the analyzes performed with either method (Table 1). We also detected some homopolymer sequence regions have resulted in erroneous readings and false positive results.

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 sizing 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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